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Biochemical Changes in Humans Upon Exposure to Ozone and Exercise

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BIOCHEMICAL CHANGES IN HUMANS UPON EXPOSURE TO OZONE AND EXERCISE

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FOREWORD

The many benefits of our modern, developing, industrial society are accompanied by certain hazards. Careful assessment of the relative risk of existing and new man-made environmental hazards is necessary for the establishment of sound regulatory policy. These regulations serve to enhance the quality of our environment in order to promote the public health and welfare and the productive capacity of our Nation's population.

The Health Effects Research Laboratory, Research Triangle Park, conducts a coordinated environmental health research program in toxicology, epidemiology, and clinical studies using human volunteer subjects. These studies address problems in air pollution, non-ionizing radiation, environmental carcinogenesis and the toxicology of pesticides as well as other chemical pollutants. The Laboratory participates in the development and revision of air quality criteria documents on pollutants for which national ambient air quality standards exist or are proposed, provides the data for registration of new pesticides or proposed suspension of those already in use, conducts research on hazardous and toxic materials, and is primarily responsible for providing the health basis for non-ionizing radiation standards. Direct support to the regulatory function of the Agency is provided in the form of expert testimony and preparation of affidavits as well as expert advice to the Administrator to assure the adequacy of health care and surveillance of persons having suffered imminent and substantial endangerment of their health.

Although animal studies have led to information which may be pertinent to the question of long-term human health effects, much more data is needed. With the increase in the atmospheric ozone levels, it is important to study the effects of ozone exposure directly to humans. This is one of only a few studies on the effects of human exposure to ozone that have been reported.

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ABSTRACT

A total of 44 human subjects were exposed to 784 $\mu g/m^3$ (0.4 ppm) ozone and 30 human subjects were exposed to air as controls. A four hour exposure was given on each of five consecutive days. Half of each group received an higher level of treadmill exercise than the other half.

Nine biochemical blood parameters were measured pre and post exposure: red blood cell glutathione reductase, vitamin E, red blood cell cholinesterase, red blood cell glucose-6-phosphate dehydrogenase, lactic acid dehydrogenase, complement C_3, and IgM. Of these variables only vitamin E, red blood cell glucose-6-phosphate dehydrogenase, and complement C_3 showed significant changes due to treatment.

This report covers a period from June 13, 1977, to May 19, 1978, and work was completed as of October 1, 1978.

INTRODUCTION

Modern manufacturing methods and the enormous increase in the number of cars and trucks on our streets and highways have lead to increased levels of atmospheric ozone, a key component of oxidant smog. Our populations, particularly in urban areas, are exposed to ozone concentrations up to 0.7 ppm at peak times and average hourly concentrations of 0.2-0.3 ppm.[1] This increase raises many questions with respect to the effects of ozone on human health. Only a few studies on the effects of human exposure to ozone have been reported.[2,3,4,5] These studies involved pulmonary function measurements on subjects from different geographic locations after exposure to ozone. Individuals living in areas with high ozone levels showed an adaptation in pulmonary function when compared to those subjects from areas of low ozone concentrations.

Species differences make generalizing data from animal studies somewhat tenuous and, for obvious reasons, the human lung is not directly available for study. However, animal studies have led to some information which may be pertinent to the question of long-term human health effects. Previous studies have shown evidence for lipid peroxidation in red blood cells exposed to ozone *in vitro* and in lungs of mice exposed to 0.7 ppm ozone *in vivo*.[6,7] Stokinger et al.[8] have reported that rabbits exposed to ozone had detectable amounts of circulating antibodies in their serum as a result of tyrosine oxidation leading to protein alteration and denaturation. Goldstein et al.[9] indicated that rats deficient in vitamin E exhibit a greater susceptibility to lethal levels of ozone. In addition, rats exposed to sublethal ozone concentrations show an accelerated

decline in serum vitamin E levels. The importance of cellular sulfhydryl moieties in maintaining cellular integrity is suggested by the fact that glutathione (GSH) levels were maintained in ozone-tolerant rats but not in non-tolerant animals.[10]

The study reported here is a part of a concerted effort to define biochemical parameters which can be used to measure an ozone pollution burden on the general population. This study is a clinical evaluation of controlled human exposure, while most other studies reported have been based on animal exposure models and on epidemiological models.

METHOD

Subjects

Healthy, Caucasian, non-smoking, male, university students characterized by an average height of 181.4 cm (std. dev. 7.0, range 160.0-194.0 cm), an average weight of 76.0 kg (std. dev. 9.6, range 57.2-109.2 kg) and an average age of 25.1 years (std. dev. 2.5, range 19.7-30.1 years) were exposed to clean air (controls) or to 784 $\mu g/m^3$ (0.4 ppm) ozone. Each group of subjects was recruited and tested separately and sequentially. In Group 1, 29 subjects (light exercise, air exposure) were tested from June 13 through August 26, 1977; in Group 2, 15 subjects (light exercise, ozone exposure) were tested from October 31 through December 9, 1977; in Group 3, 15 subjects (heavy exercise, ozone) were tested from February 27 through March 31, 1978; and in Group 4, 15 subjects (heavy exercise, air exposure) were tested from April 17 through May 19, 1978.

Experimental Design

A total of nine plasma, serum, and red blood cell biochemical measures were chosen as dependent variables. The analysis techniques

used incidentally yielded many more. The small number of subjects required that the number of variables analyzed be kept to a minimum. Consequently, variables were chosen on three bases. First, variables with large amounts of missing data were eliminated. Second, a priori biochemical judgment was used to eliminate inappropriate variables. Third, evaluation of the control day data, in combination with the logical relations of certain variables, indicated a small number of variables which were redundant (and hence eliminated) with some included in the analysis.

Subjects were exposed in groups of three for five consecutive days (always Monday through Friday) to four hours of either air or ozone. Exposures began at approximately 8:00 a.m. On Monday, all subjects received air. Groups 2 and 3 received 784 $\mu g/m^3$ (0.4 ppm) ozone on Tuesday through Friday. Groups 1 and 2 received two 15 minute exercise sessions (at 1 and 3 hours of exposure) which consisted of walking 6.44 km/hr (4 mph) on a treadmill inclined at 0°, and groups 3 and 4 received the same exercise regimen except that the treadmill was inclined at 10°. During the exposures, the temperature was maintained at 20°C with a relative humidity of 40%, and air flow through the exposure chamber of 493 cubic meters/minute.

The factors in the design include two levels of ozone (0, 784 $\mu g/m^3$), two levels of exercise (0°, 10° treadmill inclination), four levels of day (Tuesday through Friday) and two levels of pre-post (before exposure, after exposure). Ozone and exercise are between

subject factors and days and pre-post are repeated measures. A multivariate analysis of variance (MANOVA) approach was used: the eight repeated measures were taken to be eight dependent variables with a two factor design. Estimation of day effect and pre-post effect were therefore contrasts between dependent variables, as were any interactions involving either or both. This approach completely avoids the restrictive assumption necessary with a univariate random effects model for repeated measures.[11]

One MANOVA was conducted for each dependent variable. This approach is justly criticized if the observed p values are reported as "significant" when compared against nominal values of 0.05 or 0.01. As was done for this paper, that problem is solved by using the Bonferroni technique to correct the false probability levels.[12] Here that means an observed p of 0.01/9 = 0.0011 was required for the effect to be declared "significant." This approach insures that the test of a particular effect will be done at the 0.01 level for the set of nine variables taken together. Hence, appropriate multivariate p levels are used rather than the biased univariate p levels.

A serum and plasma sample were analyzed daily from each participant before and after each 4 hour exposure during the week. Samples were also taken the following Monday (a one week follow-up), at two weeks, and again at four weeks. For the purposes of significance testing, only data for actual exposure days were analyzed, which included Tuesday through Friday. Monday data is reported for control information. The follow-up data were collected to assure that any effects that occurred returned to baseline to assure subject safety.

Blood Analysis

The following enzymes were analyzed on the CentrifiChem 400 Autoanalyzer: (1) serum glutamic-pyruvic acid transaminase (SGPT) by a modified Wroblewski and LaDue technique,[13] (2) alkaline phosphatase by a modified Bessey-Lowry-Broch technique,[14] (3) lactic acid dehydrogenase (LDH) was assayed in the direction of conversion of lactate to pyruvate,[15] (4) red blood cell cholinesterase by a modification of the Ellman method,[16] and (5) red blood cell glucose-6-phosphate dehydrogenase (G6PDH) by the procedure of Catalano.[17]

Red blood cell and serum glutathione reductase were assayed by the method of Nichoalds[18] modified such that the enzyme was preincubated at 0° for 30 minutes in the presence of 60 μM flavin adenine dinucleotide (FAD) and the reaction was initiated by the simultaneous addition of 2 mM oxidized glutathione (GSSG) and 0.24 mM reduced nicotinamide adenine dinucleotide (NADPH). To measure serum vitamin E, the following technique was used. In a 15 ml, glass-stopped centrifuge tube, 2 ml of ethanol-L-ascorbic acid reagent (200 mg L-ascorbic acid in 10 ml 95% ethanol) was mixed with 0.5 ml of serum and immediately vortexed for 15 seconds. After standing at room temperature at least 2 minutes, the tubes were then placed in an 80°C water bath for 3 minutes. The samples were removed from the water bath and 2 ml of water were added to each and vortexed. After being allowed to cool, 5 ml of hexane was added to each and each sample was vortexed 75 seconds. The phases were separated by centrifuging in a clinical centrifuge at 1200 rpm for 10 minutes at room temperature. A 4 ml aliquot of the top phase was transferred to a

conical tube and evaporated to dryness in a stream of nitrogen. The evaporated sample was reconstituted in 0.1 ml benzene and 0.025 ml was spotted in duplicate on Bodman LQ6D silica gel plates. The plate was developed for 20 minutes in hexane:acetone:reagent grade anhydrous ethyl ether (80:20:10). After the plate was air dried for 5 minutes, it was sprayed with a solution of acidic cupric acetate (6 gm cupric acetate/ 100 ml 15% H_3PO_4). The spots were made visible by charring in a 160°C oven for 10 minutes. The plate was then scanned at 405 mμ with a Schoffel densitometer.

Serum proteins were determined by nephelometry.[19]

Materials

A CentrifiChem Model 400 centrifugal analyzer and CentrifiChem autopipetter (Union Carbide Corporation, Clinical Diagnostics, Rye, New York 10580) were used for assaying acetylcholinesterase, G6PDH, LDH, SGPT, and alkaline phosphatase. Manual assays for glutathione reductase were run on a Cary 118 spectrophotometer. LDH (L→P), SGPT and alkaline phosphatase reagent kits were purchased from Union Carbide Corporation, Clinical Diagnostics, Rye, New York 10580. Cholinesterase reagent kits were purchased from Bio-Dynamics/BMC, Norcross, Georgia 30093.

D,L-α-tocopherol, oxidized glutathione (free acid, grade III), FAD (disodium salt, grade III), nicotinamide adenine dinucleotide phosphate reduced form (tetra sodium salt Type 1, NADPH), nicotinamide adenine dinucleotide phosphate monosodium salt ($NADP^+$) Sigma grade, 6-phosphogluconic dehydrogenase Type V from yeast, 50 U/ml, and D-glucose-6-phosphate monosodium salt were purchased from Sigma Chemical Company, St. Louis, Missouri 63178.

RESULTS

The results are organized into two major sections. The first addresses characteristics of all of the variables simultaneously, while the second provides a detailed description of the analysis of each separate variable. Our approach has been to provide sufficient detail so that the interested reader has enough information available to examine any particular variable in detail. We have included comments on only those results of particular interest.

Overall Statistical Description

Table 1 provides summary statistics for each dependent variable, for each day at both pre and post sampling times. Means, standard deviations, minima, maxima, and skewnesses are included. Note that not all lines are based on exactly the same number of observations, and that the data for all four exposure groups have been combined. Of the approximately 75 subjects who participated in the experiment, complete data sets were obtained on 50 to 60 subjects for each test. Some subjects were excluded due to: (1) clinical reasons not related to the experiment, for example, one subject was excluded when it was discovered that he had a G6PDH deficiency, or (2) the limited quantity of blood available for each test prevented repeating the test in the case of equipment failure. These measures are included to indicate the range of the data observed. All values observed appear to fall within a "normal" range. Consideration of Table 1 and associated frequency histograms did not indicate any serious problems in using analysis of variance on these data.

Table 2 presents means for each group for pre and post measurements taken on the control day preceeding the exposure days. A number of comments need to be made. First, these measurements were taken 4 hours apart, with two 15-minute exercise periods at the beginning of the second and fourth hours. Second, each group is in the "normal range" of values. Third, it is instructive to note that the variability pre to post seems to be much less than the variability among groups. In particular, Group 3 haptoglobin results seem far higher than the other groups. These variations may reflect the way the data were collected, i.e., that all subjects in Group 1 were tested in a contiguous time period, then all subjects in Group 2, then all subjects in Group 3, and finally, all subjects in Group 4.

Table 3 presents the intercorrelations of all nine dependent variables for the control day plus height, weight, and age. Height, weight, and age were included in order to evaluate their usefulness as covariates. Since the maximum correlation of any of the three with any dependent variable is 0.29, and the next largest correlation is 0.17, it is obvious that height, weight, and age are useless as covariates. Since no dependent variables overlap to any great extent with any other dependent variable, it is possible to examine each dependent variable separately, particularly since a few variables which showed excessive overlap were eliminated from the analysis.

Analysis of Variance Summary

Tables 4.1-4.9 summarize the multivariate analyses of variance (MANOVA) for the nine dependent variables. In these source tables most

of the tests are multivariate tests. For these tests the likelihood ratio criterion was used and tested with Rao's F approximation. Where appropriate, univariate tests were computed, and therefore likelihood ratios are not presented. The actual alpha level for each family of hypotheses was 0.01. Further, note that some lower order effects which are "significant" by this standard are not marked as significant. They should not be because they are subsumed under higher order effects which were significant. These results indicate at least some effects of ozone, exercise, and time. However, not all variables show these effects. Table 4.0 summarizes the significant effects. Results for each variable will be discussed in detail later.

Tables 5.1-5.9 present means for each dependent variable for each group based on the MANOVA. The single variable results to be discussed will lead to simpler tables presented later. One simplification of these tables is presented in Figures 1.1-1.9. Since the analysis indicates little, if any, pre to post difference, the averages of each day's pre and post means were plotted. These will be referred to specifically when each variable is discussed individually.

Red Blood Cell Glutathione Reductase

Table 4.1 summarizes the MANOVA for RBC glutathione reductase. Table 5.1 provides, for each treatment condition, the mean response estimated by the analysis. These results show no indication of any effect of ozone, exercise, or pre-post factor. Figure 1.1 plots the mean response of RBC glutathione reductase for each of the groups for

each day. This figure supports the statistical analysis by also suggesting that natural variability is at least as large as any of the observed differences.

Vitamin E

Table 4.2 provides a source table for the MANOVA conducted on serum vitamin E. Only the ozone by exercise interaction produced a significant effect. This result indicates that the effect of ozone on serum vitamin E depends upon the level of exercise. Thus, a greater burden of ozone per unit time may result with increased exercise. Consideration of Table 5.2 and Figure 1.2 do not directly address this question.

Note that the effect is averaged across days and pre and post measurements. The four means tested by this hypothesis are: 5.8 μg/ml for Group 1 (light exercise, air), 7.0 μg/ml for Group 2 (light exercise, ozone), 5.8 μg/ml for Group 3 (heavy exercise, ozone) and 6.1 μg/ml for Group 4 (heavy exercise, air). Figure 2 provides a plot of these means. The plot indicates that the source of the significant effect is that Group 2 (light exercise, ozone) is different from the other three groups which, in turn, are all roughly the same. Note that Table 2 suggests that Group 2 begins close to Groups 3 and 4 relative to the difference observed. Hence, it appears that this effect did arise due to the ozone exposure experienced by the subjects. The reader should be cautioned to consider Figure 1.2 which indicates that relative to the variability between days this effect is not overwhelming.

Red Blood Cell Cholinesterase

Table 4.3, the source table for the MANOVA on RBC cholinesterase, indicates no significant effect of any treatment. Table 5.3 presents

the mean response for each group, for each point in time based on the analysis of variance. Figure 1.3 presents a plot of the means averaged across pre-post for each day for each group. Taken together these results appear to indicate no effect on RBC cholinesterase.

RBC G6PDH

Table 2 indicates that Group 3 subjects (heavy exercise, ozone) began the experiment with a G6PDH level higher than the other groups. This observation influences our interpretation of the remaining analyses. Consider Table 4.4, which summarizes the MANOVA for G6PDH, and Table 5.4 which contains means for each treatment condition. Two interactions are significant: exercise by day by pre-post and ozone by exercise. Figure 3 plots the means for exercise by day by pre-post. Only one clear pattern emerges, that of heavy exercise levels showing more variability than light exercise levels. Results of the interaction trend tests bear this out. The one degree of freedom contrast for a linear trend yielded a p value of 0.54, the contrast for a quadradic trend yield a p value of 0.0174 and the contrast for a cubic trend yield a p value of 0.0008. On this basis the cubic trend was decided to be the significant one. Since only four days are present, this only indicates that the means do differ reliably but does not indicate the nature of the relationship other than indicating that it is not a simple relationship.

Figure 4 plots the ozone by exercise means, averaged across days. The significance of this effect appears to be due to Group 3 (ozone, heavy exercise) being different from the other groups. However, it is important to note again that Group 3 began the experiment near the same

level as illustrated in Figure 4. Furthermore, the other three groups also began the experiment near the same levels as shown in Figure 4. Refer to Table 2 to compare this plot against control day measures.

Lactic Acid Dehydrogenase (LDH)

A normal range of 0-110 IU/l for LDH has been reported for the method used.[20] In contrast, our data show a range of 32-232 IU/l. Note that these two extreme values are not unique in our data. A possible factor may be the uncontrolled exercise level inherent in a subject getting from home to the facilities preceding the initial blood draw for the day.

Table 4.5 summarizes the MANOVA for LDH. The analysis indicates no effects of any of the treatments either individually or in combination. Means for each combination for LDH are presented in Table 5.5. Figure 1.5 plots mean LDH activity for each group for each day. No consistent pattern is present and the differences are small in light of the standard deviation of 20 IU/l.

α_1-Antitrypsin

Table 4.6 summarizes the MANOVA for α_1-antitrypsin. No effects were declared significant according to the decision rule discussed earlier, although some observers might argue that the ozone by exercise by pre-post or the ozone by days factors should be significant. In a situation like this, it is important to consider the means for each treatment condition reported in Table 5.6. They do not appear to indicate any particular pattern of results.

Figure 1.6 plots mean α_1-antitrypsin for each day for each group. In fact, it does appear to show a consistent pattern of an ozone-by-days interaction. Although these results appear more regular, the amount of variability present in α_1-antitrypsin causes the differences to be declared not significant.

Haptoglobin

Table 2 lists mean response of haptoglobin for each group on the control day. These results indicate two things. First, little difference is seen pre to post exposure. Second, Group 3 began the experiment with haptoglobin levels approximately 3 standard deviations higher than the other three groups, which are approximately the same.

Table 4.7 summarizes the MANOVA for haptoglobin. No results were declared significant. Given the apparent initial deviation of Group 3, it is useful to also consider Table 5.7 which lists the means for each group for each measurement point, and Figure 1.7 which plots the mean for each group for each day's haptoglobin data. Figure 1.7 and Table 2 provide a consistent picture. Group 3 began at a much higher level and remained there. Consequently, even if any result had been declared "significant," its interpretation would have had to have been strongly modified in light of the initial differences.

Complement C_3

Table 4.8, which summarizes the MANOVA for complement C_3, indicates a significant interaction of ozone by exercise by days. No other effects were declared significant. Table 5.8 provides mean response for each treatment condition. Figure 1.8 plots the means for the significant

ozone by exercise by day interaction. Tests for trends in this interaction clearly indicate a linear trend, $p \leq 0.00004$. In particular, Group 3 means increase linearly across days. However, the importance of this result is diminished in light of the change seen in Figure 1.8 in the other three groups.

IgM

Table 2 gives mean response of IgM for each group on the control day. Although the groups appear to vary, the differences are not large in comparison to the standard deviation of IgM. The standard deviation for IgM is much larger than complement C_3, haptoglobin, and α_1-antitrypsin, which are all measured on the same scale and by the same technique.

Table 4.9 indicates no significant results from the MANOVA for IgM. Table 5.9 provides mean levels of IgM for each treatment combination, while Figure 1.9 plots mean response across days for each group. Neither the table nor the figure indicate any pattern to the results.

Summary of Results

Although ozone is a highly reactive oxidizing agent, it showed no effect on several biochemical parameters, chosen for their sensitivity to an oxidative insult, as well as a pulmonary insult. These parameters included RBC glutathione reductase, RBC cholinesterase, LDH, α_1-antitrypsin, haptoglobin, and IgM. Three variables, however, serum vitamin E, RBC G6PDH, and complement C_3, appear to have shown some effect.

For serum vitamin E, the ozone by exercise interaction was significant. This was attributed to Group 2 (light exercise, ozone) being slightly higher than the other three groups in its response.

For RBC G6PDH, the ozone by exercise effect and the ozone by day by pre-post were significant. The ozone by exercise effect appears to be due to Group 3 being higher than the other three groups. It was noted that Group 3 also began the experiment at a higher level. The ozone by day by pre-post effect indicates that heavy exercise causes G6PDH levels to vary more than light exercise does.

Complement C_3 showed a significant ozone by exercise by day effect. The level for Group 3 was seen to increase linearly across time; however, Group 1 levels also increased across time. As with G6PDH, no clear pattern emerges.

DISCUSSION

In light of the highly oxidative effect of ozone, it would seem reasonable to expect a response in G6PDH, serum vitamin E and complement C_3 levels in volunteers exposed to ozone. The results, however, are not borne out by this simple relation, and in fact may be due to the variability in the data. This variability may result from the data collection scheme being sequential rather than counterbalanced. Results from a subsequent unpublished blood study, unrelated to the ozone exposure, support this hypothesis. The protocol of that study provided counterbalancing for day of the week and week of the year. Consequently, subjects were uniformly distributed across time and group. Analysis of data, including some of the same variables, indicate less variability between subjects and smaller initial differences between groups.

The positive responses that were observed are descriptive of the body's response to an oxidative challenge. G6PDH is an important enzyme

in the metabolism of glucose via the hexose monophosphate shunt. The hexose monophosphate shunt is responsible for maintaining levels of the reduced coenzyme NADPH. The NADPH is necessary to maintain sufficient levels of reduced glutathione which acts to protect cellular components from oxidation. In the presence of an oxidant such as ozone, the body may respond by increasing G6PDH activity or by increasing the levels of G6PDH. The mobilization of vitamin E, a known antioxidant, may also indicate a metabolic response to increased oxidant exposure, as demonstrated by the elevated levels of serum vitamin E.

The elevated level of complement C_3 is indicative of a mild inflammatory response. In turn, elevation of C_3 will result in elevated C_{3a} which is one of the anaphylatoxins of the complement system. This increase may be one of the reasons for the increased coughing and shortness of breath experienced by the subjects.

Taken together, these results indicate biochemical responses to ozone and exercise. The data at hand do not allow elucidation of the mechanism of such an effect. This study indicates directions which such research might profitably follow.

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TABLE 1
SUMMARY STATISTICS FOR ALL FOUR GROUPS COMBINED FOR ALL NINE VARIABLES
FOR THE CONTROL DAY

Variable	Units	Time	Mean	Std. Dev.	Min.	Max.	Skewness	N
GSH Reductase	μmoles/gm Hgb/min	Pre	7.59	1.75	4.29	11.45	0.13	54
		Post	7.59	1.95	4.06	13.20	0.46	
Serum Vitamin E	μg/ml serum	Pre	6.11	1.28	2.42	9.89	0.1	55
		Post	6.27	1.25	3.20	9.99	0.0	
RBC Cholinesterase	$mU/10^9$ RBC	Pre	1069	221	373	1595	-0.2	61
		Post	1087	234	393	1814	0.1	
G6PDH	U/gm Hgb	Pre	1.92	0.36	1.05	2.85	-0.4	50
		Post	1.97	0.42	1.06	3.64	0.8	
LDH	IU/l	Pre	87.6	20.7	51.0	155.0	0.9	54
		Post	90.3	18.4	48.5	148.	0.6	
α_1-Antitrypsin	mg/dl	Pre	175	29	90	238	-0.7	57
		Post	178	28	97	242	-0.5	
Haptoglobin	mg/dl	Pre	64	31	13	154	0.6	57
		Post	65	34	13	168	0.8	
Complement C_3	mg/dl	Pre	90	17	51	129	0.3	57
		Post	92	15	61	134	0.8	
IgM	mg/dl	Pre	112	47	22	213	0.3	57
		Post	119	53	31	255	0.7	

TABLE 2
GROUP MEANS ON CONTROL DAY

Pre Exposure

Variable	Group 1 Air Light Exercise	Group 2 O_3 Light Exercise	Group 3 O_3 Heavy Exercise	Group 4 Air Heavy Exercise
RBC Glut. Reduct. µmoles/mg Hgb/min	7.69	7.30	7.59	7.81
Serum Vitamin E µg/ml	5.45	6.34	6.29	6.36
RBC Cholinesterase mU/10^9 RBC	1086	1061	908	1202
G6PDH U/gm Hgb	1.72	1.92	2.17	1.93
LDH IU/l	92.0	85.6	77.6	92.0
α_1-Antitrypsin mg/dl	170	177	180	175
Haptoglobin mg/dl	56	58	89	57
Complement C_3 mg/dl	88	92	88	90
IgM mg/dl	105	117	100	125

Post Exposure

Variable	Group 1 Air Light Exercise	Group 2 O_3 Light Exercise	Group 3 O_3 Heavy Exercise	Group 4 Air Heavy Exercise
RBC Glut. Reduct. µmoles/mg Hgb/min	8.08	7.49	7.50	7.22
Serum Vitamin E µg/ml	5.62	6.63	6.15	6.60
RBC Cholinesterase mU/10^9 RBC	1075	1077	975	1214
G6PDH U/gm Hgb	1.75	1.95	2.31	1.96
LDH IU/l	94.2	87.0	83.5	92.9
α_1-Antitrypsin mg/dl	173	178	182	179
Haptoglobin mg/dl	54	60	86	69
Complement C_3 mg/dl	91	97	88	89
IgM mg/dl	116	127	105	126

TABLE 3
INTERCORRELATIONS OF CONTROL DAY MEASURES

	RBC Glutathione Reductase	Serum Vit. E	RBC Cholin-esterase	G6PDH	LDH	α_1 anti-trypsin	Hapto-globin	Comple-ment C_3	IgM	Ht	Wt	Age
RBC Glutathione	1.0											
Serum Vit. E	.04	1.0										
RBC Cholin-esterase	-.15	.13	1.0									
G6PHD	-.04	.12	-.21	1.0								
LDH	.11	.16	-.07	.06	1.0							
α_1-Antitrypsin	.01	.07	-.03	.30	.12	1.0						
Haptoglobin	.13	.05	-.10	.00	-.05	.07	1.0					
Complement C_3	.21	.15	.00	.00	.30	-.12	.41	1.0				
IgM	.00	.20	.17	-.18	.13	-.29	-.01	.15	1.0			
Ht	-.01	.11	-.01	.12	.02	.29	.12	.10	-.17	1.0		
Wt	.01	-.02	-.09	.17	.08	.11	.01	-.19	-.07	.43	1.0	
Age	-.01	.03	.03	-.18	-.07	.05	.13	-.13	-.14	-.24	-.09	1.0

TABLE 4.0
SUMMARY OF SIGNIFICANT EFFECTS FOR ALL DEPENDENT VARIABLES

	Variable								
	RBC Glutathione Reductase	Serum Vit. E	RBC Cholin-esterase	G6PDH	LDH	α_1 anti-trypsin	Hapto-globin	Comple-ment C_3	IgM
Ozone (O)									
Exercise (E)									
Days (D)									
Pre-post (P)									
O x E		*		*					
O x P									
E x D									
E x P									
D x P									
O x E x D								*	
O x E x P									
O x D x P									
E x D x P									
O x E x D x P				*					

TABLE 4.1
MANOVA FOR RBC GLUTATHIONE REDUCTASE

Source	Likelihood Ratio	$\tilde{F}$	Num. d.f.	Den. d.f.	P value
Ozone (O)	-	.01	1	50	.9322
Exercise (E)	-	0.0	1	50	.9772
Days (D)	.991	.15	3	48	.9324
Pre-Post (P)	-	.52	1	50	.4758
O x E	-	2.92	1	50	.0940
O x D	.874	2.30	3	48	.0889
O x P	-	.27	1	50	.6044
E x D	.918	1.42	3	48	.2483
E x P	-	.68	1	50	.4142
D x P	.892	1.94	3	48	.1352
O x E x D	.965	.59	3	48	.6277
O x E x P	-	2.94	1	50	.0925
O x D x P	.904	1.69	3	48	.1814
E x D x P	.945	.94	3	48	.4289
O x E x D x P	.939	1.03	3	48	.3870

TABLE 4.2
MANOVA FOR VITAMIN E

Source	Likelihood Ratio	$\tilde{F}$	Num. d.f.	Den. d.f.	P value
Ozone (O)	-	3.77	1	51	.0578
Exercise (E)	-	4.31	1	51	.0429
Days (D)	.993	.12	3	49	.9494
Pre-Post (P)	-	1.23	1	51	.2733
O x E	-	12.46	1	51	.0009*
O x D	.812	3.79	3	49	.0160
O x P	-	1.87	1	51	.1773
E x D	.887	2.08	3	49	.1149
E x P	-	1.39	1	51	.2434
D x P	.945	.95	3	49	.4223
O x E x D	.947	.44	3	49	.7249
O x E x P	-	.04	1	51	.8401
O x D x P	.807	3.90	3	49	.0141
E x D x P	.935	1.13	3	49	.3446
O x E x D x P	.859	2.69	3	49	.0567

*Significant at .01 level (Bonferroni).

TABLE 4.3
MANOVA FOR RBC CHOLINESTERASE

Source	Likelihood Ratio	$\tilde{F}$	Num. d.f.	Den. d.f.	P value
Ozone (O)	-	.68	1	57	.4121
Exercise (E)	-	6.77	1	57	.0118
Days (D)	.958	.81	3	55	.4959
Pre-Post (P)	-	.56	1	57	.4561
O x E	-	3.82	1	57	.0554
O x D	.967	.63	3	55	.6022
O x P	-	1.06	1	57	.3068
E x D	.937	1.23	3	55	.3074
E x P	-	.24	1	57	.6262
D x P	.882	2.45	3	55	.0724
O x E x D	.923	1.53	3	55	.2152
O x E x P	-	1.47	1	57	.2304
O x D x P	.904	1.95	3	55	.1308
E x D x P	.882	2.44	3	55	.0727
O x E x D x P	.899	2.05	3	55	.1157

TABLE 4.4
MANOVA FOR RBC G6PDH

Source	Likelihood Ratio	$\tilde{F}$	Num. d.f.	Den. d.f.	P value
Ozone (O)	-	3.25	1	46	.0779
Exercise (E)		2.74	1	46	.1046
Days (D)	.873	2.13	3	44	.1098
Pre-Post (P)	-	1.61	1	46	.2112
O x E	-	26.66	1	46	4.0×10^{-6}
O x D	.955	0.70	3	44	.5589
O x P	-	1.13	1	46	.2926
E x D	.938	.96	3	44	.4189
E x P	-	.97	1	46	.3287
D x P	.482	15.77	3	44	4.1818×10^{-7}*
O x E x D	.979	.31	3	44	.8146
O x E x P	-	.53	1	46	.4719
O x D x P	.797	3.73	3	44	.0178
E x D x P	.628	8.67	3	44	.0001*
O x E x D x P	.931	1.09	3	44	.3614

*Significant at the .01 level (Bonferroni corrected).

TABLE 4.5
MANOVA FOR LDH

Source	Likelihood Ratio	$\tilde{F}$	Num. d.f.	Den. d.f.	P value
Ozone (O)	-	.03	1	50	.8626
Exercise (E)	-	.76	1	50	.3882
Days (D)	.830	3.27	3	48	.0292
Pre-Post (P)	-	.39	1	50	.5370
O x E	-	3.14	1	50	.0825
O x D	.927	1.25	3	48	.3009
O x P	-	2.92	1	50	.0938
E x D	.880	2.19	3	48	.1019
E x P	-	.01	1	50	.9329
D x P	.942	.99	3	48	.4043
O x E x D	.938	1.05	3	48	.3770
O x E x P	-	1.03	1	50	.3155
O x D x P	.863	2.54	3	48	.0675
E x D x P	.958	0.70	3	48	.5538
O x E x D x P	.913	1.53	3	48	.2200

TABLE 4.6
MANOVA FOR α_1-ANTITRYPSIN

Source	Likelihood Ratio	$\tilde{F}$	Num. d.f.	Den. d.f.	P value
Ozone (O)	-	2.34	1	53	.1317
Exercise (E)	-	1.03	1	53	.3149
Days (D)	.798	4.30	3	51	.0089
Pre-Post (P)	-	6.67	1	53	.0126
O x E	-	.11	1	53	.7439
O x D	.801	4.21	3	51	.0098
O x P	-	2.75	1	53	.1031
E x D	.968	.57	3	51	.6433
E x P	-	1.41	1	53	.2410
D x P	.885	2.19	3	51	.0992
O x E x D	.925	1.38	3	51	.2579
O x E x P	-	7.16	1	53	.0099
O x D x P	.921	1.46	3	51	.2364
E x D x P	.930	1.27	3	51	.2941
O x E x D x P	.892	2.05	3	51	.1172

TABLE 4.7
MANOVA FOR HAPTOGLOBIN

Source	Likelihood Ratio	$\tilde{F}$	Num. d.f.	Den. d.f.	P value
Ozone (O)	-	11.29	1	53	.0014
Exercise (E)	-	5.17	1	53	.0270
Days (D)	.935	1.13	3	51	.3465
Pre-Post (P)	-	.24	1	53	.6247
O x E	-	1.85	1	53	.1789
O x D	.839	3.25	3	51	.0287
O x P	-	.01	1	53	.9270
E x D	.868	2.57	3	51	.0630
E x P	-	.44	1	53	.5109
D x P	.866	2.63	3	51	.0587
O x E x D	.972	.49	3	51	.6980
O x E x P	-	.14	1	53	.7051
O x D x P	.965	.61	3	51	.6143
E x D x P	.964	.62	3	51	.6077
O x E x D x P	.980	.34	3	51	.7958

TABLE 4.8
MANOVA FOR COMPLEMENT C_3

Source	Likelihood Ratio	$\tilde{F}$	Num. d.f.	Den. d.f.	P value
Ozone (O)	-	1.85	1	53	.1796
Exercise (E)	-	6.00	1	53	.0176
Days (D)	.964	.63	3	51	.6038
Pre-Post (P)	-	9.68	1	53	.0030
O x E	-	.02	1	53	.8866
O x D	.953	.84	3	51	.4792
O x P	-	1.48	1	53	.2284
E x D	.916	1.56	3	51	.2096
E x P	-	.61	1	53	.4368
D x P	.974	.45	3	51	.7207
O x E x D	.725	6.43	3	51	.0001*
O x E x P	-	1.83	1	53	.1812
O x D x P	.908	1.72	3	51	.1736
E x D x P	.915	1.58	3	51	.2035
O x E x D x P	.863	2.69	3	51	.0547

*Significant at the .01 level (Bonferroni corrected).

TABLE 4.9
MANOVA FOR IgM

Source	Likelihood Ratio	$\tilde{F}$	Num. d.f.	Den. d.f.	P value
Ozone (O)	-	.04	1	53	.8506
Exercise (E)	-	.30	1	53	.5880
Days (D)	.686	7.27	3	51	.0003
Pre-Post (P)	-	.11	1	53	.7429
O x E	-	.99	1	53	.3249
O x D	.875	2.42	3	51	.0752
O x P	-	.14	1	53	.7112
E x D	.934	1.20	3	51	.3173
E x P	-	1.28	1	53	.2638
D x P	.993	.13	3	51	.9391
O x E x D	.994	.11	3	51	.9506
O x E x P	-	0.0	1	53	.9832
O x D x P	.968	.56	3	51	.6492
E x D x P	.878	2.36	3	51	.0811
O x E x D x P	.963	.65	3	51	.5913

TABLE 5.1
MEAN RESPONSE FROM MANOVA FOR
RBC GLUTATHIONE REDUCTASE μmoles/mg Hgb/min

Day		Group 1 Air Light Exercise	Group 2 O_3 Light Exercise	Group 3 O_3 Heavy Exercise	Group 4 Air Heavy Exercise
2	Pre	7.82	7.31	8.68	7.38
	Post	7.37	7.95	8.35	7.65
3	Pre	7.93	7.17	7.88	7.42
	Post	8.38	7.73	8.28	7.31
4	Pre	7.78	7.64	8.11	7.78
	Post	7.85	7.53	7.19	7.77
5	Pre	8.40	7.31	7.32	7.46
	Post	8.06	7.65	7.69	7.73

TABLE 5.2
MEAN RESPONSE MANOVA
FOR VITAMIN E μg/ml serum

Day		Group 1 Air Light Exercise	Group 2 O_3 Light Exercise	Group 3 O_3 Heavy Exercise	Group 4 Air Heavy Exercise
2	Pre	6.08	7.45	5.60	5.68
	Post	5.56	8.19	5.59	5.62
3	Pre	5.82	6.78	5.72	6.85
	Post	6.02	6.48	5.80	6.53
4	Pre	5.52	6.41	5.77	6.10
	Post	6.55	6.60	5.68	6.08
5	Pre	5.62	6.89	5.78	6.13
	Post	5.27	7.35	6.27	6.02

TABLE 5.3
MEAN RESPONSE FROM MANOVA FOR
RBC CHOLINESTERASE mU/10^9 RBC

Day		Group 1 Air Light Exercise	Group 2 O_3 Light Exercise	Group 3 O_3 Heavy Exercise	Group 4 Air Heavy Exercise
2	Pre	1077	1039	1049	1288
	Post	1106	1078	1321	1272
3	Pre	1060	1118	1125	1320
	Post	1034	1200	1021	1200
4	Pre	1058	1147	1180	1252
	Post	1048	1076	1065	1235
5	Pre	1105	1186	1188	1268
	Post	1107	1105	1203	1185

TABLE 5.4
MEAN RESPONSE FROM MANOVA FOR
RBC G6PDH U/gm Hgb

Day		Group 1 Air Light Exercise	Group 2 O_3 Light Exercise	Group 3 O_3 Heavy Exercise	Group 4 Air Heavy Exercise
2	Pre	2.01	1.96	2.43	1.94
	Post	2.04	1.84	2.04	1.74
3	Pre	1.94	1.86	1.95	1.75
	Post	1.98	1.86	2.29	1.89
4	Pre	1.99	1.78	2.20	1.94
	Post	2.03	1.89	2.16	1.89
5	Pre	2.04	1.94	2.23	1.87
	Post	2.07	1.76	2.07	1.81

TABLE 5.5
MEAN RESPONSE FROM MANOVA FOR LDH IU/l

Day		Group 1 Air Light Exercise	Group 2 O_3 Light Exercise	Group 3 O_3 Heavy Exercise	Group 4 Air Heavy Exercise
2	Pre	96.3	86.6	81.5	98.9
	Post	89.7	84.6	101.8	81.4
3	Pre	87.8	85.2	78.4	76.1
	Post	89.2	91.4	89.0	75.7
4	Pre	89.6	80.1	92.2	76.0
	Post	89.7	75.4	90.6	76.9
5	Pre	91.6	72.6	78.5	75.8
	Post	95.3	84.3	80.2	74.6

TABLE 5.6
MEAN RESPONSE FROM MANOVA
FOR α_1-ANTITRYPSIN mg/dl

Day		Group 1 Air Light Exercise	Group 2 O_3 Light Exercise	Group 3 O_3 Heavy Exercise	Group 4 Air Heavy Exercise
2	Pre	165	177	186	175
	Post	171	178	185	179
3	Pre	168	183	182	177
	Post	173	167	183	175
4	Pre	168	179	191	175
	Post	173	186	196	177
5	Pre	168	178	190	174
	Post	178	184	193	172

TABLE 5.7
MEAN RESPONSE FROM MANOVA FOR HAPTOGLOBIN mg/dl

Day		Group 1 Air Light Exercise	Group 2 O_3 Light Exercise	Group 3 O_3 Heavy Exercise	Group 4 Air Heavy Exercise
2	Pre	53	59	89	60
	Post	54	60	89	59
3	Pre	50	64	89	53
	Post	52	70	90	56
4	Pre	48	70	95	59
	Post	49	67	95	60
5	Pre	53	67	94	58
	Post	52	67	91	57

TABLE 5.8
MEAN RESPONSE FROM MANOVA FOR COMPLEMENT C_3 mg/dl

Day		Group 1 Air Light Exercise	Group 2 O_3 Light Exercise	Group 3 O_3 Heavy Exercise	Group 4 Air Heavy Exercise
2	Pre	87	95	81	83
	Post	89	97	82	84
3	Pre	88	98	84	80
	Post	90	98	84	81
4	Pre	87	97	85	78
	Post	92	91	88	80
5	Pre	90	89	89	81
	Post	93	96	91	81

TABLE 5.9
MEAN RESPONSE FROM MANOVA FOR IgM mg/dl

Day		Group 1 Air Light Exercise	Group 2 O_3 Light Exercise	Group 3 O_3 Heavy Exercise	Group 4 Air Heavy Exercise
2	Pre	108	120	107	119
	Post	109	120	106	119
3	Pre	105	121	98	116
	Post	110	117	98	112
4	Pre	114	125	103	110
	Post	105	128	103	111
5	Pre	97	116	101	108
	Post	102	124	97	101

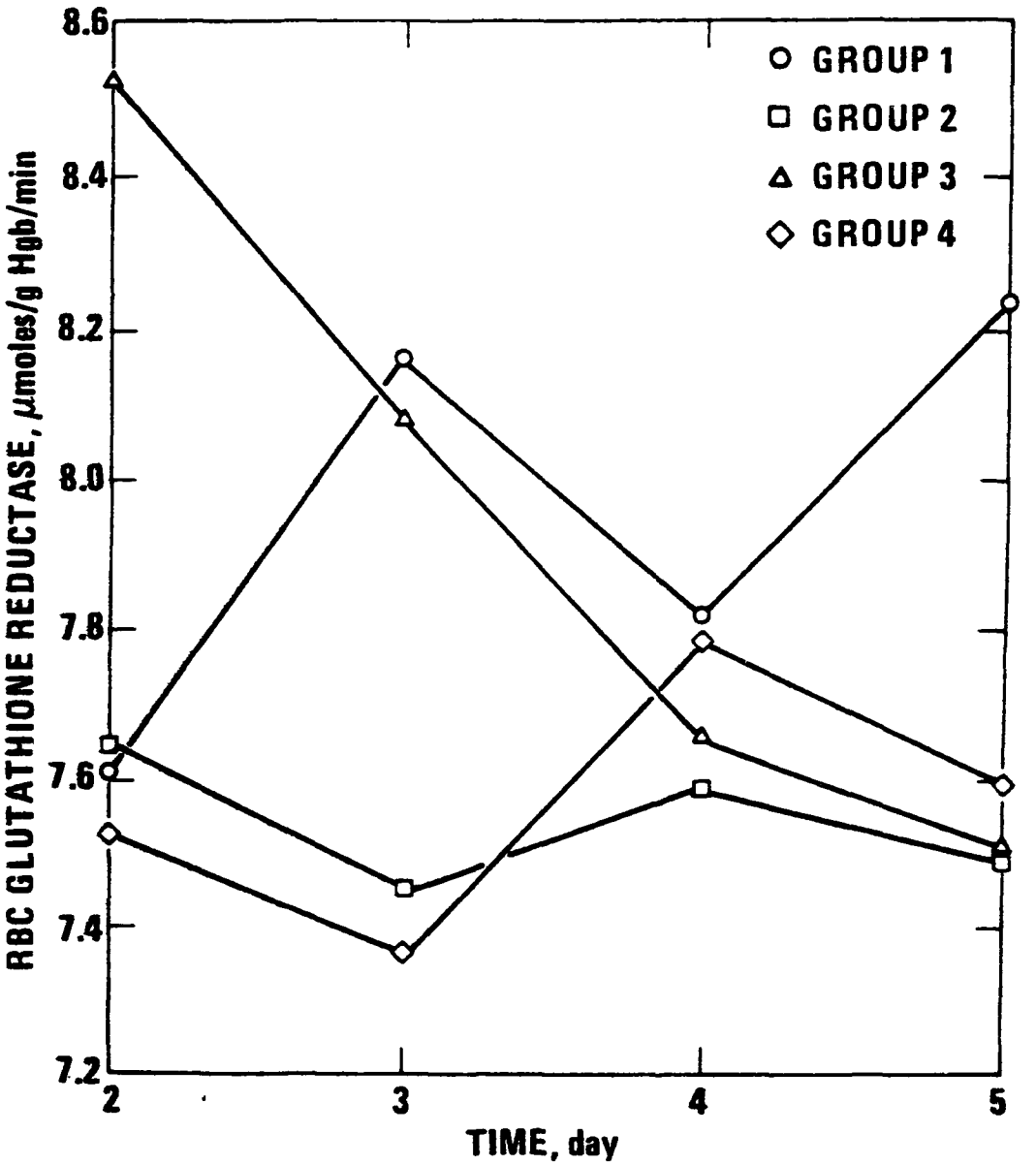

Figure 1a. Ozone by exercise by day interaction means for RBC glutathione reductase.

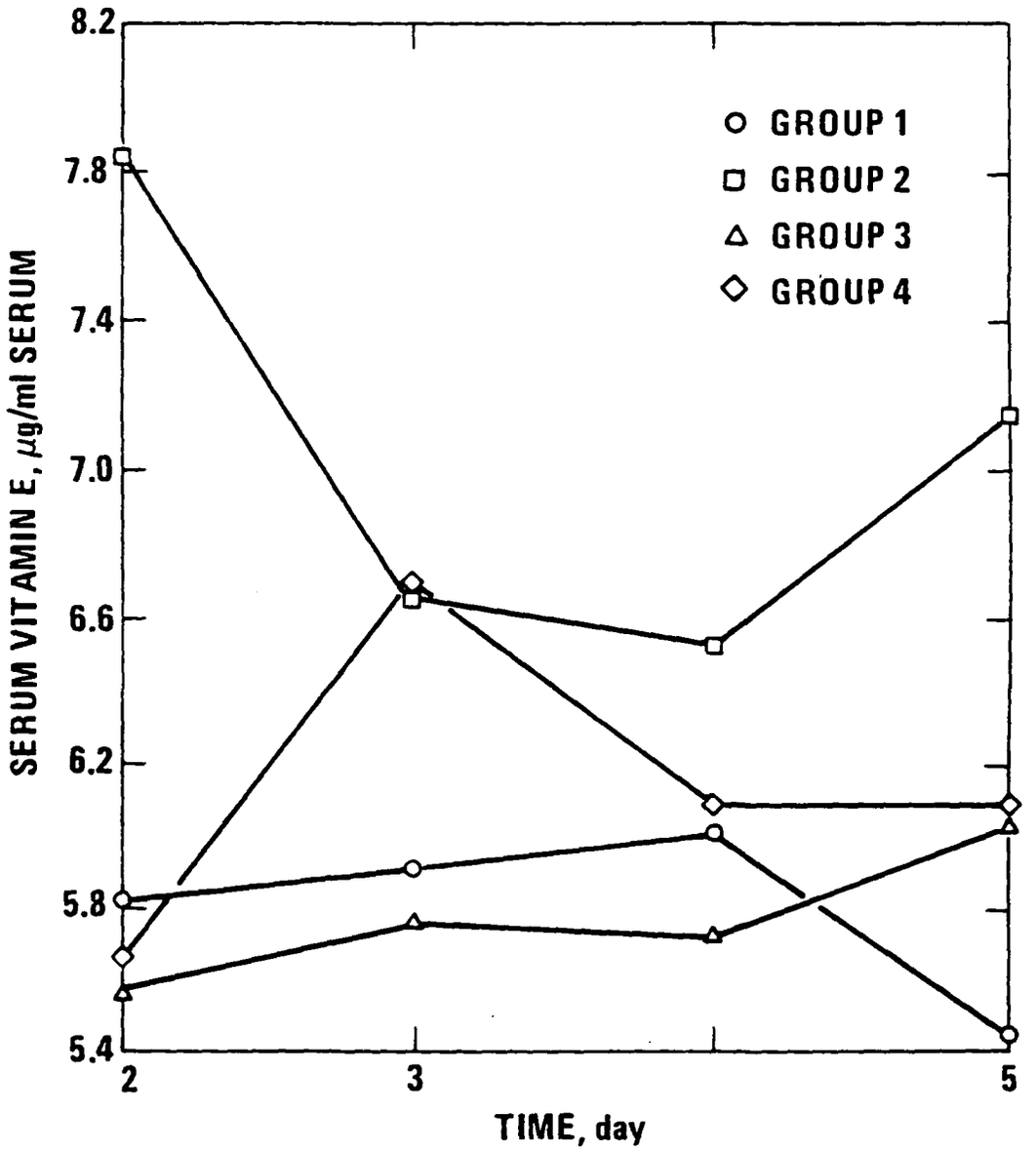

Figure 1b. Ozone by exercise by day interaction means for serum vitamin E.

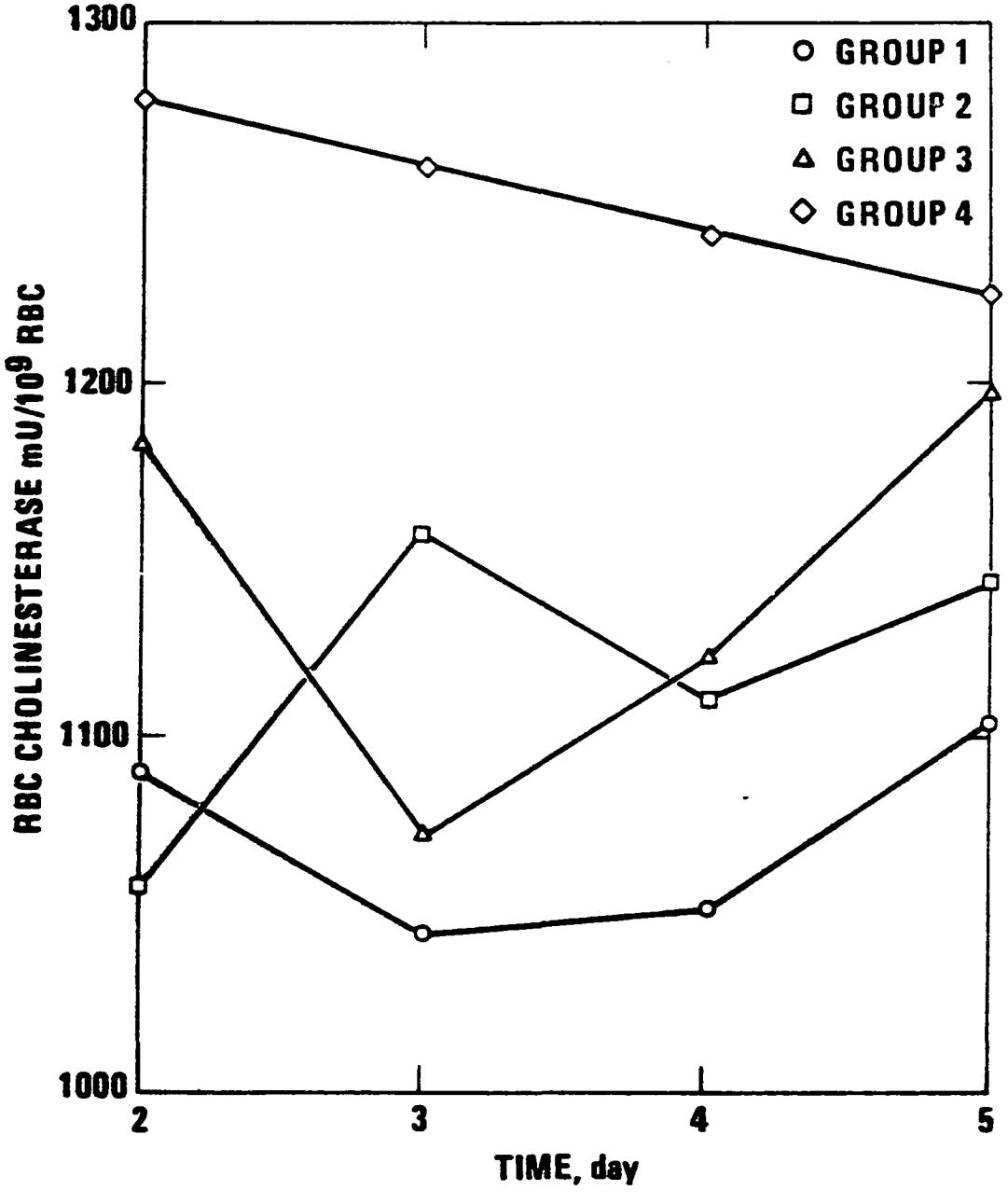

Figure 1c. Ozone by exercise by day interaction means for RBC cholinesterase.

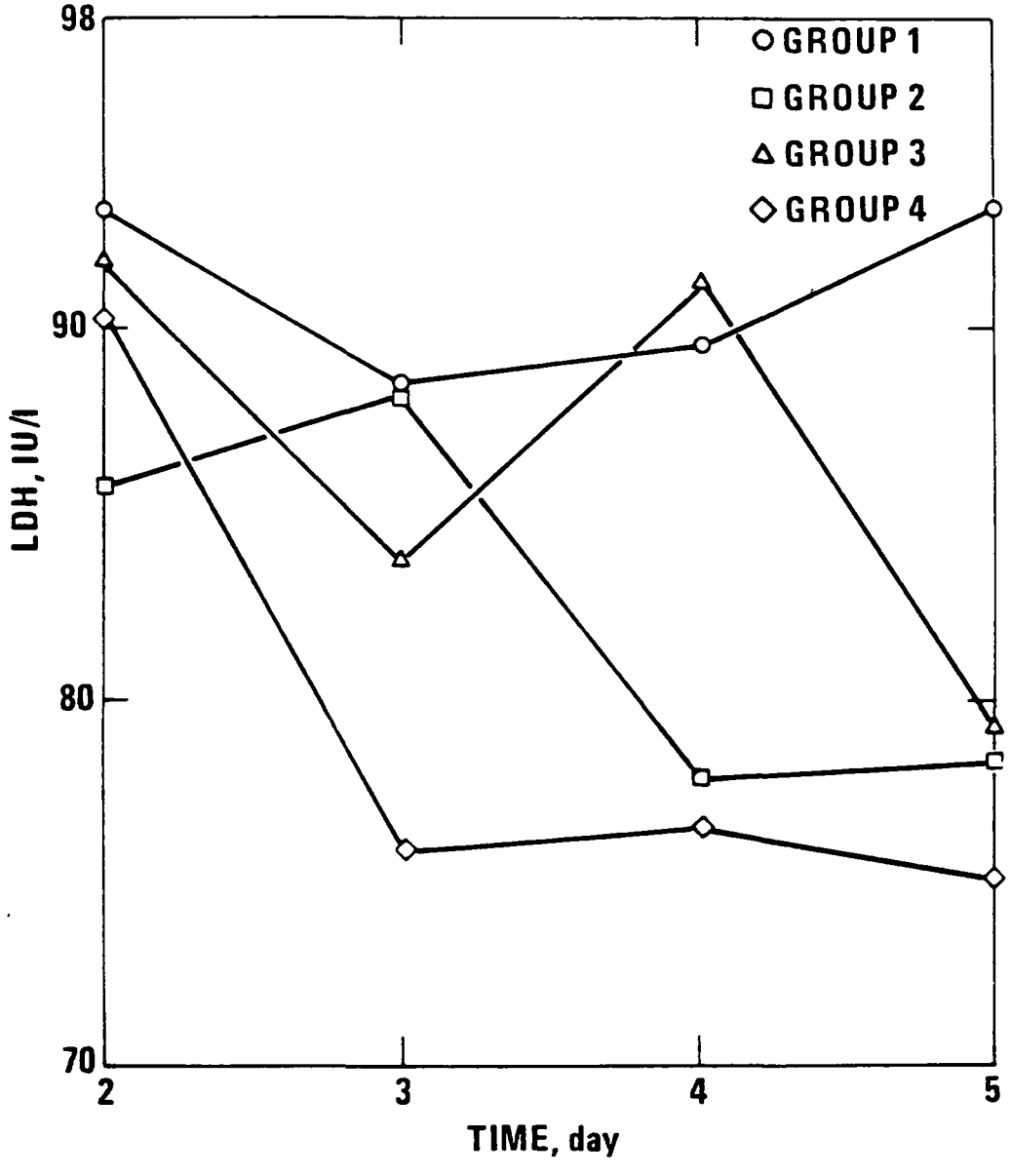

Figure 1d. Ozone by exercise by day interaction means for LDH.

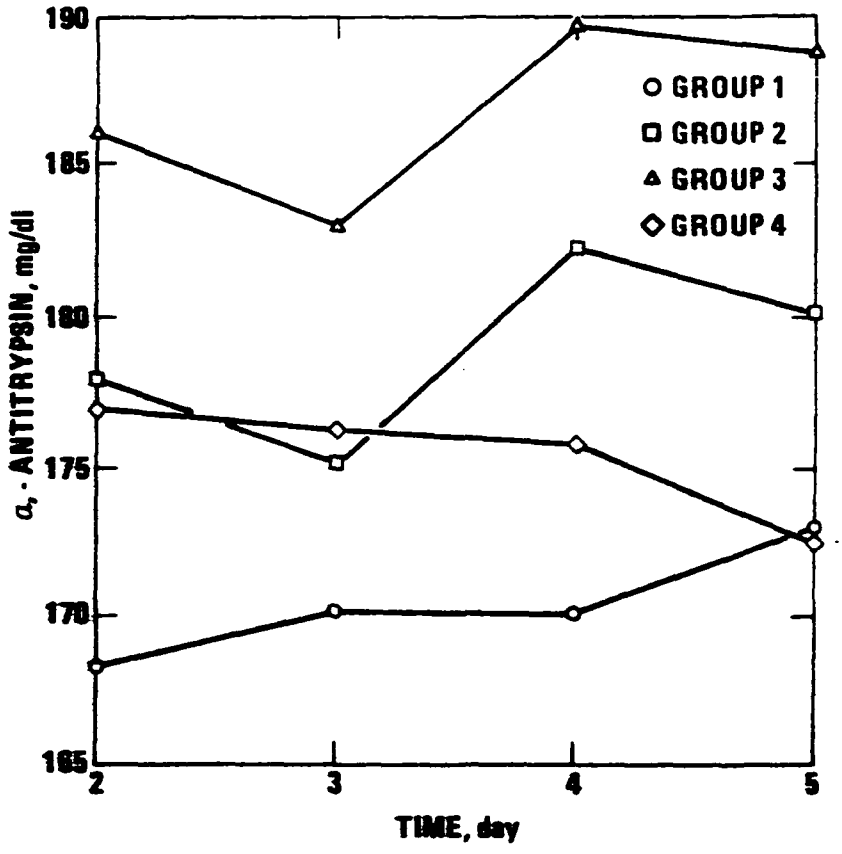

Figure 1e. Ozone by exercise by day interaction means for a_1-antitrypsin.

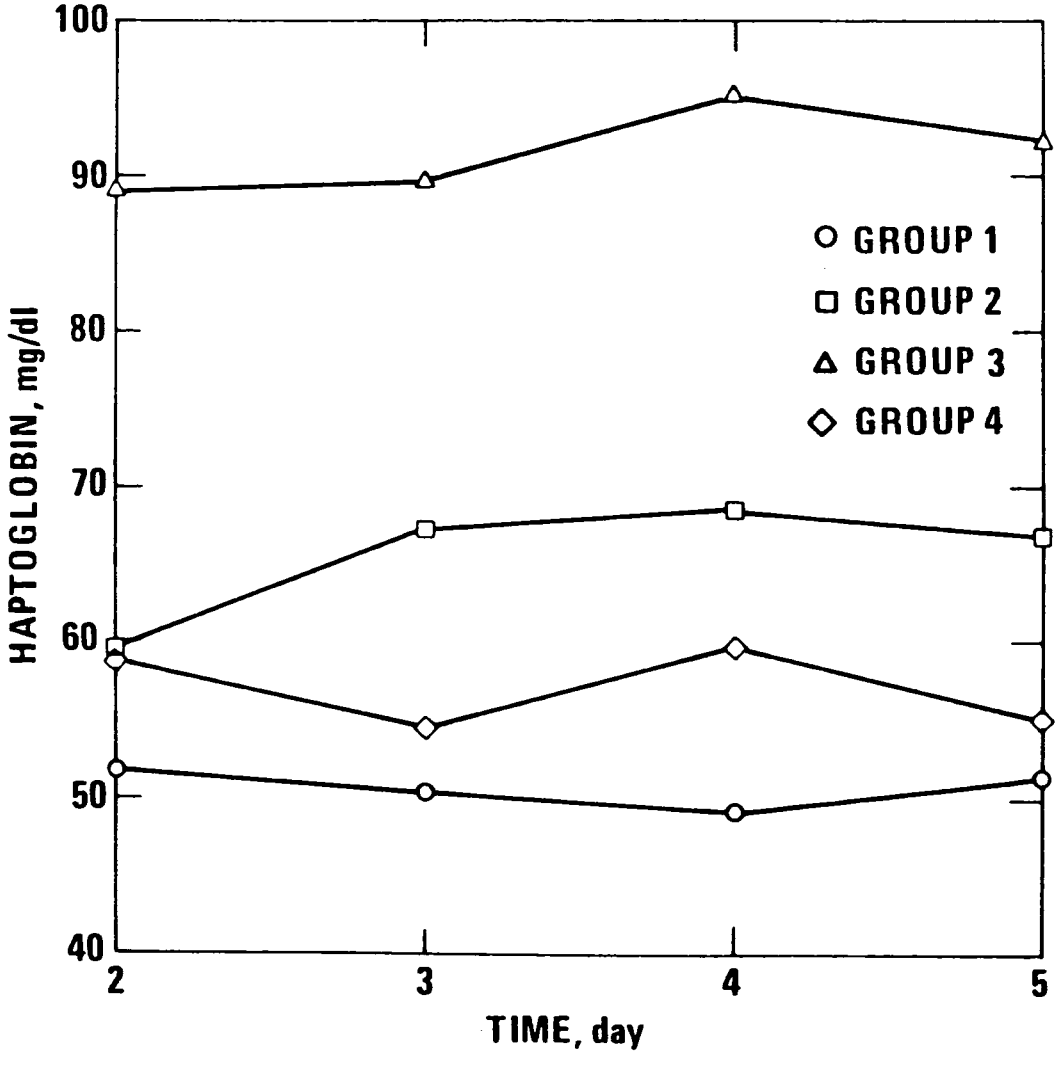

Figure 1f. Ozone by exercise by day interaction means for haptoglobin.

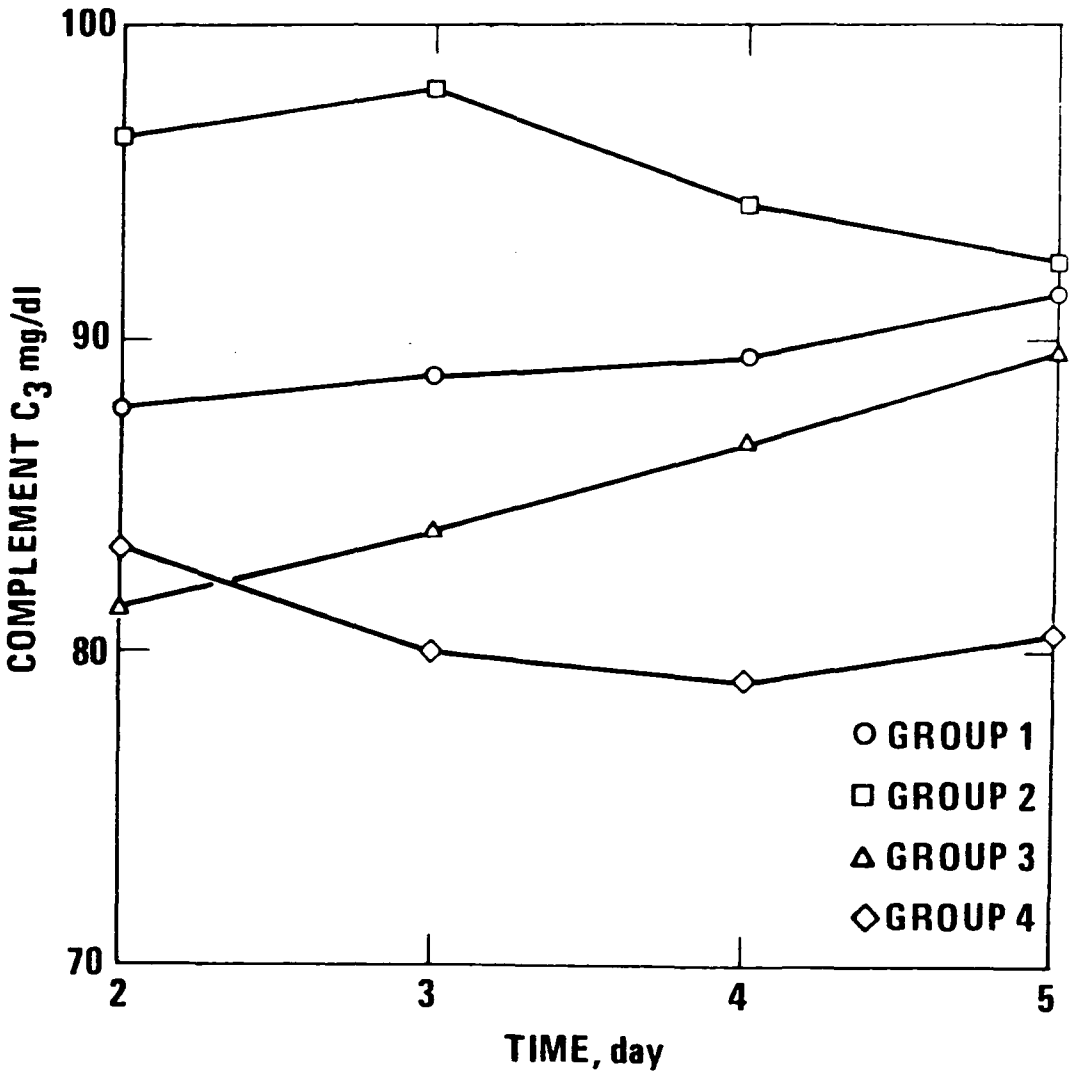

Figure 1g. Ozone by exercise by day interaction means for complement C_3.

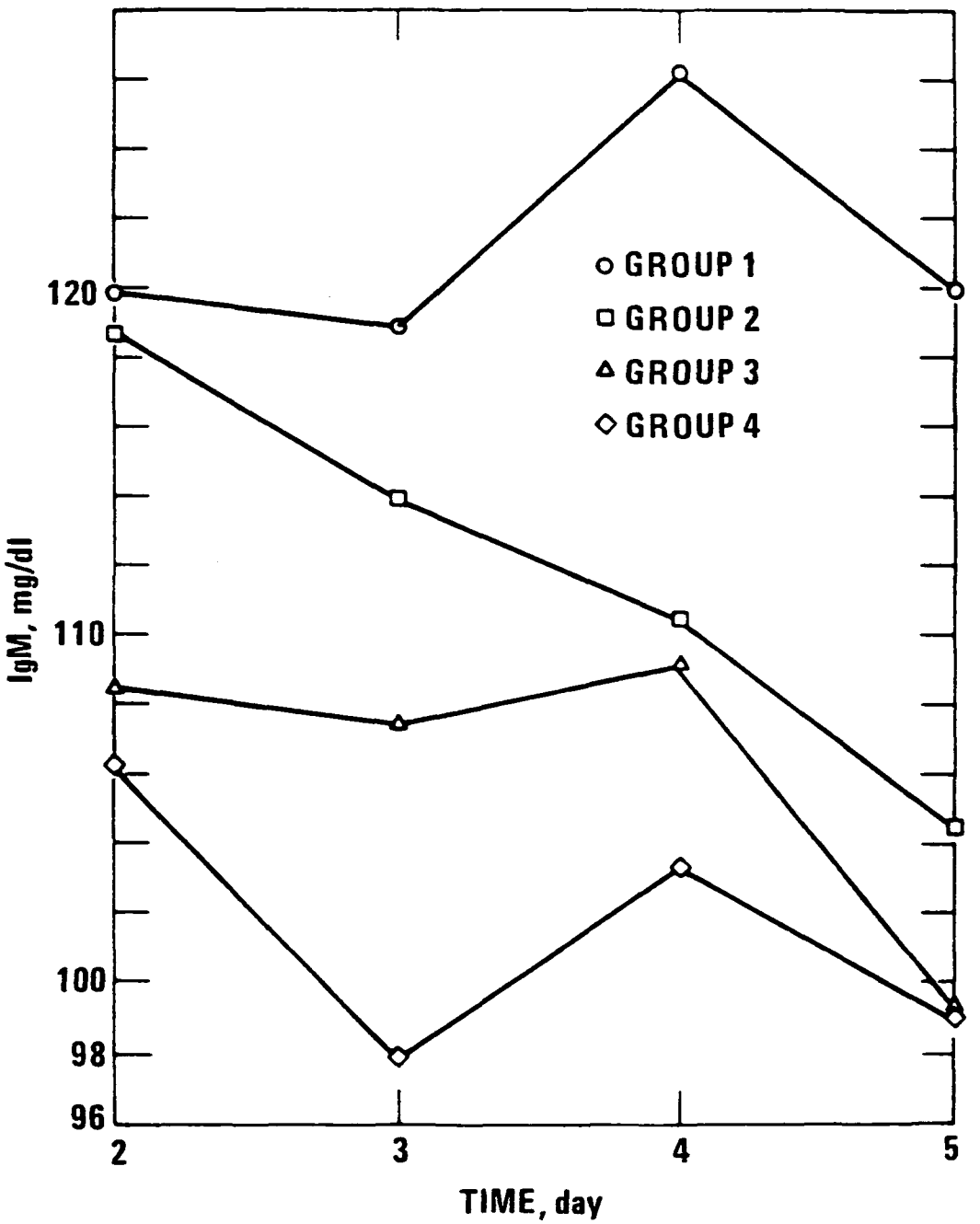

Figure 1h. Ozone by exercise by day interaction means for IgM.

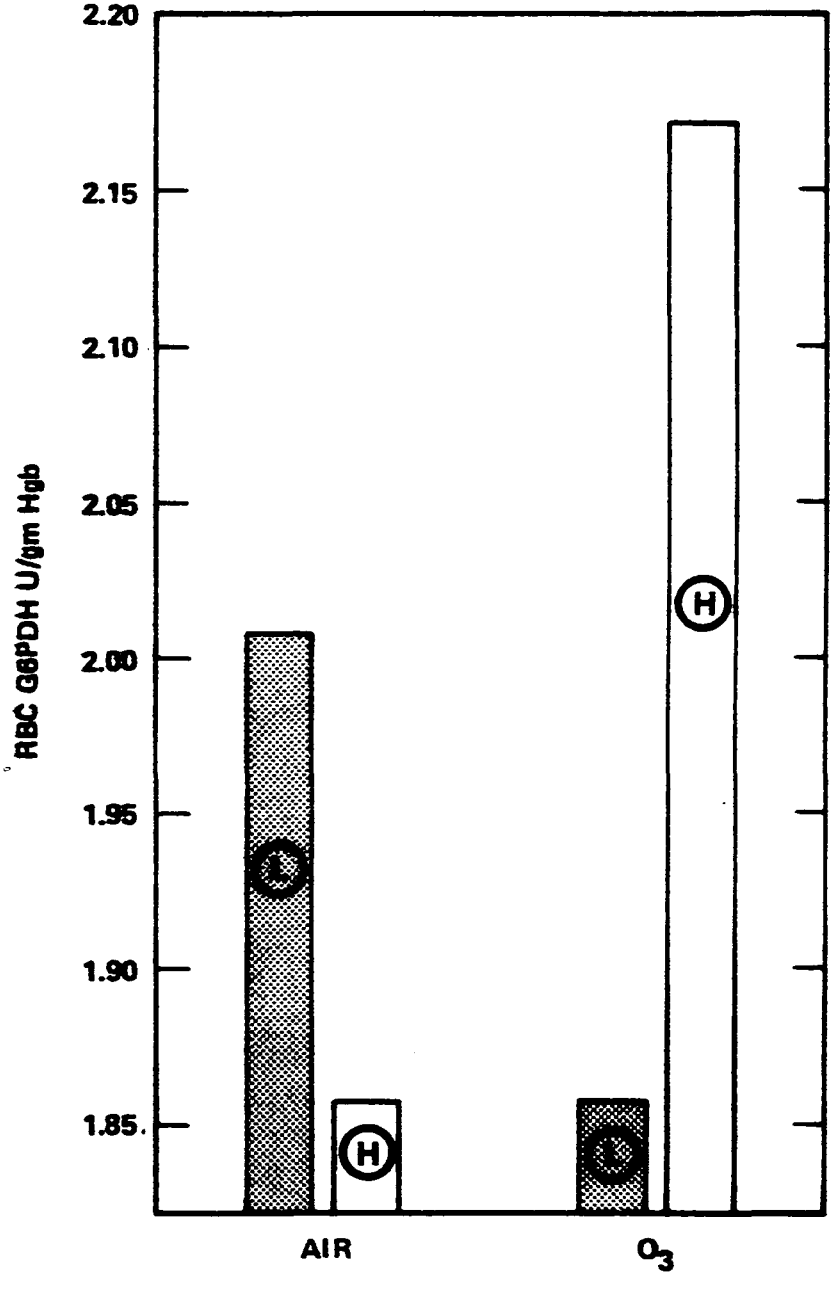

Figure 2. Ozone by exercise means for serum vitamin E.

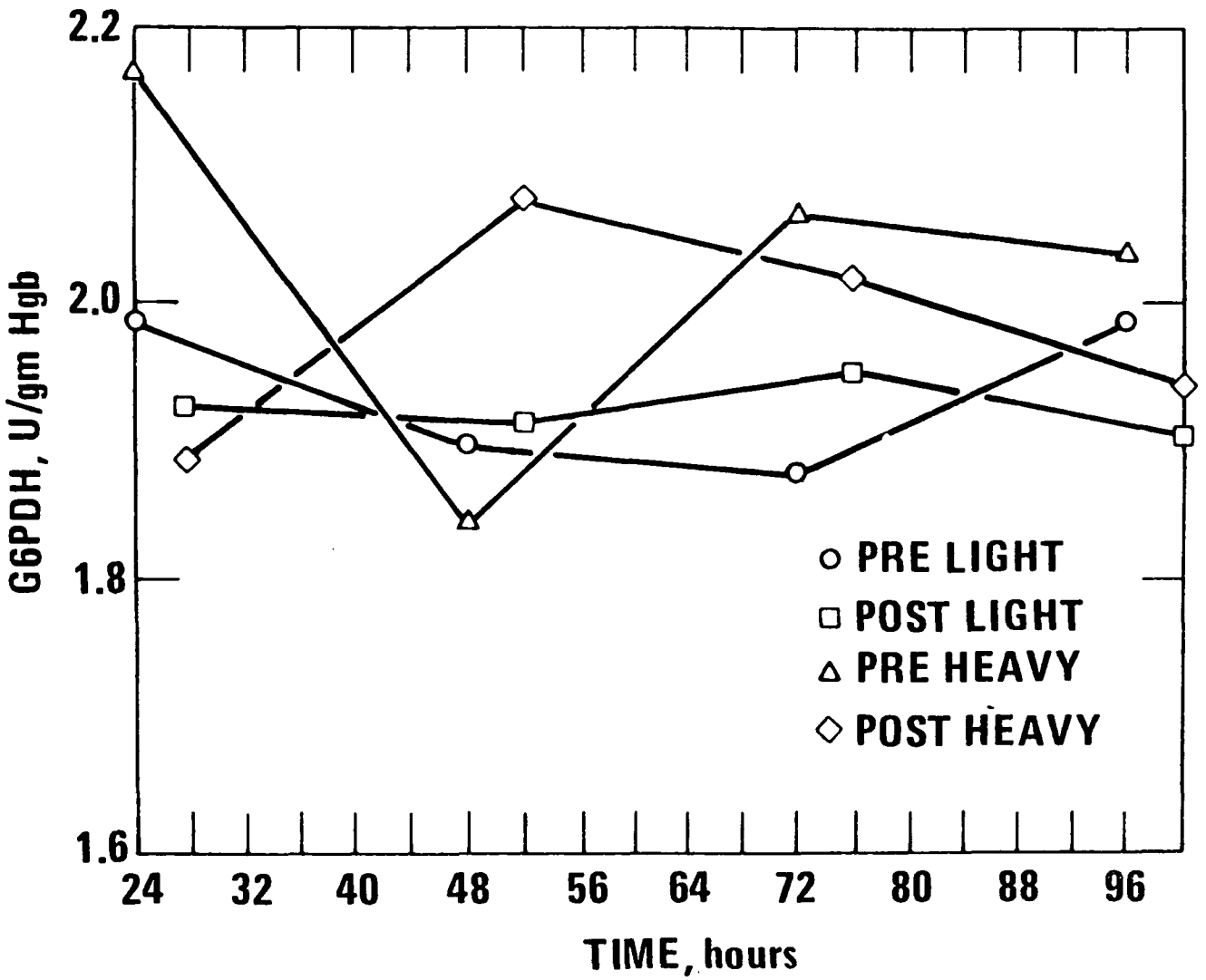

Figure 3. Pre-post by exercise by days means for G6PDH.

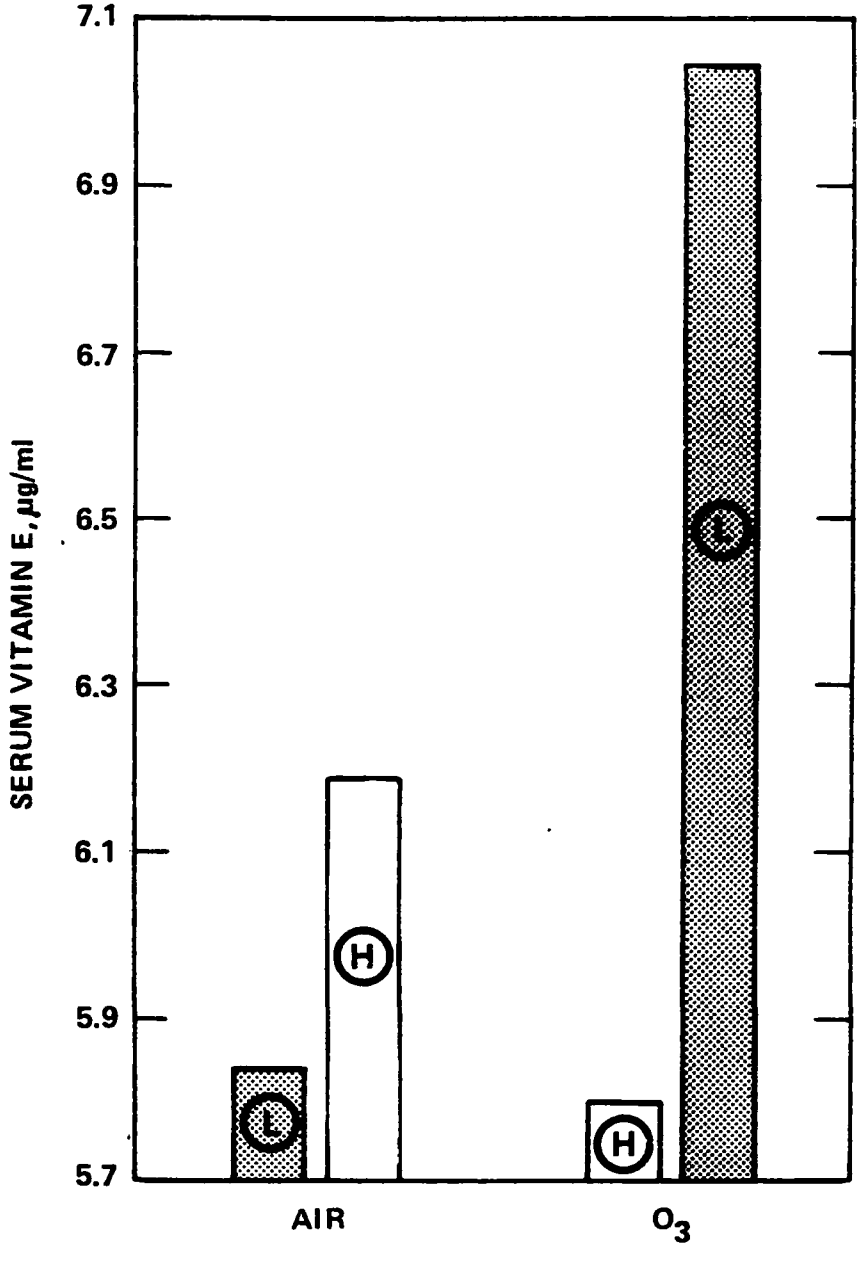

Figure 4. Ozone by exercise means for G6PDH.

TECHNICAL REPORT DATA
(Please read Instructions on the reverse before completing)

1. REPORT NO. EPA-600/1-79-026	2.	3. RECIPIENT'S ACCESSION NO.
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		6. PERFORMING ORGANIZATION CODE
7. AUTHOR(S) Suzanne Chaney, Paulette DeWitt, Wendy Blomquist, Keith Muller, Robert Bruce, George Goldstein		8. PERFORMING ORGANIZATION REPORT NO.
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15. SUPPLEMENTARY NOTES

16. ABSTRACT

A total of 44 human subjects were exposed to 784 ug/m^3 (0.4 ppm) ozone and 30 human subjects were exposed to air as controls. A four hour exposure was given on each of five consecutive days. Half of each group received an higher level of treadmill exercise than the other half.

Nine biochemical blood parameters were measured pre and post exposure: red blood cell glutathione reductase, vitamin E, red blood cell cholinesterase, red blood cell glucose-6-phosphate dehydrogenase, lactic acid dehydrogenase, complement C_3, and IgM. Of these variables only vitamin E, red blood cell glucose-6-phosphate dehydrogenase, and complement C_3 showed significant changes due to treatment.

17. KEY WORDS AND DOCUMENT ANALYSIS

a. DESCRIPTORS	b. IDENTIFIERS/OPEN ENDED TERMS	c. COSATI Field/Group
Blood biochemistry Human exposure Oxidizing pollutant Ozone	Ozadapt 1-4 Pollutant insult screening Reductive detoxification	06,A

18. DISTRIBUTION STATEMENT RELEASE TO PUBLIC	19. SECURITY CLASS *(This Report)* UNCLASSIFIED	21. NO. OF PAGES 51
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