

Assessment of Lifestyle Effects on the Overall Antioxidant Capacity of Healthy Subjects

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Oxidative damage is increasingly recognized as playing an important role in the pathogenesis of several diseases such as cancer and cardiovascular diseases. Using a biologic test based on whole blood resistance to free-radical aggression, we sought to evaluate lifestyle factors that may contribute to the normal variability of the overall antioxidant status. We assessed this global antiradical defense capacity in 88 men and 96 women in relation to information on lifestyle obtained by questionnaire. In our relatively young, healthy population, we found a weak negative relation between male sex or aging and the resistance to oxidant stress. Among the factors studied, non-smoking, vitamin and/or mineral supplementation, and regular physical activity were closely associated with an increased overall antioxidant capacity. Conversely, the antioxidant potential was negatively related to tobacco smoking; psychologic stress; alcohol consumption; moderate vegetable, low fruit, and low fish consumption; and, to a lesser extent, high natural ultraviolet light exposure. Thus, we were able to determine "unhealthy" and "healthy" lifestyle patterns that truly contributed to the variation of individual antioxidant capacity. We conclude that lifestyle determinants of cancer and cardiovascular risks were associated with a decreased overall antioxidant status as dynamically measured by means of a biologic test. Thus, the evaluation of the total human resistance against free-radical aggression, taking into account nutritional habits, lifestyle, and environmental factors, may be useful in preventive medicine as a precocious diagnosis to identify healthy subjects who are at risk for free-radical-mediated diseases. *Key words:* alcohol consumption, dietary habits, free radical, hemolysis, lifestyle factors, physical activity, psychologic stress, tobacco smoking, vitamin intake. *Environ Health Perspect* 110:479–487 (2002). [Online 2 April 2002] <http://ehpnet1.niehs.nih.gov/docs/2002/110p479-487/lesgards/abstract.html>

Evidence is increasing that free-radical-mediated damage is involved in aging and in the genesis of many chronic diseases such as cancer, cardiovascular diseases, diabetes, and inflammatory diseases (1–3).

Free radicals are highly reactive species produced under normal biologic conditions, mainly during oxygen consumption in redox reactions required to generate energy and to eliminate xenobiotic and pathogenic organisms. An excess of free radicals may induce a pronounced impairment of the cellular metabolism and significant damage of tissues. The organism is naturally protected against this excessive free-radical attack by enzymatic and chemical detoxification systems (4). Thus, under normal physiologic conditions, a balance state is established between free-radical production and antiradical defenses. Nevertheless, various lifestyle, nutritional, environmental, and genetic factors may induce an abnormal increase in free-radical production and/or a decrease in antioxidant defenses that could alter this balance state and conduct to the so-called oxidant stress (5). This impairment of the individual overall defense capacity may then favor aging as well as the genesis and development of many degenerative diseases. The aim of our study was to examine selected nutritional habits and lifestyle and environ-

mental factors that may influence the overall antioxidant defense capacity of healthy subjects. We measured this antioxidant capacity using a biologic test that allows the assessment of the overall individual resistance against free-radical aggression (6–13).

Subjects and Methods

Subjects and study design. We recruited 184 subjects from among healthy blood donors from the Central Blood Center in Marseille, France. Before enrollment, trained personnel conducted a standardized interview regarding the medical history of the volunteers. The recruited subjects consisted of 88 women and 96 men between 19 and 63 years old. They had no history of metabolic disorders such as liver or kidney dysfunction, no history of cancers, and no clinical manifestations of cardiovascular diseases, diabetes, or hypertension. Additional exclusion criteria included chronic disorders requiring medication. Finally, we confirmed their health status by results of routine biochemical analyses. On the examination day, we submitted to each subject an anonymous questionnaire that asked questions regarding lifestyle, environmental factors, and nutritional habits during the last 12 months. We divided these factors into those of endogenous origin (age, sex, exercise,

and psychologic stress) and those of exogenous origin [nutritional diet, vitamin intake, alcohol consumption, cigarette smoking, and ultraviolet (UV) radiation]. Trained personnel and nurses from the Central Blood Center performed the examination and collected the blood samples from the volunteers, who had not fasted beforehand. In accordance with French law, we requested a written informed consent from all participants before the beginning of the experiment. Because we collected blood samples from tubes of systematic control subjects, approval of the study protocol by a human investigation committee was not necessary (14).

Data collection. We obtained information about each endogenous and exogenous factor from the anonymous questionnaires. To perform statistical analyses, we classified qualitative information into several categories or modalities corresponding to the proposed answers. For quantitative information, we created categories according to different subject classes.

Endogenous factors. We classified total subjects as well as both men and women into three age groups: < 35 years old (category 1); 35–49 years old (category 2); > 49 years old (category 3). Concerning physical exercise, we asked the subjects to mark one of the following categories that best fit their degree of physical activity in leisure time: sedentary or no activity (category 1); occasional sport activity (category 2); regular sport activity for at least 4 hr per week (active exercise) (category 3).

We measured stress at work using a self-administered five-item questionnaire concerning workplace demands and intrusion of work concerns into home life. The items were the following: a) "Does your job imply high mental exigency?" b) "Do you have a weak decision power?" c) "Do you perceive hostile attitudes from people you work with?" d) "Do

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you ever have difficulty sleeping because you are thinking about work-related concerns?" *e*) "Do work concerns weigh on your mind at home?" The first three questions are intended to measure the perceived "demand" aspect of work situations. These items are similar to those in the "job strain" model proposed by Karasek et al. (15), and the remaining two questions are similar to the "effort-reward" imbalance model proposed by Siegrist (16).

We measured stress at home using a two-item questionnaire: *f*) "Considering all your efforts and achievement, are you receiving the respect and prestige you deserve at home?" *g*) "Do you ever have difficulty sleeping because you are thinking about home life concerns?" We then scored each item using a three-point scale from "never/hardly never" to "often" or "very often/all the time." According to the sum of item scores, we then classified psychologic stress intensity in the work place and at home into three categories: no stress or weak stress (category 1); moderate stress (category 2); high stress (category 3).

Exogenous factors. We obtained information on dietary habits on the basis of intake frequency. We categorized vegetable and meat consumption according to the number of intakes per week. Category 1 included subjects who never ate the specified food or had one dietary intake per week; subjects of category 2 had 2–4 intakes per week; category 3 included subjects who ate the food 5–7 times (or more) per week. We recorded fish consumption in three categories: < 1 intake per week (category 1); 1–2 intakes per week (category 2); and > 2 intakes per week (category 3). Concerning fruit consumption, we have considered the number of intakes per day: < 1 fruit intake per day (category 1); 1 intake per day (category 2); > 1 intake per day (category 3). Finally, we classified subjects according to the use of vitamin supplements: nonconsumers (category 1); regular consumers (category 2). We asked subjects who were currently using supplements to indicate the type of vitamins and/or trace elements.

Regarding smoking habits, we asked subjects to report the average number of cigarettes smoked per day. On the basis of this information, we classified them into three categories: never smoked, light smokers (1–4 cigarettes/day), and heavy smokers (5–50 cigarettes/day). We recorded individual irradiation by UV light in three categories according to sun exposure frequency: no exposure, occasional exposure, and regular exposure. Finally, we asked subjects about their strong alcohol drinking frequency: no alcohol consumption (category 1); 1–2 aperitifs per week (category 2); > 2 aperitifs per week (category 3).

Blood sample collection and assessment of the global antiradical defense potential. We collected nonfasting blood samples from

systematic control subjects into evacuated tubes containing EDTA (final concentration, 3 mmol/L) and kept at 4°C. We evaluated the total antiradical potential of each individual by using a biologic test based on free-radical-induced hemolysis (6,7). We submitted blood samples, diluted to 1/50 in isotonic saline solution, to organic free radicals produced at 37°C under air atmosphere from the thermal decomposition of a 27 mmol/L solution of 2,2'-azobis(2-amidinopropane) dihydrochloride (Spiral, Dijon, France). The extracellular and intracellular antioxidant defenses contribute to maintaining blood cell membrane integrity and function until cell lysis. We recorded hemolysis using a 96-well microplate reader by measuring the optical density decay at 450 nm. We expressed results as the time that is required to reach 50% of maximal hemolysis [half-hemolysis time (HT₅₀) in minutes], which refers to the whole blood resistance to free-radical attack. The measurement of HT₅₀ is very reproducible and has been shown to be representative of the overall defense against free radicals in humans and animal models (6–9,11–13).

Statistical analysis. We report all data as mean ± SD. In addition to descriptive statistics, we performed bivariate and multivariate analyses to determine the importance of each studied factor for HT₅₀ data. We determined the effects of these expected determinants of the antioxidant capacity (age, sex, lifestyle, and environmental and nutritional

factors) using analysis of variance (ANOVA) performed with the statistical software SPSS (SPSS Science Inc., Chicago, IL, USA). We determined interactions between some potential determinants of antiradical defense variability using two-way ANOVA. We then analyzed the significance of differences between means by comparisons using the Fischer's test or the Newman Keuls' test. All tests were two-tailed, and we considered a *p*-value < 0.05 to be significant.

To explain the variation of the resistance to oxidant stress and to assess the simultaneous relationships among the various potential predictors, we performed multivariate analysis using the multiple correspondence analysis (MCA). This model, which is equivalent to multiple regression for categorical variables, is meaningful when the population under investigation is described by numerous qualitative variables classified according to self-questionnaire answers. It allows the processing of data matrices in the form of contingency tables based on the hypothesis of the total independence of matrix rows and columns that corresponds to a hypothetical equal distribution of variable categories among the population. We assigned each recorded category of studied factors given by the questionnaire to one modality in the matrix. To explain the contribution of predictive factors to individual antiradical resistance, we entered HT₅₀ data, divided into quartiles, as categorical variables in the analysis. This analysis provides a

Table 1. Effects of endogenous factors on the overall antioxidant capacity of 184 healthy subjects (mean age, 42 years).

Endogenous factors, category or frequency	No. of subjects	HT ₅₀ (mean ± SD)
Sex*		
Women	88	125.83 ^a ± 20.97
Men	96	133.59 ^b ± 20.27
Age (years)		
Women		
< 35	20	125.15 ± 22.81
35–49	47	126.03 ± 20.94
> 49	21	126.02 ± 20.24
Men		
< 35	19	136.34 ± 20.88
35–49	52	133.15 ± 20.81
> 49	25	132.42 ± 19.26
Exercise (week frequency)*		
Never	46	128.02 ^a ± 21.20
Occasionally	66	124.89 ^a ± 20.98
Regularly	72	135.63 ^b ± 19.51
Stress at work (intensity)*		
Weak	70	135.98 ^a ± 21.00
Moderate	81	131.24 ^a ± 19.78
High	26	111.83 ^b ± 14.11
Stress at home (intensity)*		
Weak	92	132.98 ^a ± 21.21
Moderate	81	129.35 ^a ± 19.87
High	11	107.85 ^b ± 11.37

Sex, age, exercise, and psychologic stress effects on free-radical-mediated hemolysis were evaluated by the HT₅₀. For each factor, the significance of difference between HT₅₀ results was determined by multiple comparisons of the means using ANOVA, followed by Fischer's test. For each factor, means with superscript *a* are significantly different from those with superscript *b* (*p* < 0.05).

*Factor with significant effect.

diagram of variance proportions explained by factorial axes, which we ascribed to statistical factors. It additionally provides the relative contribution of statistical factors to the rate of variance explained by each modality and the absolute contribution of modalities to the rate of variance explained by each statistical factor. This analysis points out the proximity, the independence, and the opposition between lifestyle modalities and HT₅₀ quartiles that, regarding multiple regression analysis, can be interpreted as positive or negative correlations. It thus reveals the predictive potential of endogenous and exogenous factors for individual resistance against free radicals.

Results

Descriptive and Bivariate Analysis

Effect of endogenous factors. Table 1 summarizes the effects of sex, age, physical exercise, and psychologic stress on individual resistance to oxidant stress. In the population studied (mean age, 42 years; 52% men and 48% women), men had higher whole blood resistance to free-radical aggression than did women ($p = 0.0116$). However, we found no significant differences between HT₅₀ means of the three age groups for both men and women combined.

Moreover, results showed that physical activity greatly enhanced blood resistance to free radicals. Indeed, HT₅₀ was significantly higher in subjects who had a regular physical activity than in those who practiced an occasional activity ($p = 0.0024$) or did not perform exercise ($p = 0.050$). Regarding psychologic stress, 50% and 60% of participants reported stress at home and stress at work, respectively. We found no difference between HT₅₀ of nonstressed and moderately stressed subjects. Nevertheless, having high stress at work or at home induced a strong decrease in blood antiradical resistance (having high stress vs. moderate or no stress at work, $p < 0.0001$ and $p < 0.0001$, respectively; having high stress vs. moderate and no stress at home, $p = 0.0011$ and $p < 0.0001$, respectively).

Effect of exogenous factors. Table 2 summarizes the effects of nutritional habits, alcohol consumption, tobacco smoking, and natural UV light exposure on the individual defense capacity. Concerning dietary habits, results indicated that fish or meat consumption had no significant effect on the whole blood antiradical resistance of subjects. However, < 1 fruit intake per day and < 2 intakes of vegetables per week strongly impaired the antioxidant resistance. We found no difference between the HT₅₀ of subjects

consuming 1 fruit per day and the HT₅₀ of those consuming > 1 fruit per day. Nevertheless, results actually indicated that whole blood resistance to oxidant stress was considerably lower in subjects eating < 1 fruit per day than in those eating 1 fruit or > 1 fruit per day ($p = 0.0006$ and $p = 0.001$, respectively). We obtained similar results for vegetable consumption. We found no difference between the HT₅₀ of subjects who had 2–4 vegetable intakes per week and that of subjects who had > 4 vegetable intakes per week. Nevertheless, the antiradical defense capacity was lower in subjects having < 2 vegetable intakes per week than in those having 2–4 intakes or > 4 intakes per week ($p = 0.0193$ and $p = 0.0140$, respectively). Among the participants, 8% consumed vitamin and/or trace element supplements. Subjects most often used vitamins C, B, and E and magnesium supplements. Results showed that whole blood resistance to free-radical aggression was significantly higher in these vitamin consumers than in nonconsumers ($p = 0.0012$).

Regarding smoking habits, we found a great decrease in the whole blood resistance against free-radical aggression in heavy smokers compared with light smokers (1–4 cigarettes per day) and with nonsmokers ($p = 0.0026$, $p < 0.0001$, respectively). We observed no significant difference between light smokers and nonsmokers. The decrease in the antiradical defense capacity observed in subjects who were regularly exposed to UV radiation compared with those who were never or occasionally exposed was not quite significant (overall ANOVA, overall $p = 0.086$). Finally, alcohol consumption had no significant effect on the individual resistance against free radicals.

Combined effects of psychologic stress and tobacco smoking. To compare smoking and psychologic stress effects according to subject age and to determine whether these effects were independent of each other, we studied their combined effects in the three age groups (Figure 1). Of the subjects under investigation, 21% were < 35 years old, 54% were 35–49 years old, and 25% were > 49 years old. Additionally, 37% of subjects were nonsmokers without stress, 26% were nonsmokers with stress, 17% were smokers without stress, and 20% were smokers with stress. Two-way ANOVA results showed that the main effect of stress was significantly higher than that of smoking in the studied population as a whole and in the youngest, middle, and oldest age groups (stress main effects, $p < 0.0001$, $p = 0.0018$, $p < 0.0001$, and $p < 0.0001$, vs. tobacco main effects, $p = 0.0004$, $p = 0.0295$, $p = 0.1094$, and $p = 0.0733$, respectively). The overall tobacco effect was not quite significant in subjects 35–49 years old and in older subjects. Moreover, in 35–49-year-old subjects,

Table 2. Effects of exogenous factors on the overall antioxidant capacity of 184 healthy subjects (mean age, 42 years).

Exogenous factors, consumption or exposure	No. of subjects	HT ₅₀ (mean ± SD)
Vitamin (week intake frequency)*		
Never	165	128.20 ^a ± 20.37
Regularly	19	144.48 ^b ± 20.37
Fruit (no./day)*		
< 1	53	120.76 ^a ± 17.92
1	77	133.39 ^b ± 20.80
> 1	54	133.82 ^b ± 21.40
Vegetables (no. of intakes/week)*		
< 2	26	120.05 ^a ± 22.36
2–4	113	130.85 ^b ± 20.41
> 4	45	132.86 ^b ± 19.21
Fish (no. of intakes/week)		
< 1	20	125.10 ± 22.07
1–2	108	129.13 ± 18.80
> 2	56	133.75 ± 23.38
Red meat (no. of intakes/week)		
< 2	42	130.06 ± 23.69
2–4	117	130.44 ± 20.51
> 4	25	126.92 ± 18.23
Strong alcohol (no. of intakes/week)		
0	28	128.42 ± 22.39
1–2	144	130.63 ± 20.78
> 2	12	120.98 ± 23.22
Tobacco smoking (no. of cigarettes/day)*		
0	115	134.11 ^a ± 20.71
1–4	25	132.46 ^a ± 19.86
> 4	44	117.35 ^b ± 17.09
Sunlight (frequency of exposures/week)		
Never	26	136.19 ± 17.92
Occasionally	134	129.70 ± 21.62
Regularly	24	123.10 ± 18.35

For each factor, the significance of difference between HT₅₀ results was determined by multiple comparisons of the means using ANOVA, followed by Fischer's test. Means with different superscript letters are significantly different ($p < 0.05$).

*Factor with significant effect.

the stress-mediated untoward effect was lower in smokers than in nonsmokers (HT₅₀ decrease of 11% in smokers vs. 18% in nonsmokers). In addition, tobacco smoking had no negative effect on stressed subjects, whereas it decreased the antioxidant resistance of nonstressed subjects (HT₅₀ decrease of 1% in stressed vs. 8% in nonstressed subjects). However, we found no interactive effects between stress and smoking in subjects as a whole and in the youngest, middle, and oldest subjects (two-way ANOVA: $p = 0.4685$, $p = 0.8216$, $p = 0.1788$, and $p = 0.9724$, respectively). Therefore, “age-adjusted” psychologic stress and tobacco-smoking-mediated decrease in the antioxidant capacity would be independent of each other. Additionally, psychologic stress would be the most determining factor for the decrease in the individual resistance against free radicals.

Furthermore, regarding aging, we found no significant differences between the HT₅₀ of subjects from the three age classes in each group of subjects divided according to stress and tobacco behaviors. Nevertheless, as expected, we observed the lowest HT₅₀ in the oldest stressed smokers and the highest HT₅₀ in the youngest nonstressed, nonsmokers. Results showed that being > 49 years old enhanced the stress-mediated impairment of individual defense capacity in both smokers and nonsmokers compared with being either 35–49 or < 35 years old (in smokers, HT₅₀ decrease of 23% vs. 11% and 16%, respectively; in nonsmokers, HT₅₀ decrease of 21% vs. 18% and 16%, respectively). However, being > 49 years old did not enhance smoking-induced impairment of individual defense capacity in both stressed and nonstressed subjects compared with being < 35 years old (HT₅₀ decrease of 11% vs. 11% in stressed subjects; HT₅₀ decrease of 8% vs. 11% in nonstressed subjects). By contrast, compared with being < 35 years old, being 35–49 years old reduced the tobacco-mediated decrease in the antiradical resistance in stressed subjects (HT₅₀ decrease of 1% vs. 11%) and decreased the psychologic stress negative effect in smokers (HT₅₀ decrease of 11% vs. 16%). Thus, both smoking and psychologic stress-mediated reduction of the individual antiradical resistance would partially depend on age.

Multivariate Analysis

We performed a multivariate analysis using the MCA method to determine the absolute contribution of each lifestyle factor to the variation of the individual antiradical resistance, taking into account the contribution of all other factors. We performed the MCA on 177 subjects who completely answered to 13 questions about their lifestyle, nutritional habits, and environmental influences. We divided subjects into three age groups and categorized

them into four groups according to HT₅₀ quartiles: $93 \leq \text{HT}_{50} < 112$ min; $112 \leq \text{HT}_{50} < 129$ min; $129 \leq \text{HT}_{50} < 148$ min; $148 \leq \text{HT}_{50} < 173$ min (mean \pm SD, 130 ± 21 min; 95% confidence interval, 127–133 min). By adding HT₅₀ variables that we sought to explain, we obtained 14 categorical variables with a total of 41 modalities as defined above. We entered data as a design matrix of 177 rows (individual cases) and 41 columns (modalities) containing the codes 0 or 1 for the absence or the presence of the modality. This analysis enabled us to obtain a typology of individuals according to their lifestyles and HT₅₀ results on the assumption that two individuals are similar when they have the same lifestyle profile. It also enabled us to study the relationships between all modalities and especially between HT₅₀ modalities and all other modalities. Several modalities form a group, which can be highlighted, when they are often expressed by a great number of subjects with the same lifestyle. The relationships between HT₅₀ variable and endogenous and exogenous factors resulting from the MCA are synthetically illustrated on Figure 2.

We obtained most of the information through the first two-factorial axis, which account for 29% of the overall inertia given by the MCA (data not shown). This percentage of variance proportion gives a satisfactory explanation considering the great number of modalities. By using absolute contributions of modalities to the inertia of each axis, statistical factors of the strongest modalities have been clearly stated. We ascribed factor 1 to the x -axis and factor 2 to the y -axis. In Figure 2, boldface type indicates the modalities whose contributions are the strongest for the first factor, and italic indicates those that are the strongest for the second factor. We omitted modalities weakly represented on the two axes and nearer the intersection of both axes, which corresponds to the mean profile. Furthermore, vitamin intake and low fish consumption, which are distant from both axes, are badly represented modalities because of low numbers of individuals ($n = 19$ and $n = 20$, respectively).

MCA results show that the HT₅₀ variable contributed to 21.9% of the rate of variance explained by factor 1. Therefore, individual resistance against free-radical aggression was highly represented on the x -axis of Figure 2. Modalities that have negative coordinates on this axis belonged to subjects who had the same lifestyle profile. These subjects have reported high stress both at work and at home; they consumed strong alcohol (> twice per week) and were heavy smokers. Additionally, they had moderate intakes of vegetables (2–4 per week), ate < 1 fruit per day, and had < 1 intake of fish per week. The MCA synthetic representation

showed that these lifestyle factors, which are close to a HT₅₀ of < 112 min, highly contributed to a low individual resistance against free radicals. In opposition, a large proportion of subjects whose lifestyle modalities had positive coordinates on the x -axis were highly resistant to oxidant stress (HT₅₀ > 148 min). The fact that they consumed vitamin and/or mineral supplements, practiced sports regularly, and were nonsmokers thus partly accounts for their high antioxidant capacity independently of the other studied factors.

On the other hand, the MCA results indicate that the HT₅₀ variable contributed to only 1.4% of the variance proportion explained by factor 2. Nevertheless, several factor modalities had a significant weight that indicates their probable nonlinear association with the individual defense capacity. We observed that some subjects who were weakly resistant to oxidant stress (HT₅₀ < 112 min) were regularly exposed to UV radiation, did not have regular physical activity, and had moderate fish consumption (1–2 intakes per week; see italicized modalities on the left side of the y -axis). In opposition, modalities associated with a high HT₅₀ (> 148 min) were shared by subjects who were never exposed to UV light, had low stress at work and at home, ate more than one fruit per day, and had a moderate intake of meat (2–4 per week). It is noteworthy that taking into account all other factors, male sex did not show a positive contribution to the antiradical defense capacity. On the contrary, being male was weakly associated with a low HT₅₀, whereas being female was weakly related to a high HT₅₀. In addition, the MCA revealed a slight contribution of aging in the decrease of the individual defense capacity. Indeed, being > 49 years old was partially related to a low

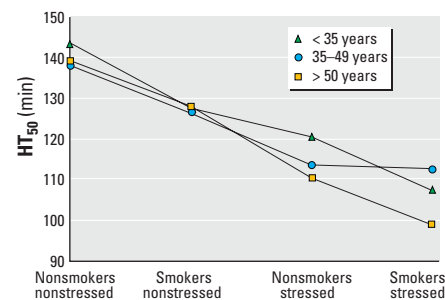


Figure 1. Combined effects of psychologic stress and tobacco smoking on the overall antioxidant capacity of 184 healthy subjects in three age groups. The effect of tobacco smoking on the antiradical defense capacity of nonstressed and stressed subjects in the three age groups, evaluated by HT₅₀, are shown on the left and right sides of the figure, respectively. We determined the main effects of both psychologic stress and tobacco smoking and their interactions using two-way ANOVA. After ANOVA, we analyzed significant differences between means by multiple comparisons using the Newman Keuls' test.

antiradical resistance, whereas being < 35 years old was partially related to a high antiradical resistance.

Taken together, MCA results emphasized that male sex and old age as well as moderate fish intake, no regular physical activity, and regular sun exposure were nonlinearly related to a decreased antioxidant potential. On the other hand, having high stress at work and at home, heavy smoking, strong alcohol drinking, moderate vegetable intake, low fruit consumption, and no fish consumption were strongly related to a reduced defense capacity independently of other lifestyle factors. Conversely, female sex and young age as well as no stress at work or at home, no UV exposure, high fruit consumption, and moderate meat intake contributed to a high individual defense capacity but to a lesser extent than did vitamin intake, regular physical activity, and not smoking. These latter factors have a strong positive relation with the overall antioxidant defense capacity of individuals of the others.

Discussion

Numerous studies increasingly have suggested that “unhealthy” lifestyle factors are involved in an unbalanced antioxidative homeostasis leading to an oxidant stress that may increase the incidence of diseases (3–5). Nevertheless, to our knowledge, relationships between intrinsic, lifestyle, and environmental factors

and the individual antioxidant defense capacity have not been yet investigated. In the present human population-based study, we have evaluated lifestyle profiles that account for changes in the overall antioxidant capacity of healthy subjects measured by means of their whole blood resistance against free-radical aggression.

Several studies have indicated that the assessment of free-radical-mediated hemolysis, caused by lipid peroxidation and protein oxidation together, reflect overall individual antiradical defense. We have previously shown that biologic antioxidant compounds such as albumin, uric acid, bilirubin, glutathione, vitamins E and C, carotenoids, and polyphenols increased blood cell resistance against free-radical attack (6–10). Conversely, cellular depletion has been demonstrated in catalase activity or in glutathione, and oxidized compounds such as oxidized albumin and irradiated collagen shorten hemolysis compared with controls (6–11). In addition, we have reported that in opposition to folic acid deficiency-mediated hyperhomocysteinemia, oral contraceptive treatment, or vitamin E nutritional deficiency, vitamin E supplementation decreased blood susceptibility to free-radical aggression in animal models (6–9, 12). Furthermore, in a human population study, Girodon et al. (13) found that 2-year supplementation with a low dose of vitamin C, vitamin E, and β -carotene,

where plasma levels were positively correlated with HT₅₀, improved blood cell antiradical resistance of elderly people. Finally, Blache and colleagues (8,9) have found that both cigarette smoking and prolonged physical activity in trained healthy subjects, which are known to produce circulating free radicals, significantly shortened free-radical-mediated hemolysis and that blood antiradical resistance of atherosclerotic patients and diabetic rats was highly reduced compared with controls.

These findings favor assaying individual antiradical defense capacity by means of the free-radical-mediated hemolysis tests. Moreover, this measurement can be a physiologically relevant alternative to the selective assessment of free-radical scavenging or reducing activity of plasma or to the selective analysis of biologic antioxidant compounds. Because they do not take into account cellular environment and the numerous interactions between biologic antioxidants, these latter assays would not be highly relevant to the determination of the physiologic antioxidant state. Thus, the present study enabled us to determine the ability of the antiradical resistance measurement to predict factors that further account for an increased or decreased antioxidant defense system.

Our findings emphasize that nonsmoking, vitamin and/or trace element supplementation, regular physical activity, and no UV light exposure contributed to an enhanced antioxidant defense potential and thus may be ascribed to a “healthy” lifestyle profile. Conversely, tobacco smoking, high psychologic stress, strong alcohol drinking, and moderate vegetable, low fruit, and little fish consumption contributed to a decreased antioxidant potential and thus may be ascribed to an “unhealthy” lifestyle profile.

Concerning the contribution of sex to the overall antioxidant capacity, multivariate analysis showed that being male was weakly related to a decreased antioxidant potential, whereas being female was weakly related to an increased antioxidant potential. Because ANOVA results indicated that men had a significant higher antioxidant capacity than did women (Table 1), this probably nonlinear negative relation suggests that other factors may contribute to the sex-related variation of the blood antiradical resistance. Indeed, the MCA representation highlighted that although the negative effects of UV light would be higher, those of psychologic stress, tobacco, and aging would be lower in women than in men. Nevertheless, because multivariate analysis did not account for the total variation of blood cell resistance to oxidant stress, we believe that unevaluated factors such as sexual, genetic, and/or hormonal differences may play a major role in the variation of the individual antioxidant capacity according to

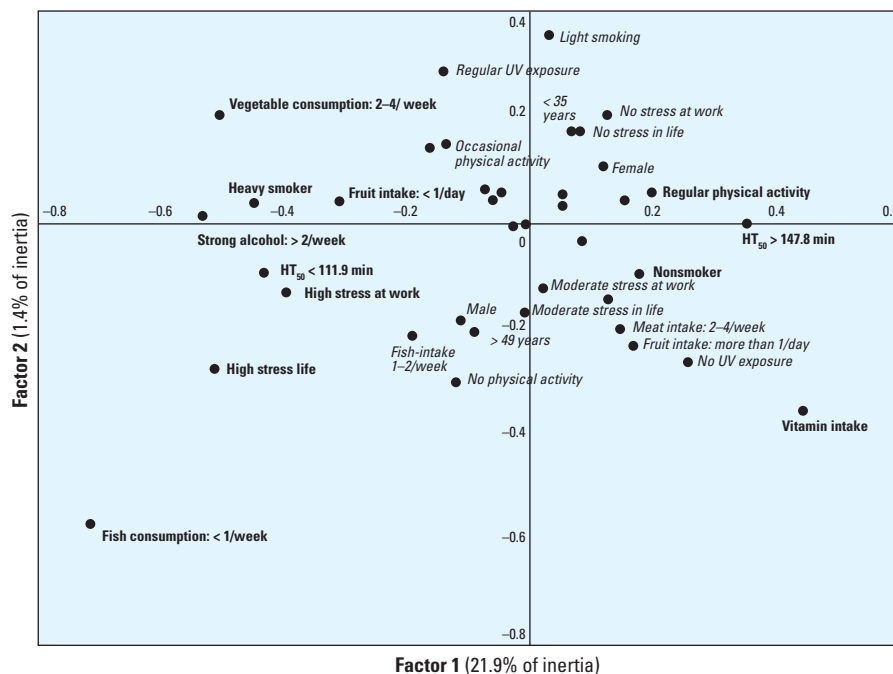


Figure 2. Relationships between lifestyle factors and the overall antioxidant capacity of 177 healthy subjects. Modalities whose contributions are strongest for factor 1 (x-axis) are shown in boldface, and those that are strongest for factor 2 (y-axis) are italicized. Both factors together account for 29% of the overall inertia. The HT₅₀ variable contributed to 21.9% of the total inertia explained by the factor 1 and 1.4% of the total inertia explained by the factor 2. Thus, boldface modalities on the negative or positive side of the x-axis are negatively or positively related to the overall antioxidant defense capacity, respectively. On the other hand, italicized modalities on the left or right side of the y-axis are nonlinearly negatively or positively related to the individual defense capacity, respectively.

sex. This suggestion is in accordance with clinical studies, which have shown that partly because of estrogen influence, women are more protected than men from atherogenic events, which are increasingly related to oxidant stress (17).

Although the causal role of oxidant stress in aging remains unclear, aging has been associated with increased oxidant generation, a decline in the robustness of defenses and repair, and an accumulation of end products of oxidative damage (18). Some putative mechanisms underlying senescence related to oxidant stress have been proposed. Age-associated oxidative stress may lead to a progressive loss of functional capacity and may influence the cellular differentiation-like processes involved in gene regulation or compromise the effectiveness of mitochondria in generating energy (19). As expected from the relatively young age of the studied population, we found no significant differences in the overall antioxidant capacity between subjects of three age groups. Nevertheless, multivariate analysis revealed a slight negative association of aging with the overall antioxidant capacity that argues in favor of the age-associated oxidative stress theory. Being > 49 years old was indeed partially related to a low antiradical resistance, whereas being < 35 years old was partially related to a high antiradical resistance. In agreement with the involvement of an impaired antioxidative homeostasis in aging, an earlier investigation has clearly shown using the free-radical-mediated hemolysis test that elderly subjects (mean age, 83 years) have a reduced blood antiradical resistance compared with that of young people (mean age, 42 years) (13). Apart from the young mean age of our population, the partial negative association observed in the present study may depend on other factors whose negative contributions increased with age. Indeed, although the negative effect of UV light exposure and low fruit consumption tended to be stronger, the negative contributions of male sex, sedentary, and psychologic stress would be lower in the youngest age group than in the oldest age group (Figure 2).

Because the individual capacity to enhance antioxidant defenses depends partly upon the diet, we have evaluated the effect of nutritional habits on the overall antioxidant capacity. The positive or negative effects of multivitamin supplements on the plasma antioxidant capacity of healthy subjects and on the incidence of degenerative diseases are still discussed (13,20–22). Our results show that the blood resistance against free radicals was higher in subjects who consumed multivitamin and/or mineral supplements, which were especially in the form of vitamins C, E, and B and magnesium. Furthermore, this vitamin intake was closely associated with a high antioxidant

capacity independently of other factors. Despite a low percentage of subjects taking vitamin supplements, these data suggest that increasing the micronutrient diet and probably the micronutrient status could improve the effectiveness of the antioxidant defense system.

Besides classical antioxidant molecules such as vitamins E, C, and A, flavonoids and other phenolic compounds contained in fruits and vegetables are increasingly suggested to play important roles in enhancing plasma antioxidant capacity of humans (23). Free-radical scavenger activities of many of these polyphenols are several times stronger than those of vitamins E and C (24). Additionally, many foods contain synthetic antioxidant molecules such as butylated hydroxyanisole, butylated hydroxytoluene, propyl gallate, and tertiary butylhydroquinone, which are used as additives to inhibit food lipid and protein oxidation and whose antioxidant efficiency could extend to the body (25). Furthermore, despite conflicting results, some epidemiologic evidence is in favor of the potential beneficial effect of dietary antioxidants in the reduction of the incidence of cancer and cardiovascular diseases (26,27). In accordance with a positive implication of dietary antioxidants, our results point out that high vegetable and fruit consumption significantly increases blood resistance to free radicals compared with low vegetable and fruit consumption.

However, whereas low fruit consumption and moderate vegetable intake are probably linearly related to a decreased individual antioxidant capacity, we found only a weak relation between high fruit consumption and an increased antioxidant potential. This result suggests that it is easier to damage the regulated antioxidant defense system by reducing dietary antioxidants than to strengthen this system by increasing dietary antioxidants. Nevertheless, vitamin supplementation was effective in enhancing the overall antioxidant defense capacity, and vitamin tablets may have been transformed to ameliorate their absorption. In addition, we did not take into account disparity between studied subjects in micronutrient absorption; and finally, the negative contributions of tobacco smoking tended to be lower in high fruit consumers than in low fruit consumers (Figure 2). In the light of these observations, we thus may have underestimated positive influences of fruit and vegetable antioxidants.

Along with fruit and vegetable, the type of diet as well as the total caloric intake may play a relevant role in the regulation of the antioxidative homeostasis *in vivo*. In contrast to meat consumption, which was associated with an increased risk for cancer and cardiovascular diseases that was not exclusively explained by the meat fat content, fish consumption has been reported to decrease the incidence of

colorectal cancer (28). This slight association may be caused by DNA, protein, and lipid oxidation in subjects on low caloric diets, which may reportedly increase longevity. In favor of an increased antioxidant defense system resulting from a fish-enriched diet low in meat, our results indicate that little fish eating and to a lesser extent moderate fish eating were negatively related to the antioxidant defense capacity, whereas moderate meat intake was weakly related to an increased antioxidant potential. Nevertheless, the negative effect of little fish consumption remains to be confirmed because of the low sample size of subjects who had reported low fish intake (modality very distant from both axes; see Figure 2). Taken together, these findings support the interest of the Cretan Mediterranean diet based on fruit, vegetables, fishes, and little red meat, which appears to prevent coronary heart disease and seems to be responsible for the favorable life expectancy of the Cretans (29).

Regarding alcohol consumption, whereas ethanol may improve micronutrient transport, chronic ethanol consumption has been reported to affect antioxidant defenses and to induce oxidant stress, probably through ethanol hepatic redox cycling. In particular, heavy alcohol consumption alters liver metabolism, thereby promoting vitamin deficiencies as well as toxic interactions with key nutrients (30,31). In accordance with these reports, we found a tendency toward a decrease in the total antiradical defense in heavy spirit consumers compared with that in occasional consumers and nonconsumers. Despite the small sample size of regular spirit consumers, probably due to their own intake underestimation, we observed a close negative association between heavy alcohol consumption and individual antioxidant defense capacity that strengthened our primary results. Regular consumers were especially male, were older, and had less intake of vegetables and fruits than did occasional consumers and nonconsumers (data not shown). Therefore, independently of other nutritional habits such as diminishing fruit and vegetable intake, chronic alcohol consumption may truly impair the physiologic antioxidant status.

Regarding physical exercise, which is recognized as “healthy” behavior, we found that regular physical activity at least 4 hours per week significantly increased the whole blood resistance to free radicals. This regular activity was strongly positively related to the overall antioxidant defense capacity, whereas occasional activity had no positive effect. On the contrary, occasional physical exercise as well as sedentary habits was partially associated with a decreased antioxidant potential. These results are consistent with other studies that have reported a positive association between regular

physical activity and cardiovascular circulation capacity, antioxidant enzyme activity (32,33), and clearance of both oxidant stress products and loosely bound iron (34). Nevertheless, strenuous exercise could promote free-radical production that may lead to local inflammation and even to tissue damage (35). Our findings support the view that an increased antioxidant defense system may result merely from physiologic adaptations to regular physical exercise, whereas decreased individual defenses may result from occasional strenuous training favoring free-radical generation and hence oxidative damage.

Psychologic stress is supposed to be one of the major ailments that undermine individuals in their professional and private life. Nervous exhaustion has become a cause of work intermission and sick leave affecting twice as many women (20%) as men (10%). In our study population, women reported higher stress in their private lives than did men (results not shown). The participants came from various professional sectors and had various working and marital statuses, and analysis showed no effect of these groups. Although we assured participants that their answers to the questionnaire would not be submitted to their management, subjectivity in answering a questionnaire on stress remains inevitable (36). Nonetheless, our results showed that psychologic stress was the lifestyle factor the most markedly associated with a decreased antioxidant capacity (Figures 1 and 2). This close negative relation with the antioxidant potential would be stronger for professional than for private stress. Nevertheless, because of small sample size of subjects who reported high stress in their private lives, further investigation should be performed to support this latter finding. Conversely, our results indicate that having no stress at work or at home is weakly related to a high antioxidant defense capacity.

These findings thus suggest that psychologic stress could induce oxidative damage by impairing the overall antioxidant system. In agreement with this suggestion, in healthy subjects, remaining awake all night was responsible for an important increase in urine level of thiobarbituric acid–reactive substances, which are a known indicator of lipid peroxidation (37). Other experimental studies have demonstrated that psychologic stress decreases DNA repair (38) and inhibits radiation-induced apoptosis (39) in human blood leukocytes. Furthermore, despite the scarcity of investigations on the potential involvement of psychologic stress in human disease, a recent clinical trial emphasized that men with high work-related stress have an increased risk for atherosclerosis that is consistent with the occurrence of oxidant stress (17). Nevertheless, mechanisms underlying

psychologic stress-related oxidant stress remained to be thoroughly investigated. Because it is rare that physiologic markers can validate high stress levels evaluated by subjective techniques, our results additionally indicate that the determination of overall antioxidant capacity in whole blood may be of significant use in detecting and remedying potential psychologic stress damage.

Concerning the combined effects of psychologic stress and tobacco smoking, despite higher tobacco consumption in subjects bearing high psychologic stress (data not shown), we found no significant synergistic or antagonist influences on the decrease in the antioxidant capacity of subjects from three age groups. The untoward effects of both psychologic stress and tobacco smoking would then be independent of each other. However, whereas the stress effect tended to increase with age, the tobacco negative effect tended to decrease with age (see Figures 1 and 2), probably because of an age-related reduction in the reported daily number of cigarettes smoked. In addition, the negative contributions of low fruit consumption, sedentary habits, male sex, and regular UV light exposure tended to be higher in smokers than in nonsmokers (Figure 2). Nevertheless, despite these potential partial interactive effects, as found for high psychologic stress, multivariate analysis showed that heavy smoking was strongly related to a decreased antioxidant defense capacity independent of other lifestyle factors. Furthermore, nonsmoking was highly positively related to the antioxidant potential, indicating a strong negative correlation between smoking and the overall individual antioxidant capacity. Therefore, these results provide evidence that blood cells of smokers may have undergone oxidative aggression caused by inhaled chemical compounds probably responsible for a deregulated antioxidative homeostasis. Thus, it is conceivable that whereas nonsmoking strengthened the overall antioxidant defense system, smoking impaired this antiradical defense system.

These suggestions are consistent with the observations of a lower antioxidant status and of higher lipid peroxidation product levels in plasma of smokers than in that of nonsmokers (40). Furthermore, prooxidant compounds as well as nicotine in cigarette smoke have been involved in atherogenesis through oxidative modification of plasma low-density lipoproteins (41) and in lung cancer through oxidation and nitrosation of DNA and other cellular components (42). Moreover, whereas psychologic traits appear to be the most important determinant for a decreased antioxidant capacity, nonsmoking as well as regular physical activity would be the most relevant factors for an increased antioxidant potential (Figure 2). Nevertheless, the fact

that the positive contribution of nonsmoking, regular physical activity, and high fruit consumption tended to be stronger in nonstressed subjects than in highly stressed subjects reveals possible interactive effects that may have partially masked the positive contribution of no psychologic stress.

Finally, solar radiation is one of the major damaging environmental agents for human skin, causing sunburn, premature skin aging, and skin carcinogenesis (43,44). In the present study, regular UV light exposure, which tended to decrease the blood antiradical resistance, was partially associated with a decreased antioxidant capacity. In contrast, lack of UV exposure was weakly related to an increased antioxidant capacity. Apart from the increased negative contributions of low fruit consumption and tobacco smoking in subjects who had reported regular UV light exposure (Figure 2), the weak relations between UV light exposure and the antioxidant defense capacity may be ascribed to various pigmentation characteristics and freckling in the studied population. Therefore, additional studies should be conducted in the UV-exposed phototype groups as defined by Fitzpatrick (45). It may determine whether the UV light–mediated impairment of blood antiradical resistance reflects the harmful effect of UV light on the skin antioxidant system, especially on phototypes I, II, and III. It may additionally evaluate the involvement of biologic antioxidants such as β -carotene in human skin defenses against UV radiation (46).

Consequently, regarding the maintenance of an antioxidative homeostasis *in vivo*, our findings on individual behaviors enabled us to define a “healthy” and “unhealthy” lifestyle pattern (Figure 2). Because the “unhealthy” lifestyle pattern that we have found in the present study is consistent with lifestyle profile recognized to put an individual at risk for cancer and cardiovascular disease, the free-radical–mediated hemolysis test is likely able to predict factors associated with an increased risk for free-radical–mediated disease.

Nevertheless, because of low sample size—especially for vitamin intake, heavy alcohol drinking, high life-related stress, and little fish consumption—additional clinical designs using a great number of healthy subjects are needed to confirm these findings. The use of the hemolysis test, initiated by free-radical generation at water-soluble or lipid-soluble sites, in combination with analytic dosages of specific biomarkers could be helpful for this purpose. It should especially allow the evaluation of the relevance of biologic water- and lipid-soluble antioxidants and of enzymatic and nonenzymatic systems in the blood resistance against free-radical aggression. It should additionally give support to the ability of the free-radical–mediated

hemolysis test to determine factors associated with an increased or decreased antioxidant defense system. This test might then serve as a practical approach to antioxidant therapy based on correcting a decreased antioxidant defense potential. To our knowledge, this practical approach has not been yet developed. It appears somewhat promising regarding some prevention trials (21,26) and regarding the potential beneficial effect of vitamin supplement intake observed in the present healthy population and in elderly people (13,22).

In conclusion, our results emphasize that not smoking, vitamin and/or mineral supplementation, and regular physical activity, and to a lesser extent weak psychologic stress, moderate meat and high fruit consumption, and no sun exposure are positively related to individual antioxidant capacity. These behaviors may then be ascribed to a "healthy" lifestyle pattern. Conversely, tobacco smoking; high psychologic stress; heavy alcohol, moderate vegetable, low fruit, and little fish consumption; and to a lesser extent regular UV light exposure and no regular physical activity were negatively related to the individual blood antiradical resistance. These "unhealthy" lifestyle and environmental factors combined with unevaluated factors such as genetic predisposition may thus truly contribute to a decreased antioxidant defenses. Furthermore, our findings indicate that, whereas regular physical activity and non-smoking would be the most relevant factors in improving the antioxidant defense system, psychologic stress and tobacco smoking would be the most relevant factors in impairing the overall antiradical defense capacity independently of each other and of other factors. Therefore, the free-radical-mediated hemolysis assessment could be useful as a precocious diagnosis to identify apparently healthy subjects who are at risk for free-radical-mediated diseases such as cancer and atherosclerosis. On the other hand, among patients bearing free-radical-mediated pathology, it could help to detect, for each individual, the positive or negative development of a therapy on the overall antioxidant capacity.

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