Free radical and antioxidant status in urban and rural Tirupati men: interaction with nutrient intake, substance abuse, obesity and body fat distribution

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Rapid growth in urbanisation and industrialisation causes exposure to toxicant pollution which may contribute to increased incidences of non-communicable diseases. The present study reports on plasma lipid peroxides (LPO), lymphocyte free radicals, antioxidants and DNA damage in relation to life-style, obesity and body fat distribution measures among 56 urban men and 45 age matched rural men. Significant increases in plasma LPO, free radical generation (superoxide anion and hydrogen peroxide), and DNA damage indicated by malondialdehyde (MDA) levels were observed in urban compared to rural men. *In vitro* assay of DNA damage showed a higher level of MDA in samples of urban men than those of rural men. There were no significant differences in antioxidant enzymes between urban and rural men. Neither body mass index nor fat distribution had a significant influence on free radical generation, while the habits of smoking and alcohol consumption were associated with increased levels of free radicals, plasma LPO and DNA damage and reduced levels of antioxidant enzymes such as glutathione-S-transferase (GST), superoxide dismutase (SOD) and catalase in urban men. Dietary energy and fat intakes were positively correlated with free radical generation. Both superoxide anion and hydrogen peroxide were positively correlated with LPO and DNA damage, and negatively correlated with antioxidant enzymes in urban men. The marked elevation of free radical generation, LPO, DNA damage and depletion in antioxidant levels in urban men may suggest that exposure to environmental toxicant pollution is a risk factor for oxidative damage. It was of interest in this study that, whilst BMI was not greater in urban than rural men, abdominal fatness was. Hypothetically, fat distribution could be altered by the process of oxidative damage if it altered regulation of metabolically active omental fat.

Key words: free radicals, antioxidants, Tirupati, India, rural, urban, nutrient intake, alcohol, cigarette smoking, obesity, body fat distribution

Introduction

Populations exposed to toxicants from industrialisation and urbanisation reveal increased risk of heart disease, cancers, age associated degenerative diseases and genetic damage[1-5]. Free radicals are capable of inducing DNA damage and mutagenesis[6]. Mutagens may be either free radicals directly involved in reactions or generate free radical intermediates[7]. Human tissue free radical concentrations in industrial environments are much higher than those who live and work in rural environments[3]. Free radical reactions and lipid peroxidation products may play a significant role in physiological impairment and in various pathological conditions[8]. The intracellular concentration of both free radicals and non-radical lipid peroxidation products are physiologically controlled by scavenger molecules and protective enzymatic mechanisms. Inadequate physiological antioxidant defence systems may thus lead to oxidative damage[9].

Differences in geographical location, inadequate attention to confounding factors such as age, sex and life style factors, and disease all contribute to oxidative stress[10]. Oxidative damage correlates with smoking habits[11]. Frei *et al*[12] have shown that tobacco smoking induces lipid peroxidation and lipoprotein oxidation by diminishing antioxidant concentration. A higher metabolic rate among lean persons may promote an increase in oxidative damage[13,14], unless matched by increased antioxidant capacity. Depletion of total antioxidants among the obese may also elevate levels of lipid peroxides[15].

Although studies have been conducted in different animal and human populations to show effects of free radical mediated oxidative stress, human populations in developing countries, such as India, have not been studied in relation to modernisation of life style and industrialisation, which are occurring rapidly. Therefore, the present study has been undertaken to assess the levels of lymphocyte free radicals and antioxidants in relation to life style, obesity and body fat distribution in an urban and rural community.

Materials and methods

56 healthy male urban subjects were selected by a purposive sampling technique. All urban subjects lived in densely populated market and business centres of Tirupati. 32 worked in the sugar industry, 19 with dry cell batteries and

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the remaining 5 worked as metal welders, in the industrial zone of Tirupati. For controls, 45 age matched healthy male agricultural farm workers, 40km away from the Tirupati industrial zone were selected. Subjects were asked, but not compelled to participate or subjected to any risk, other than that usually entailed in their occupation. Informed consent was given. Age ranged from 25 to 48 years.

The participants were interviewed about smoking habits, alcohol usage and dietary intake. All subjects were involved in heavy manual labour. Dietary information was collected, using a 7-d prospective survey. After examination, each person received a 7-d dairy to record his daily food intake and its quality, quantity, origin and method of preparation. In the morning of eight day, a dietitian interviewed each subject for details and evaluated the quantity of food ingested per day. From this 7-d collection of information, daily intake of energy and other nutrients were calculated from the food composition tables, based on Gopalan *et al*[16].

The *physical assessment* included height, weight, circumferences of the waist and hip according to the method specified by Shimokata *et al*[17]. The body mass index (BMI) was calculated as BMI = weight in kg/height in metres^2 (kg/m^2). Obesity was defined as BMI > 25. Waist hip ratio (WHR) was calculated from the circumferences of waist and hip.

Venous *blood* (10 ml) was collected in the morning from all subjects into disposable vials containing EDTA. Plasma was separated from the whole blood on centrifugation at 3000 rpm. Lymphocytes were separated from the whole blood by dextran sedimentation technique[18].

Plasma *lipid peroxidation* products: Thiobarbituric acid was added to plasma sample under acidic condition, and the absorption of colour that developed after heating was estimated spectrophotometrically at 535nm[19]. 1,1,3,3-tetra-ethoxy propane (TEP) was used as internal standard. Superoxide anion: Superoxide anion can reduce nitroblue tetrazolium (NBT) to the insoluble formazan[20]. Hydrogen Peroxide: Hydrogen peroxide released by lymphocytes was estimated by the horse-radish peroxidase method[21]. 1 x 10^6 lymphocyte cells/assay were taken for both Superoxide anion and H_2O_2 assay.

Lymphocyte Antioxidants: Glutathione-S-transferase: GST activity was measured by following the increase in absorbance at 340 nm using 1-chloro-2,4 clinitrobenzene (CDNB) as a substrate as described by Habig *et al*[22]. Superoxide dismutase: SOD activity was assayed according to the method of Misra and Fridovich[23]. Catalase: Catalase assay was carried out by the method of Beer and Sizer[24]. The decomposition of H_2O_2 was followed directly by measuring the decrease in absorbance at 240 nm.

DNA damage: DNA was extracted from lymphocytes by the procedure of Hoar *et al*[25]. The study also carried out a simple analysis for DNA damage, and *in vitro* DNA damage with the addition of TEP, an analogue for MDA, in samples from both urban and rural communities.

TBA assay for DNA damage: The principle of the assay is that sugar fragments consists of compounds that carry one or several carbonyl functions[26]. Mix one aliquot of the DNA solution with one aliquot of 0.6% 2-thiobarbituric acid. The contents were heated at 90°C for 20 min and the developed red colour was measured at 537 nm. The values were expressed as nmol MDA equivalents/mg DNA.

In vitro DNA Damage: Aliquots of DNA solution prepared in tris buffer (0.1 M, pH 7.4) in the absence and presence of MDA were analysed to evaluate *in vitro* DNA damage[27]. MDA was obtained from acid hydrolysis of tetraethoxypropane (Reaction mixture containing 50 µl TEP and 100 µl HCl (0.1 N) in 100 ml doubly distilled water was incubated at 50°C for 1 hr. The hydrolysed product MDA was characterised by its wave length maxima at 245 nm and $\in$ value of 1.3 x 10^4 M^{-1} Cm^{-1}). The UV absorption of native DNA solution was first recorded. Next the DNA solution was incubated with MDA (50 nmol) for ½ hr in a water jacketed incubator maintained at 37°C and UV absorption recorded. Δ OD was calculated for each sample as an increase after MDA treatment. The concentration of DNA extracted from each individual was 25 µg.

Statistical analysis included multiple regression analysis and partial correlations. $P<0.05$ was regarded as statistically significant.

Results

Body mass index, weight and height were not significantly different between urban and rural men, whereas, WHR or central (trunk) fat distribution was higher in urban men than rural men. Urban men consumed more total energy, protein, fat, carbohydrate and ascorbic acid than did rural men (Table 1). Levels of both LPO and lymphocyte free radicals (superoxide anion and hydrogen peroxide) were significantly higher in urban men than rural men (Table 2). The level of DNA damage was 4.51 nmol MDA equi/mg DNA in urban compared to 1.98 nmol MDA equi/ mg DNA in rural men (Table 2). Lymphocyte antioxidant levels were not significantly different between urban and rural men.

Table 1. Mean values for anthropometry and dietary variables amongst urban and rural males.

Variable	Urban n = 56 Mean ± SD	Rural n = 45 Mean ± SD	t value
Height (cm)	164.5 ± 5.9	165.3 ± 6.1	0.67
Weight (kg)	58.5 ± 7.3	57.1 ± 7.6	0.89
BMI (kg/m^2)	21.7 ± 2.6	20.9 ± 2.4	1.55
Waist circum (cm)	76.9 ± 5.6	74.7 ± 4.7	2.14*
Hip circum (cm)	89.8 ± 3.9	88.0 ± 3.2	2.59*
WHR	0.86 ± 0.04	0.85 ± 0.03	2.01*
Total energy (K cal)	2445 ± 283	2037 ± 336	6.5*
Protein (g)	62.4 ± 7.9	52.7 ± 9.7	5.44*
Fat (g)	24.1 ± 7.1	15.0 ± 5.9	7.11*
Fibre (g)	2.7 ± 0.5	2.8 ± 0.6	1.06
Carbohydrate (g)	529.3 ± 73.0	453.1 ± 81.8	4.88*
Ascorbic Acid (mg)	8.3 ± 2.1	5.3 ± 1.9	7.62*

* Difference between populations significant at $p < 0.05$

When urban men were divided into 3 groups based on body mass index (< 20, 20-24.9, 25-29.9 considering >25 as obese), body fat distribution, plasma LPO, free radical generation and DNA damage were not different within the BMI groups (Table 3). But, WHR and LPO were significantly higher in obese subjects than those from other group. A significant increase in the activities of GST and Catalase were observed from the group of <20 to 24.9 units of BMI, and a decreased activity of GST alone was significant among obese individuals.

Based on habits, urban men were divided into smokers and non-smokers and alcohol users and non-users and the

results are presented in Table 3. There was a significant increase in plasma LPO, lymphocyte free radicals and DNA damage, while a decrease in lymphocyte antioxidant levels in smokers and alcoholics compared to non-smokers and non-alcoholics respectively.

Table 2. Mean values for plasma lipid peroxidation, lymphocyte free radicals and enzymatic antioxidant concentrations and lymphocyte DNA damage among urban and rural men.

Variable	Urban n = 56 Mean ± SD	Rural n = 45 Mean ± SD	t value
Lipid peroxides nmol/ml plasma	3.31 ± 1.29	2.61 ± 1.29	2.71*
Superoxide anion μ moles/1 x 10^6 cells	4.00 ± 2.13	2.14 ± 1.18	5.56*
Hydrogen peroxide μ moles/1 x 10^6 cells	2.76 ± 1.7	1.23 ± 0.87	5.84*
DNA damage nmol MDA equiv/mg DNA	4.51 ± 3.12	1.98 ± 1.6	5.27*
Glutathione - S transferase μ moles/mg protein	100.01 ± 27.62	92.20 ± 25.9	1.46
Superoxide dismutase U /mg protein	66.55 ± 25.75	64.4 ± 22.99	0.44
Catalase U / mg protein	48.32 ± 22.80	45.93 ± 19.25	0.57

*Difference between populations significant at $p < 0.05$

Partial correlation coefficients for free radical generation ie, superoxide anion and H_2O_2 to plasma LPO, DNA damage, lymphocyte antioxidants, BMI, WHR and dietary intake were calculated (Table 4). Both superoxide anion and H_2O_2 were positively associated with plasma LPO and DNA damage, and negatively related with the antioxidant enzymes GST, SOD and catalase. Free radical concentrations were not correlated with BMI or WHR. Among dietary variables energy and fat intake alone positively correlated with lymphocyte free radical concentrations.

Regression equations for free radical generation in urban men may be used to predict either superoxide anion or H_2O_2 taking into account age, smoking habits, alcohol use, BMI, fat distribution, plasma LPO, DNA damage and antioxidant enzymes (Table 5). 83% of the variance in free radical generation was explained by independent variables. Antioxidant enzymes (GSTs, SOD and catalase), plasma LPO and DNA damage account for a high percentage of the variance in either superoxide anion or H_2O_2 generation indicating that increase in plasma LPO or decrease in antioxidant enzymes may elevate the free radical generation. Age contributed significant variance while smoking was a significant source of positive variance for free radical generation. For dietary composition, fat alone provided significant positive prediction of free radical concentration. Both body mass index and body fat contributed negatively but not significantly to free radical concentration.

Table 4. Partial correlation coefficients for free radical generation with serum LPO, DNA damage, GST, SOD, Catalase, BMI, body fat and dietary intake for urban males controlled for age.

Variable	Superoxide anion	Hydrogen peroxide
LPO	0.8188*	0.8479*
DNA damage	0.8293*	0.8111*
GST	-0.4406*	-0.4991*
SOD	-0.5501*	-0.5533*
Catalase	-0.4873*	-0.4831*
BMI	0.0882	-0.1408
WHR	-0.0004	-0.0460
Energy	0.1906*	0.1981*
Protein	-0.0648	-0.1237
Fat	0.1823	0.1988*
Fibre	-0.1532	-0.1250
Carbohydrate	-0.0850	-0.0813
Ascorbic acid	-0.0212	0.0427

* Significant at $p < 0.05$

Table 5. Prediction of free radical generation in multiple regression analysis for urban males.

Dependant variable	Superoxide anion Coefficient	t value	Hydrogen peroxide Coefficient	t value
Age	-0.0142	-0.38	-0.0367	-1.30
Smoking	0.5742	1.98*	0.4986	1.03
Alcohol	0.0132	0.03	-0.1822	-0.55
Energy	0.0004	0.41	0.0003	0.43
Protein	0.0227	0.66	-0.0064	-0.25
Fat	0.0766	2.02*	-0.0528	-1.87
Fibre	0.0704	0.21	0.0784	0.31
Carbohydrate	-0.0005	-0.18	-0.0001	-0.03
Ascorbic acid	0.0609	0.69	0.0634	0.98
BMI	-0.0116	-0.18	-0.0133	-0.27
WHR	-0.5064	-0.10	2.4921	0.65
GST	0.0228	1.70	-0.0005	-0.05
SOD	-0.0590	-2.19*	-0.0339	-2.10*
Catalase	0.0283	1.32	0.0350	2.21*
LPO	1.2243	6.08*	0.9891	6.61*
Intercept	0.9327	0.22	0.1231	0.04
Multiple r^2	0.8330		0.8547	

* Significant at $p < 0.05$

Mean Δ optical density values presented in Tables 6 & 7 show the difference in OD values of native DNA before incubation and after incubation with 50 nmol of TEP at 37°C for 20 min. Significant differences were observed

Table 3. Plasma lipid peroxidation (LPO), lymphocyte free radicals and enzymatic antioxidant concentrations and lymphocyte DNA damage in urban men with different BMI, smoking habits and alcoholism.

	WHR	LPO	Superoxide anion	Hydrogen peroxide	DNA damage	GST	SOD	Catalase
BMI < 20 (n=17)	0.84±0.04	2.86±1.17	3.49±1.80	2.32±1.16	4.24±2.86	91.47±20.14	61.76±20.03	41.29±19.34
BMI 20-24.9 (n=31)	0.85±0.04	3.27±1.21	4.06±2.13	2.75±1.73	4.12±3.01	108.52*±28.51	72.25±26.18	54.61*±22.73
BMI 25-29.9 (n=8)	0.88*±0.04	4.42*±1.38	4.90±2.66	3.74±2.25	6.58±3.63	85.63*±29.12	54.63±31.48	38.88±24.84
Smokers (n=20)	0.86±0.05	4.40±0.93	5.81±1.81	4.19±1.42	7.33±2.20	74.70±15.84	41.80±19.90	25.15±14.37
Non-Smokers (n=36)	0.85±0.04	2.71*±1.06	3.01*±1.55	1.97*±1.27	2.94*±2.36	114.17*±22.14	80.31*±16.78	61.19*±15.08
Alcoholics (n=10)	0.85±0.04	4.17±0.94	5.22±2.21	3.80±1.46	6.75±2.87	80.90±16.28	49.00±21.88	29.00±17.52
Non- alcoholics (n=46)	0.85±0.04	3.13*±1.29	3.74*±2.03	2.53*±1.67	4.01*±2.98	104.20*±27.92	70.37*±25.12	52.52*±21.75

* Significant difference within the groups at $p < 0.05$

between rural and urban men. In urban men, smokers and alcoholic users possess significant higher OD values.

Table 6. Δ UV absorption spectra of urban and rural males

	Urban (n=56)	Rural (n=45)	
	Δ OD values Mean ± SD	Δ OD values Mean ± SD	t value
Total Sample	0.052 ± 0.024	0.031 ± 0.021	4.74*

ΔOD values = DNA in presence of MDA (50 nmol) - native DNA
* Significant at $p < 0.05$

Table 7. Δ UV absorption spectra of urban men by BMI, smoking and alcoholism

Variable	n	Δ OD values Mean ± SD
BMI		
1. < 20	17	0.052 ± 0.027
2. 20-24.9	31	0.047 ± 0.02
3. 25 >	8	0.057 ± 0.032
Smokers	20	0.069 ± 0.031
Non-smokers	36	0.04 ± 0.01*
Alcoholics	10	0.061 ± 0.032
Non-alcoholics	46	0.047 ± 0.03*

Δ OD values = DNA in presence of MDA (50 nmol) - native DNA
n = sample size; * Significant at $p < 0.05$

Discussion

Industrialisation and urbanisation cause exposure to environmental pollution, which contains reactive compounds of smog induces free radicals causing lipid peroxidation and DNA damage[5]. Significantly higher levels of LPO and lymphocyte free radicals in urban men probably indicate that urban men in Tirupati, India are exposed to a higher pollutive environment and less antioxidant capacity than rural men. We have previously observed increased concentrations of plasma lipid peroxides among a population exposed to toxicants[5]. Other studies demonstrate an increased generation of superoxide anion and H_2O_2, with elevated oxidative damage products such as protein carbonyls, lipofuscin and n–pentane[28]. Disease states and chemotherapy also increase free radical generation and lipid peroxidation[29]. Our results extend these findings to urbanisation.

Populations exposed to various *industrial toxicants* exhibit higher levels of hydroxyl radicals, DNA damage and sister chromatid exchange[30-31]. The results of the present study reveal an increased DNA damage measured as nmol of MDA equivalents per mg DNA in urban men, suggesting that free radicals and lipid peroxidation products induce DNA damage. The *in vitro* study demonstrated that DNA damage was more pronounced in the presence of MDA in samples from urban men than in those from rural men. Rongliang *et al*[3] and Popp *et al*[32] suggested that free radicals and sister chromatid exchange induce DNA protein cross links in metal exposed populations. However, Brambilla *et al*[8] observed no relationship between MDA production and DNA radioactivity either in controls or with prooxidant stimulation. They suggested that interaction of lipid peroxidation products with DNA is limited.

Depletion of antioxidant enzymes in *urban* men and a negative correlation between free radical generation and antioxidants may allow DNA damage. Epidemiological and experimental studies show that antioxidants may protect against free radical-mediated damage[33]. Antioxidants reduce neutrophil free radical production and serum lipid peroxides in myocardial infarction[34].

There was no correlation between *BMI or body fat distribution* with free radical concentration but antioxidant status in obese individuals was decreased with elevated levels of lipid peroxidation products. The data suggest that free radical concentration and DNA damage should take into account BMI in comparison of individuals along with other factors such as smoking and alcoholism.

Smoking influences more the levels of free radicals and DNA damage[35]. *In vitro* study has demonstrated that tobacco smoke and several of its constituents, such as hydroquinone and cathecol generate free radicals and induce oxidative DNA damage[30]. Mazette *et al*[36] found higher lipid peroxide levels and lower antioxidant capacity among smokers compared to non-smokers. Higher concentration of free radicals, LPO and DNA damage, and lower antioxidant status in urban men who smoke and/or drink alcohol in the present study indicates that usage of cigarette and alcohol augments free radical generation. The apparent effect of tobacco smoking on free radical generation and DNA damage may also increase basic metabolic rate[37], since the majority of non-obese individuals in the present study were smokers whose energy intake was higher.

Dietary composition was not found to have an effect on free radical generation or DNA damage, except for dietary fat which was positively related to free radical generation.

The positive relations between free radicals and LPO and the negative relations between free radicals and antioxidants provide evidence of oxidative stress with urbanisation or industrialisation. Tobacco smoke and alcohol may magnify the oxidative stress of industrialisation. Hence, occupational oxidative stress may be reduced through healthy life style. A large-scale intervention study in Tirupati with oxidative status as an end-point would be worthwhile.

Editor's Note

It was of interest in this study that, whilst BMI was not greater in urban than rural men, abdominal fatness was. Hypothetically, fat distribution could be altered by the process of oxidative damage if it altered regulation of metabolically active omental fat.

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Free radicals and antioxidants: influence of obesity, body fat and life styles
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自由基與抗氧化劑：肥胖症，體脂和生活方式的影響

摘要

城市化和工業化的迅速增長，帶來了有毒物質的污染，致使非傳染性疾病增加．本研究選用56位城市男性居民和45位年齡配對的農村男性居民爲對象．比較了肥胖症，體脂和生活方式與血漿脂類過氧化物（LPO）淋巴細胞自由基，抗氧化劑和DNA損害的關系．結果從丙二醛（MDA）水平顯示，城市男性血漿LPO，自由基（超氧化物陰離子和過氧化氫）和DNA損害較農村男性明顯增加．體外DNA損害的分析也顯示了城市男性的MDA水平較農村男性爲高．而抗氧化劑酶類在倆組人群無明顯差异．吸烟和酗酒可引起城市男性自由基，血漿LPO水平增加和DNA損害．并降低抗氧化劑酶類如谷胱甘肽－S－轉移酶（GST），超氧化物歧化酶（SOD）和過氧化氫酶．但體重指數與自由基的形成無明顯的影響．膳食脂肪和能量的攝取與自由基的生成呈正相關．城市男性的超氧化物陰離子和過氧化氫與LPO和DNA損害呈正相關，但與抗氧化劑酶類呈負相關．作者從城市男性自由基的形成，LPO，DNA損害的增加和抗氧化劑水平的下降，提出了環境污染也許是氧化損害的危險因素．

References:

1. Mandal PK. Cytogenetic damage in workers exposed to fertilisers. J Ecotoxicol Environ Monit 1991; 4: 317-320.
2. Reddy KK, Bulliyya G, Ramachandraiah T, Kumari KS, Reddanna P, Thyagaraju K. Serum lipids and lipid peroxidation pattern in industrial and rural workers in India. Age 1991; 14: 33-38.
3. Rongliang Z, Xiaoxuan W, Huping H, Zhanru F, Yuzhan Z. Sister chromatid exchange and DNA damage in human lymphocytes induced by air-dust in Lanzhou city: involvement in free radicals. J Environ Scineces 1991; 3: 69-74.
4. Perara FP, Hemminkt K, Gryzbowska E, Motyklewicz G et al.,. Molecular and genetic damage in humans from environmental pollution in Poland. Nature 1992; 360: 256-258.
5. Reddy KK, Ramachandraiah T, Reddanna P, Thyagaraju K. Serum lipid peroxides and lipids in urban and rural Indian men. Arch Environ Health 1994; 49: 123-27.
6. Devasagayam TPA, Steenken S, Obendorf MSW, Schulz WA, Sies H. Formation of 8-hydroxy (deoxy) guanosine and generation of strand breaks at guanine residues in DNA by singlet oxygen. Biochemistry 1991; 30: 6283-89.
7. Zheng R, Lesco SA, Trpis L, Ts'o OP. The verification for free radical mechanism of benzo (a) pyrene carcinogenesis. Acta Biochemica et Biophysica 1987;19:317-21.
8. Brambilla G, Martelli A, Mancini UM. Is lipid peroxidation associated with DNA damage? Mutation Res 1989; 214: 123-27.
9. Halliwell B, Gutteridge JMC. Free Radicals in Biology and Medicine. Oxford, Claredon Press 1989, 1-543.
10. Bunker VW. Free radicals, antioxidants and ageing. Med Lab Sci 1992; 49: 299-312.
11. Loft S, Fischer-Nelsen A, Jeding JB, Vistesen K, Poulsen HE. 8-hydroxyquanosine as a urinary biomarker of oxidative DNA damage. J Toxicol Environ Health 1993; 40: 391-404.
12. Frei B, Forte JM, Ames BN, Cross CE. Gas phase oxidants of cigarette smoke induce lipid peroxidation and changes in lipoprotein properties in human blood plasma. Biochem J 1991; 277: 133-138.
13. Shah M, Miller DS, Geissler CA. Lower metabolic rate of post-obese versus lean women: Thermogenesis, basal metabolic rate and genetics. Eur J Clin Nutr1988; 42: 741-52.
14. Chung MH, Kasai H, Nishimura S, Yu BP. Protection of DNA damage by dietary restriction. Free Radical Biol Med 1992; 12: 523-5.
15. Olinescu R, Radaceanu V, Nita S, Lupeanu E. Age dependent variations of the plasma peroxides and total antioxidants in women with obesity and hypothyroidism. Rom J Intern Med 1992; 30: 285-90.
16. Gopalan G, Ramasastri BV, Balasubramanian SC. Nutritive values of Indian foods. NIN, Indian Council of Medical Research, Hyderabad, India, 1985.
17. Shimokata H, Tobin JD, Muller DC, Elahi D, Coon PG, Andres R. Studies in the distribution of body fat: 1. Effects of age, sex and obesity. J Gerontology 1989: 44: 66-73.
18. Sandhya P, Das UN. Modification of normal human leucocyte alkaline phosphotase activity by colchicine and imidazole: Relevance to thromboxane A_2 and prostaglandin involvement. IRCS Med Sci 1981; 9: 207-208.
19. Ohkawa M, Ohishi N, Yagi K. Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. Analyt Biochem 1979; 95: 357-358.
20. Das UN, Begin ME, Ells G, Huang YS, Horrobin DF. Polyunsaturated fatty acids augment free radical generation in tumor cells in Vitro. Biochem Biophys Res commun 1987; 145: 15-21.
21. Pick E, Keisari Y. A simple calorimetric method for the measurement of H_2O_2 produced by cells in culture. J Immunol Methods 1980; 38: 161-170.

22. Habig WH, Pabst MJ, Jacoby WB, Gtutathione S-transferase. First enzymatic step in mercapturic acid formation. J Biol Chem 1874; 249:7130-7139.
23. Misra HP, Fridovich I. The role of superoxide anion in the autoxidation of epinephrine and a simple assay for superoxide dismutase. J Biol Chem 1972; 247:3170-5.
24. Beer RF, Sizor IW. A spectrophotometric method for measuring the breakdown of hydrogen by catalase, J Biol Chem 1952; 195:133-140.
25. Hoar DI. Molecular diagnosis; New horizons in medicine. Can Fam Physician 1987; 33:401-404.
26. Langfinger D, Von Sonntag C. g - radiolysis of 2-deoxyguanosine. The structure of the malondialdehyde like product. Z naturforch C Biosci 1985; 40C:446-448.
27. Srivastava VK. Malondialdehyde induces DNA denaturation. Ind J Biochem Biophys 1984; 21:340-342.
28. Sohal RS, Dubey A. Mitochondrial oxidative damage, hydrogen peroxide release and aging. Free Rad Biol Med 1994; 16:621-626.
29. Sangeetha P, Das UN, Koratkar R, Suryaprabha P. Increase in free radical generation and lipid peroxidation following chemotherapy in patients with cancer. Free Rad Biol Med 1990; 8:15-19.
30. Leanderson P, Tagesson C. Cigarette smoke induced DNA damage in cultured human lung cells: role of hydroxyl radicals and endonuclease activation. Chem Biol Interac 1992; 81:1997-2008.
31. Weitberg AB, Cornese D. Oxygen radicals potentiate the genetic toxicity of tobacco specific nitrosamines. Clin Genet 1993; 43:88-91.
32. Popp W, Vahrenholz C, Schmieding W, Krewet E, Norpoth K. Investigation of the frequecny of DNA strand breakage and cross-linking and of sister chromatid exchange in the lymphocytes of electric welders exposed to chromium and nickel containing fumes. Int Arch Occup Envirn Health 1991; 63:115-120.
33. Van Antwerpen VI, Theron AJ, Richards GA et al: Plasma levels of beta- carotene are inversely correlated with circulating neutrophil counts in young male cigarette smokers. Inflammation 1995; 19:405-414.
34. Herbaczynskacedro K, Klosiewiczwasek, B, Cedro K et al. Supplementation with vitamins C and E suppresses leucocyte oxygen free radical production in patients with myocardial infarction. Eur Heart J 1995; 16:1044-1049.
35. Tagesson C, Kallberg M, Leanderson P. Determination of urinary 8-hydroxydeoxyguanosine by coupled column high performance liquid chromatography with electrochemical detection: a noninvasive assay for inVivo oxidative DNA damage in humans. Toxicol Methods 1992; 1:242-251.
36. Mazzetti A, Lapenna D, Pierdomenico SD et al. Vitamin E, C and lipid peroxidation in plasma and arterial tissue of smokers and non-smokers. Atherosclerosis 1995; 112:91-99.
37. Moffatt RJ, Owens SG. Cessation from cigarette smoking: changes in body weight, body composition and energy consumption. Metabolism 1991; 40:465-470.