Exercise-Induced Genetic Damage: A Review

G. Gandhi* and Gunjan

*Department of Human Genetics, Guru Nanak Dev University,
Amritsar 143 005, Punjab, India
Telephone (O): +91-183-2258802-09 Extn. 3444, Fax (Univ.):+91-183-2258820
E-mail: gsbau@hotmail.com; gandhig07@hotmail.com*

ABSTRACT Physical activity improves the quality of life and well being in healthy persons and has been demonstrated to have beneficial effect on quality of life during and after therapy in cancer patients. Considerable evidence on the other front has shown an association between physical activity and genetic damage and also implied a possible role of physical activity on cancer incidence. Diverse biologic mechanisms have sought to explain the complex relationship between energy balance, physical activity and genetic damage. Primary and secondary reactions have been implicated. These include free radical damage, immune dysfunction, mechanical injury, sex hormones, growth factors, cytokines, etc. Alterations in pro-and anti-apoptotic proteins during long-term physical activity can possibly explain why exercise training relates to inconsistence in cancer causing. Future research is needed in this direction for possible intervention trials. The value of antioxidant supplementation for attenuating post-exercise tissue damage cannot be undermined though more conclusive studies are required for exact recommendations.

INTRODUCTION

Frequent participation in physical activity contributes to better health and has been a recurring theme in medicine and education. The physical activity of a person or group is frequently categorized by the context in which it occurs (U.S. Department of Health and Human Services 1996). Exercise and physical activity are often used synonymously but exercise has been defined (Caspersen et al. 1985) as a physical activity that is planned, structured, repetitive, and purposive for the improvement or mainte-nance of one or more components of physical fitness; exercise training is the physical activity performed for enhancing physical fitness only. Physical fitness is the ability to carry out daily tasks with vigor and alertness, without undue fatigue, and with ample energy to enjoy leisure-time pursuits and to meet unforeseen emergencies (Park 1989). It includes cardio-respiratory and skeletal muscular endurance, skeletal muscular strength and power, speed, flexibility, agility, balance, reaction time and body composition. These attributes differ in their importance to athletic performance versus health and so performance-related fitness and health-related fitness are distinct (Pate 1983; Caspersen et al. 1985). Health-related fitness includes cardio-respiratory fitness, muscular strength and endurance, body composition and flexibility; the importance of an attribute depends on the particular performance or health goal.

Exercise fads and trends have come in vogue and have gained popularity among the youth in order to keep fit and healthy. Regular exercise has multiple benefits yet strenuous exercise generates free radicals (reactive oxygen species, ROS) which cause oxidative stress. ROS are formed continuously in the body but physical exercise leads to their increased generation. In case free radicals are not adequately removed by antioxidant defenses, they react with membranes, proteins, nucleic acids and cellular components to initiate cellular damage and degeneration.

Free Radicals: These possess one or more unpaired electrons, which make them highly reactive; they have a short life span, are auto-catalytic with diverse chemical reactivity and low specificity. They can be generated both *in vitro* and *in vivo* by radical formation via electron transfer and by hemolytic fission or by ion formation via heterolytic fission (Suresh and Tiwary 1999). Most of the free radicals are derived from oxygen being constantly generated by metabolic and biochemical reactions involving patho-physiological conditions, endogenous systems, exogenous xenobiotics or during exposure to physiochemical agents. However, this defense mechanism despite its vital role can be hazardous. Diseases such as rheumatoid arthritis and inflammatory bowel disease are accompanied by inappropriate phagocytic activation and damage to the

associated tissues occurs by the release of ROS. Also, ROS originated from stimulated neutrophils cause damage to the DNA of other cells in their vicinity. The release of superoxide and its dismutation production of hydrogen peroxide from non-phagocytic mammalian cells (endothelial cells and fibroblasts) occur upon stimulus from specific cytokines or polypeptide growth factors, though the rate of superoxide generation is compa-ratively low. Oxygen gas gets converted to its more stable chemical state of water; during this process intermediates like superoxides (O_2^-*) and hydroxyl (*OH) species are produced; these and derivatives like hydrogen peroxide (H_2O_2) and hypochlorous acid (HOCl) participate in reactions giving rise to free radical species (Devasagayam and Kamat 2000), the reactive oxygen species (ROS) and another group, the 'reactive nitrogen species'(RNS) which has both, nitrogen and oxygen and includes nitric oxide (NO*) and toxic peroxynitrite ($ONOO^-$).

Concept of Oxidative Stress

The generation of pro-oxidants in the form of ROS and RNS are constantly and effectively kept in check by the antioxidant defense system in a normal healthy human body. An imbalance between overproduction of free radicals or a decrease in the level of the antioxidant defenses can lead to oxidative stress.

Approximately 1-5% of ROS escape electron transport chain and damage cellular components (c.f. Kelly et al. 1998). Normally, there are 1.5×10^5 oxidative adducts per cell and this corresponds to about 0.005% of the total number of nucleotides that comprise the human genome (Burdon 1999). The primary site of oxidative damage (Hartmann and Niess 1999) is DNA leading onto ROS-induced single-and double-strand breaks, base alterations, damage to deoxyribose, formation of DNA-protein cross-links, etc. *OH predominantly causes strand breaks while $^1O_2$ mainly induces base alterations like 8-hydroxydeoxyadenine, 8-hydroxydeoxy guanine and thymine glycol.

Maximal Oxygen Uptake (VO$_2$ Max): It is the maximal capacity for oxygen consumption by the body during maximal exertion. It is also known as aerobic power, maximal oxygen consumption, and cardio-respiratory endurance capacity. Various factors can affect VO_2 max, viz.

Mode of Exercise: Different forms of exercise reflect the quantity of muscle mass activated and so exhibiting variations in VO_2 max (Blomqvist et al. 1981; Lewis et al. 1983).

Heredity: It has an estimated genetic effect of about 25 to 40% for VO_2 max, 50% for maximum heart rate, and 70% for physical working capacity (Perusse et al. 1989 ; Bouchard and Perusse 1994; Bouchard et al. 1994). About 40% of the variation in muscular strength among individuals probably results from genetic factors (Perusse et al. 1988).

State of Testing: An individual's state of aerobic training contributes significantly to the VO_2 max, which normally varies between 5 and 20% depending on individual's fitness at the time of testing (McArdle 2002).

Gender: Women VO_2 max scores are 15 - 30% below values of male counterparts (Veeger et al. 1991); even among trained endurance athletes, gender differences vary between 15 and 20% (Bergh et al. 1987). The gender difference in VO_2 max has generally been related to difference in body composition and hemoglobin concen-tration. On an average, a male generates more total aerobic energy because he possesses more muscle mass and has less fat than the average female. Although trained athletes have lower percentage of fat than average individuals, yet trained women still possess significantly more body fat than their male counterparts. Also, because of higher testosterone levels, men have a 10 to 14% greater hemoglobin concentration than women and this difference in the blood's oxygen-carrying capacity enables men to circulate more oxygen during exercise, thus increasing their aerobic capacities above that of women (Woodson et al. 1984).

Body Size and Composition: Nearly 70% of the differences in VO_2 max scores among individuals are due to variations in body mass (Wyndham et al. 1969). The VO_2 max of the female remains about 20% lower than that of the male when expressed per unit of body mass (ml/kg/min).

Age: For children, the relative values for boys VO_2 max values remain level at about 52 ml/kg/min from ages 6 to 10 and for girls, the VO_2 max value is 40 ml/kg/min at age 16, a value 32% below their male counterparts; for adults, VO_2 max declines steadily after age 25 at a rate of about 1% per year, however active adults retain a relatively high VO_2 max at all ages (McArdle 2002).

Various tests to estimate VO_2 max include: walking tests, endurance runs, predictions based on heart rate, step test and prediction from non-exercise data.

Molecular Damage Induced by Reactive Species

Both oxygen- and nitrogen-derived reactive species are capable of altering biological macromolecules like lipids, proteins and DNA.

Damage to Lipids: Free radicals attack lipids very readily by attacking polyunsaturated fatty acids (PUFAs) present in the cellular membrane by lipid peroxidation (oxidative destruction of PUFA). Toxic aldehyde products released at the end of the reaction are hazardous to cellular components including DNA, because they form covalent aldehyde-DNA adducts (Suresh and Tiwary 1999).

Damage to Proteins: ROS also induce significant alterations in proteins with the most susceptible being sulphydryl groups in amino acids and lead to fragmentation of proteins and cross-links (Devasagayam and Kamat 2000). Certain enzymes such as catalases, peroxidases and [4Fe-4s]-containing dehydratases (e.g. aconitase, fumarase) get inactivated and release Fe^{2+} after an encounter with Fe^{2+}. There is also an aggregation of peroxidized lipids and proteins and of lipofuscin, which accumulates in lysosomes of aged cells and in brain cells of patients with Alzheimer's disease.

Damage to DNA: The 'steady state' level of oxidative DNA damage from the 'natural' sources is high. In humans, there are 1.5×10^5 oxidative adducts per cell and this corresponds to about 0.005% of the total number of nucleotides that comprise the human genome (Burdon 1999). The primary site of oxidative damage (Hartmann and Niess 1999) is DNA leading onto ROS-induced single-and double-strand breaks, base alterations, damage to deoxyribose, formation of DNA-protein cross-links, etc.

Exercises–Induced Reactive Oxygen Species (ROS)

Mechanisms by which exhaustive exercise can induce DNA damage include mitochondrial electron transport chain, ischemia-reperfusion mechanism, xanthine oxidase catalyzed reaction, inflammatory mechanism and production of catecholamine (Hartmann and Niess 1999).

Mitochondrial Electron Transport Chain: In the exercising muscle, chemically bound energy is converted to mechanical energy and this energy comes from ATP (which is limited in cells and has to be regenerated). The most efficient pathway of ATP regeneration is via oxidation of glycogen and fat stored in the musculature. Molecular oxygen is the final acceptor of electrons in the respiratory chain located in mitochondria. Therefore, mitochondria represent potential sites for elevated oxygen free radical formation during oxidative stress as from exhaustive exercise. Exercise-increased mitochondrial ROS production is based on the fact that tissue and whole-body O_2 consumption increases dramatically during strenuous exercise. Whole body O_2 consumption (VO_2) increases up to 20-fold, whereas VO_2 at the muscle fiber level is elevated by as much as 100-fold during maximal exercise (Ames et al.1995). The percentage of O_2, which becomes O_2^- remains the same but ROS production is increased proportionally. Mitochondrial oxidative damage supports this hypothesis that mitochondria are the primary site of ROS generation. After exhaustive exercise, respiration increases in muscle and liver mitochondria (Grollman and Moriya 1993) and in heart mitochondria (Tanaka et al. 1996), indicating a possible inner membrane leakage. Mitochondrial lipid peroxidation is also enhanced after exercise and is accompanied by loss of thiol protein content and the inactivation of oxidative enzymes (Hayakawa et al. 1993).

Ischemia-reperfusion Mechanism: During exercising the blood flow is restricted in some areas (kidneys and splanchnic region) and is deviated to the active muscles. This selective distribution of oxygen and nutrients produces a status of transient hypoxia in the restricted areas, which is directly related to the magnitude of the exercise (Adams and Best 2002). Besides this, muscle fibers undergo relative hypoxia during exercise performed at intensities above maximal oxygen uptake (VO_2max), as O_2 supply cannot match the energy requirements. Reoxygenation of these tissues occurs after the cessation of exercise – completing the ischemia – reperfusion cycle with the subsequent ROS generation. Furthermore, reperfusion leads to an increased ROS generation through the conversion of xanthine dehydrogenase to xanthine oxidase (Ji 1999).

Xanthine Oxidase Catalyzed Reactions: The major source of free radical generation in the ischemia and reperfused (I-R) heart are xanthine oxidase (XO)-catalyzed reactions (Kuppasamy and Zweier 1989; Downey 1990). During ischemia, due to the energy demand of contracting myocardium, ATP is degraded to ADP and AMP. AMP is continuously degraded to hypoxanthine, which is converted to xanthine and uric acid by XO coupled with one-electron reduction of O_2, giving rise to O_2^-, if O_2 supply is insufficient. High intensity exercise produces a cellular environment in favour of activating the XO pathway (Hellsten 1994). After intense muscular contraction hypoxanthine gets accumulated; uric acid concentration gets increased in both, contracting muscle and in the plasma, suggesting that XO gets activated (Norman et al. 1987; Hellesten et al. 1993). Also, blood hypoxanthine and xanthine concentrations have been reported to increase dramatically in human subjects after intense exercise (Sahlin et al. 1991).

Inflammatory Mechanism: Blood-borne polymorphoneutrophils (PMNs) play a critical role in defending tissues from virus and bacterial invasions. Muscle or soft-tissue damage, caused by ROS-induced oxidative processes or simply stretching or mechanical forces, results in the activation of PMN. PMN migrate to the injury site and release two primary factors for phagocytosis i.e. lysozymes and O_2^-. Lysozymes facilitate the breakdown of damaged protein and cell debris and O_2^- is converted to H_2O_2 by cytoplasmic superoxide dismutase (SOD). H_2O_2 thus produced is further converted to OH by metal ions or to hydrochloric acid (HCl). This inflammatory response is considered critical in removing damaged proteins and preventing bacterial and viral infections but ROS and other oxidants released from neutrophils cause secondary damage such as lipid peroxidation (Meydani et al. 1992). However, it is the body's immune system which responds to an acute bout of intense exercise much the same as to sepsis wherein they share a common mediator, ROS (Camus et al. 1994). An acute bout of exhaustive exercise in humans also resulted in increased cell counts of leukocytes, lymphocytes and neutrophils (Hack et al. 1992).

Production of Catecholamine: Prolonged exercise elevates the levels of the circulating catecholamine hormone, the auto-oxidation of which produces free radicals. Catecholamine enhances myocardial and skeletal muscle oxidative metabolism via activation of β-adrenergic receptor thereby potentially increasing ROS production (Ji 1999).

Exercise-induced Damage to Cellular DNA

Strenuous exercise induces oxidative stress, which can cause DNA modifications. Among the DNA modifications, strand breaks, abasic sites, and DNA-protein cross links can be measured using biochemical methods. Double and single stranded breaks as well as alkali-labile sites can be detected using SCGE while clastogenic action can be assessed using cytogenetic techniques.

The 'steady-state' levels of DNA-oxidation can be assessed from chemical hydrolysis of DNA isolated from cells using Gas Chromato-graphy coupled with Mass Spectrometry (GC-MS). High Pressure Liquid Chromatography (HPLC) analysis has been carried out for the detection of 8-OHdG, the most important and extensively used biomarker of oxidative DNA damage because it represent 5% of the total oxidized bases in the DNA and is present in quantities that are sufficient to be readily detected (Helbock et al. 1999). The promutagenic base, 8-OHdG in DNA is formed from oxygen radical's (hydroxyl radical) attack on 2'-deoxyguanosine (dG) as a result of oxidative stress, resulting in a hydroxyl moiety replacing the hydrogen atom. This 8-OHdG is a potentially important factor in carcinogenesis because 8-OHdG preferentially base pairs with adenine rather than cytosine, generating G to T transversions, which are frequently found in tumor relevant genes. Thus, increases in level of 8-OHdG can have important implications for mutagenesis and the induction of tumors (c.f. Remmen et al. 2003). However these lesions can also be repaired and so removed from nuclear DNA. The repair of 8-OHdG in DNA involves base-excision repair yielding 8-hydroxyguanine (8-OHGua) and nucleotide excision repair, yielding 8-OHdG, which is excreted in urine (Shigenaga and Ames 1991; Germadnik et al. 1997; Suzuki et al. 1995; Pilger et al. 1997). A relationship between oxygen consumption and urinary excretion of this base modification and concomitantly elevated levels of 8-OHdG in the urine of athletes after strenuous exercise has been suggested (Loft et al. 1994; 1995).

Cytogenetic methods give a specific indication of mutagenic effects but only in proliferating cells. Sister chromatid exchanges (SCE) are reciprocal exchanges between DNA molecules of a replicating chromosome. Micronuclei (MN) are small chromosome fragments or whole chromosomes, which are not integrated into the daughter cell during cell division. A number of studies have reported the assessment of SCE and MN in physically active individuals (Hartmann et al. 1994; Schiffl et al. 1997; Umegaki et al. 1998; Gandhi and Kumar unpublished data; Gandhi and Mahajan unpublished data).

The Single Cell Gel Electrophoresis (SCGE/ comet) assay has also been used to demonstrate elevated DNA damage in leukocytes of human subjects after various forms of exercise (Hartmann et al. 1995; Niess et al. 1996, 1998; Mastaloudis et al. 2004; Gandhi and Gunjan unpublished data). The assay is a microgel electrophoresis technique that enables the detection of DNA damage and DNA repair in individual cells with high sensitivity. Oxidized bases can also be detected with the comet assay in conjunction with lesion-specific endonucleases such as *E. coli* endonuclease III and formami- dopyrimidine -DNA-glycosylase (FPG). These enzymes, applied to the microscopic slides for a short time after lysis, nick DNA at sites of base alteration and the resulting single-strand breaks are quantified. The free radical-induced oxidative DNA base modifications after hyperbaric oxygen therapy and exercise were detected *in vitro* with high sensitivity using a modification of the comet assay (Dennog and Speit 1996; Hartmann et al. 1998).

Antioxidant Defense System

Aerobic organisms possess a variety of enzymatic and non-enzymatic mechanisms that counteract the detrimental effects of free radicals. Antioxidants are capable of neutralizing free radicals or their actions (Suresh and Tiwary 1999) and include enzymes (Catalase, Glutathione Peroxidase, Peroxidase, Superoxide dismutase (SOD), Cytochrome oxidase system to detoxify hydrogen peroxide and superoxides) and other chemicals (aqueous phase-Glutathione Urate, Bilirubin, Cysteine, Ceruloplasmin ,Transferrin, Mannitol to scavenge H_2O_2, O_2^-, OH^- ; lipid phase- α-Tocopherol , β-Carotene against lipid peroxidation).

Nutritional Supplements

On one hand, strenuous physical exercise enhances free radical production, which can result in oxidative damage to cellular material. On the other hand, the antioxidant defense system plays a vital role in protection against oxidative stress. It is important to enhance cellular antioxidant capacity through nutritional means since the body contains an elaborate antioxidant defense system that depends on dietary intake of antioxidant vitamins and minerals and on the endogenous production of antioxidant compounds such as glutathione. Whether the body's natural antioxidant defense system is sufficient to counteract the increase in ROS with exercise or whether additional exogenous supplements (Bompa et al. 2003) are needed are important queries.

Genetic Damage Investigations in Physically Active Individuals

An effort has been made to consolidate the available literature of over the last few years on genetic damage investigations in physically active individuals or in those doing exercises. This detailed literature is reviewed here and a synoptic view of various studies that have demonstrated exercise-induced DNA damage using different techniques and assays and in various tissues in individuals doing various forms of physical activity or exercises is depicted in Table 1.

Tice et al. (1990) were the first to report an effect of exercise by the comet assay. After jogging 5km, one of three individuals had an increased level of DNA damage in blood sample obtained 5 min after the run. Inoue et al. (1993) carried out a study to find the effect of exercise on the formation of the oxidative DNA damage biomarker, 8-OHdG and of purine metabolites such as hypoxanthine, xanthine and uric acid. Venous blood and urine samples were collected from distance runners and swimmers before and after the usual training and the observed amount of 8-OHdG obtained from nuclear DNA of lympho-cytes decreased remarkably after intermittent swimming as well as after distance running. The plasma concentrations of hypoxanthine, xanthine and uric acid rose significantly after each exercise. However though

Table 1: Reports Documenting Genetic Damage From Exercising

Reference	Assay/endpoint	Tissue/specimen investigated	Exercise protocol	Results	Inference
Tice et al. (1990)	SCGE/DNA breaks	Lymphocytes	Jogging (5 km)	- Increased DNA damage 5 min after exercise	-DNA damage was increased after exercise but no record concerning the intensity of the run was reported.
Inoue et al. (1993)	HPLC/8-OHdG	Venous blood/Urine	Distance runners/ swimmers	- Nuclear 8-OHdG levels slightly decreased after running and swimming - Urinary excretions of xanthine, uric acid decreased whereas that of hypoxanthine increased after exercise - Plasma concentration of hypoxanthine, xanthine and uric acid decreased after exercise - 8-OHdG to creatine ratio in urine increased after running and swimming	Repair of oxidative DNA damage was augmented by exercise.
Hartmann et al. (1994)	SCGE/DNA breaks Blood cultures/SCE	Lymphocytes	Treadmill running	- Increased DNA damage 6 min after exercise, further increased after 6h and reached maximum 24h after the run.	The higher level of DNA damage in hours after exercise was an effect caused by the DNA repair system i.e., excisions of damaged nucleotides as there was no evidence of cytogenetic damage.
		Lymphocytes	Treadmill running	- DNA migration level reached to about control level after 72h - No evidence of SCE frequency	
Sen et al. (1994)	FADU	Leukocytes	Submaximal exercises	Induction of DNA strands breaks directly after the runs	
Hartmann et al. (1995)	SCGE/DNA breaks	Lymphocytes	Exhaustive treadmill running	- Increased DNA strand breakage 24h after the run - Less DNA damage in individuals receiving vitamin E supplementation 14 days before the exercise	Vitamin E prevented exercise – induced DNA damage and DNA breakage occured in WBCs after exhaustive exercise as a consequence of oxidative stress.
Niess et al. (1996)	SCGE/DNA breaks	Lymphocytes	Incremental Treadmill running	Increased DNA migration 24h after exercise in untrained as compared to trained	Adaptation to training was capable of reducing free radical associated DNA damage.
Poulsen et al. (1996)	HPLC/ oxidized DNA bases	Urine	Vigorous exercise (10 h a day for 30 days)	Increased urinary excretion of 8-OHdG (an oxidatively modified deoxynucleoside originating from nuclear DNA repair (or from cell turnover)	Oxidative stress to DNA points to a risk of development of cancer and premature ageing from extreme exercise.

Table 1: Contd....

Reference	Assay/endpoint	Tissue/specimen investigated	Exercise protocol	Results	Inference
Sumida et al. (1997)	HPLC/8-OHdG	Urine	Treadmill running - Bicycle erogmeter - 20 km run	No effect on the 8-OHdG excretion from day 1 to 3 after exercise regimen	Oxidative stress during a single bout of intensive exercise did not result in accumulation of oxidative DNA damage.
Pilger et al. (1997)	HPLC/8-OHdG	Urine	Running	No difference in the mean 8-OHdG excretion levels between runner and untrained healthy	Physical exercise in trained individuals did not induce a disturbance of the oxidant- to-antioxidant balance.
Okamura et al. (1997)	HPLC/8-OHdG	Urine	8-day training	- Urinary 8-OHdG levels increased - Plasma TBARS, CK and myoglobin levels increased after the training	Repeated exercise augmented oxidative stress and the DNA was injured by exercise-induced ROS. But did nott accumulate by consecutive exercise-sustained as long as the exercise was repeated.
Schiffl et al. (1997)	MN assay	Lymphocytes	Exhaustive sprints	Micronuclei increased 24-48 h post exercise	Exhaustive physical exercise causes severe mutations at the chromosome level in blood lympocytes.
Hartmann et al. (1998)	SCGE/DNA breaks	Leukocytes	Triathlon race	- Increased DNA damage in well trained athletes - No alternation is urinary 8-oxodG levels, FPG-sensitive sites, MN frequency	DNA damage in the comet assay did not result from direct effect of ROS and did not result in chromosomal damage.
Umegaki et al. (1998)	MN assay blood samples were subjected to x-ray irradiation *in vitro* to examine the modification of exercise induced chromosomal damage by a secondarily induced oxidative stress	Lymphocytes	Intensive treadmill running	- No altered MN frequency until 30 min post-run both in trained and untrained - Increased X-ray induced chromosomal damage in untrained	Intensive exercise induced slight chromosomal damage in untrained group, intensified by the secondarily induced oxidative stress.

Table 1: Contd...

Reference	Assay/endpoint	Tissue/specimen investigated	Exercise protocol	Results	Inference
Niess et al. (1998)	SCGE/DNA breaks	Leukocytes	Half marathon (HM)	Correlation between increased DNA migration 24h after HM and PMN count 1h post exercise	DNA damage in leukocytes occurred after intensive endurance exercise and was due to ROS released from PMN
Mars et al. (1998)	SCGE/DNA breaks	Lymphocytes	Exhaustive Treadmill running	- Single strand DNA damage in 10% of lymphocytes immediately after exercise - Lymphocyte apoptosis in 63% of lympho-cytes immediately after exercise and in 86.2% 24 h post exercise	- Mechanism behind immune dysfunction after exhaustive exercise. - Lymphocyte apoptosis accounted for exercise induced lympho-cytopenia and reduced immunity.
Asami et al. (1998)	HPLC/8-OHdG	Leukocytes	Physical exercise	- Lower level of 8-OH-Gua in trained as compared to untrained athletes before exercise - Levels of 8-OH-Gua decreased in untrained after exercise - Mean levels of 8-OH-Gua repair activity increased in untrained subjects after exercise	Physical exercise caused both rapid and long range reduction of oxidative DNA damage in human leukocytes.
Leaf et al. (1999)	Measurement of lipid peroxidation	Leukocytes	Cardiopulmonarus exercise stress testing	- Physical exercise training increased physical work capacity - No alteration in lipid peroxidation expired markers (ethane, pentane) and in plasma MDA	Physical exercise training reduced potential chronic health effect associated with daily activities by contributing to an overall reduction in exercise-induced free radical production.
Moller et al. (2001)	SCGE (with lesion specific endonucleases) DNA breaks and oxidized DNA bases	Lymphocytes/urine	Bicycle running	- Increased DNA strand breaks in altitude hypoxia, level further increased immediately after exercise - Increased FPG and endonuclease III sensitive sites at altitude hypoxia but no alteration after exercise	- DNA strand breaks and oxidative DNA damage produced by ROS was generated by leakage of mitochondrial respiration or during a hypoxia induced inflammation.. - Hypoxia depleted the antioxidant system's capacity.
Tsai et al. (2001)	SCGE (with lesion specific endonuclease /DNA breaks	Leukocytes	42 km Marathon race	- Increased urinary 8-OHdG levels - Oxidized pyrmidines (endonuclease III-sensitive sites) contributes to most of the post exercise nucleotide oxidation	Correlation between oxidative DNA damage and plasma CK levels and lipid peroxidation metabolites lasted for more than one week post-race.

Table 1: Contd....

Reference	Assay/endpoint	Tissue/specimen investigated	Exercise protocol	Results	Inference
Sato et al. (2003)	HPLC/8-OHdG RT-PCR to identify and quantitate hMTHI mRNA	Leukocytes	Bicycle ergometer	- Basal 8-OHdG levels were lower in physically active subjects as compared to sedentary subjects - 8-OHdG levels decreased 30min post exercise in sedentary subjects - hMTH1 mRNA levels increased in sedentary subjects after exercise	Mild exercise had beneficial effect on the maintenance of low levels of 8-OHdG by keeping the sanitization system at higher level.
Mastaloudis et al. (2004)	SCGE/DNA breaks	Leukocytes	50 Km ultramarathon run	- Percentage DNA damage increased but returned to baseline value 2h post-race-62% less DNA damage in women taking antioxidant as observed one day postrace	Endurance exercise resulted in DNA damage as shown by SCGE assay and antioxidants (1000 mg vitamin C and 400 IU RRR-a-tocopheryl acetate enhanced recovery in females.
Orhan et al. (2004)	Lipid peroxidation and acetone(GC-ECD) & O, O'-dityrosine ,8-OHdG (HPLC-APCI-MS/MS & ELISA)	Urine fractions	Cycle ergometer	- oxidative damage in moderately trained individuals - urinary biomarkers elevated	One hour of exercise induced oxidative damage in moderately trained individuals. Urinary biomarkers were sensitive to monitor damage.
Vincent et al.(2006)	Oxidative stress (inflammation, blood lipids)	Blood	Exercising- overweight young adults with after antioxidant (AOX) supplementation	- attenuated post-exercise oxidative stress	- AOX-supplementation attenuated post-exercise oxidative stress and contributors to oxidative stress .
Pittaluga et al.(2006)	Redox homeostasis, oxidative damage, lymphocyte cell death and repair systems	Blood	Maximal test in subjects with different training levels	- high baseline values of oxidized glutathione,micronuclei and hemolysis in agonist trainers. - a well balanced profile at rest, but were more susceptible to exercise-induced variations in non-agonists (NA).	- Agonistic training led to a chronic oxidative insult - NA with the lowest level of training frequency more susceptible to exercise-induced variations.Most parame-ters employed showed inter-individual variations
Demirba et al.(2006)	Oxidative/ antioxidativeparameters and DNA damage	Blood	TET test on untrained healthy subjects	- TET increased oxidants, decreased TAC and vitamin C, no DNA damage	- A balance shift towards oxidative side, but not enough to produce DNA damage.

Table 1: Contd...

Reference	Assay/endpoint	Tissue/specimen investigated	Exercise protocol	Results	Inference
Fatouros et al. (2006)	Free plasma DNA along with C-reactive protein (CRP), creatine kinase (CK), and uric acid (UA)	Blood	Resistance training regimen to stress the entire musculature	- plasma DNA concentrations, CRP, CK,UA increased	- Plasma DNA concentrations increased in proportion to training load. - Plasma DNA a sensitive marker for overtraining-induced inflammation.
Peters et al.(2006)	% apoptotic and necrotic cells(flow cytometry - Annexin V-FITC and propidium iodide uptake;SCGE)	Blood samples	Prolonged, sub-maximal exercise	- exercise-induced changes in total blood lymphocyte counts and cortisol concentrations did not result in a significant change in % apoptotic lymphocytes or DNA strand breaks .	No significant change in apoptotic lymphocytes or DNA strand breaks in the endurance-trained athletes.
Gandhi and Mahajan; Gandhi and Kumar unpublished	The capillary blood in vivo micronucleus test in lymphocytes	T- lymphocytes	Bodybuilding, Wrestling	- elevated frequencies of MN	Elevated MN frequencies indicating the clastogenic/aneugenic consequences of strenuous exercises
Gandhi and Gunjan, unpublished	SCGE	Leukocytes	Individuals maintaining a physical fitness regimen by work-outs in gymnasiums	- percent cell damage and DNA damage significantly increased	Significantly increased cell damage and DNA migration length

the urinary excretion of hypoxanthine increased, that of xanthine and uric acid decreased after exercise. After swimming or running, the 8-OHdG to creatinine ratio in urine increased. The authors thus concluded that the repair of oxidative DNA damage is augmented by exercise.

Hartmann et al. (1994) performed the SCE and SCGE assay to study the effect of physical activity on DNA damage in the lymphocytes of volunteers (n=3) after a 6-hr run on a treadmill. The individuals were noted for BP (blood plasma), ECG, lactate concentration and creatine kinase (CK) activity. Increased DNA migration in WBC's of these individuals was observed. However there was no significant increase in SCE. The DNA migration reached its maximum after 24h but it gradually decreased to that of the control level after 72h.

Sen et al. (1994) detected an association between a single bout of exercise and the induction of DNA strand breaks in leukocytes using a fluorometric analysis of DNA unwinding. In this study, two sub-maximal exercises were carried out seven days apart, each lasting 30 min. Blood samples were taken before and 2 min after each exercise and an induction of DNA damage in 13 out of 18 samples was observed.

Hartmann et al. (1995) also applied the SCGE assay to study DNA damage in PBL of human subjects after a single bout of exhaustive exercise and also to study the effect of vitamin E supplementation. Blood samples were taken before and 24h after the run and they found increased DNA strand breakage in all samples. A clear reduction in exercise-induced DNA damage was however observed after the supplementation with vitamin E (1200mg) among volunteers. Malondialdehyde (MDA) concentration decreased following this supplementation but its concentration did not alter significantly 15min and 24h after a run, suggesting that vitamin E prevented exercise-induced DNA damage.

Sandri et al. (1995) reported a possible role of apoptosis in normal and dystrophin-deficient muscles after running. For the study, normal and dystrophin-deficient mouse muscles were analyzed after a night of spontaneous wheel running followed by two days of rest. Terminal deoxynucleotidyl transferase-mediated end labeling (TUNEL) of DNA in nuclei in tissue sections and after gel electrophoresis of extracted DNA showed the presence of fragmented DNA.

Moreover, ubiquitin, a protein whose appearance is related to apoptosis, increased in muscles of dystrophic and normal runner mice. This study confirmed that DNA damage was absent in muscles of sedentary mice but was present in muscles of runner mice.

Niess et al. (1996) used the SCGE assay and found DNA damage in PBL of trained (TR, n=6) and untrained men (UTR, n=5) after they completed an incremental treadmill test until exhaustion (maximal lactate: 12.9±1.7 in TR and 12.2±2.5 mmol: 1-1 in UTR). A significantly increased DNA migration value from 2.31±0.20(TR) and 2.22±0.66 (UTR) at rest to 2.65±0.30(TR) and 3.00±0.41(UTR) tail moment, respectively, was found 24h after exercise. However, increase in DNA damage was, significantly lowered in TR (+18.7±6.8%) as compared to UTR (+35.7±8.9%) individuals but the plasma levels of MDA were not significantly increased in TR and UTR after exercise. When these values were checked at rest and 15 min after exercise, they were significantly lowered in TR as compared to that in UTR, which suggested that adaptation to training resulted in reduced free radical generation associated-effects such as DNA damage.

Poulsen et al. (1996) studied the effect of extreme exercise and the oxidative DNA modification resulting from it since extreme exercise increases oxygen uptake with a potential for increased formation of reactive oxygen species and damage to biomolecules (if such an increase exceeds the protective capacity of the antioxidant defense mechanism). The authors reported that vigorous exercise, approximately 10h a day for 30 days, increased the rate of oxidative DNA modification by 33% (95% confidence limits, 3-67%; p<0.02) in 20 men owing to the urinary excretion of 8-OHdG, an oxidatively modified deoxynucleoside originating from nuclear DNA repair, oxidation of the nucleotide pool from mitochondrial DNA and/or from cell turnover. They suggested that oxidative stress of DNA led to increased risk for the development of cancer and pre-mature ageing from extreme exercise.

Sandri et al. (1997) conducted a study to determine the role of apoptosis in muscular dystrophy, dystrophin-deficient (*mdx*) mice. For the study, the mice were subjected to spontaneous exercise and skeletal muscles were analyzed for apoptosis and ubiquitin. After exercise, the

increase of apoptotic myonuclei was detected by TUNEL, by electron microscopy, and by DNA analyses for high molecular weight and for ladder fragments. They observed that expression of ubiquitin correlated with exercise and with positive myonuclei for apoptosis. The biochemical analysis also confirmed a high level of ubiquitination, both in sarcoplasmic and myofibrillar proteins. However muscles from sedentary congenit control mice (C57B) were negative for apoptosis though after exercise, some nuclei were positive. After exercise, western blots for *bcl*-2, *fas*-2, and *bag*-1 also showed a significant decrease of *bcl*-2 product in mdx mice. It was suggested that spontaneous exercise resulted in the increase of ubiquitin expression and in the reduction of *bcl*-2 tightly related to programmed cell death in *mdx* mice. Rather, DNA fragmentation was absent in muscles of sedentary normal mice but was present in *mdx* mice at rest which dramatically increased after exercise and suggested that there was an exercise-induced muscle damage and its progression in dystrophinopathies.

A study (Pilger et al. 1997) was conducted to examine the effect of regular running exercise on the urinary levels of 8-OHdG in 32 long-distance runners and in a group of untrained healthy subjects. The range of 8-OHdG in urine was 0.12-6.45 mµ mol/mol creatinine in both groups. There were however no significant differences in the mean excretion levels between the runners and controls. The authors concluded that physical exercise in trained individuals did not induce a disturbance of the oxidant-to-antioxidant balance.

Schiffl et al. (1997) carried out the MN test on PHA-stimulated blood lymphocytes of six healthy volunteers before and after two exhaustive sprints. The lactate concentrations in peripheral blood were between 9.6 and 12.4 mmol/1 and the number of micronuclei was significantly increased after 24-48h in all six subjects. The mean values of the total group also increased from 37 MN per 3000 binucleated cells before exercise to 56 and 55 MN 24h and 48h after exercise, respectively. These results led to the conclusion that exhaustive physical exercise caused severe mutations at the chromosome level in blood lymphocytes.

Okamura et al. in the same year (1997) conducted a study to find out the effect of repeated exercise on oxidative damage to DNA in ten, well-trained long distance runners who participated in an eight-day training camp where the average running distance was 30±3 km/day. The oxidative DNA damage (urinary 8-OHdG) was estimated. Blood and urine samples were collected after overnight fasting, before and after the camp, and for both, a three-day control period as well as throughout the camp. It was observed that urinary 8-OHdG increased during the camp period compared to that in the control period (265.7±75.5 vs. 335.6±107.4 pmol/kg/ day, p<0.05) whereas the content of 8-OHdG in the lymphocyte DNA on the day after finishing the camp did not differ from that before the camp. Plasma thiobarbituric acid reactive substances (TBARS), lactate dehydrogenase (LDH), CK, and myoglobin however rose after the camp period (p<0.05). Moreover, the plasma beta-carotene levels tended to rise after the camp, while the plasma alpha-tocopherol levels increased significantly after the camp (p<0.05). These results suggested that repeated exercise augmented oxidative stress; DNA was also damaged by exercise-induced reactive oxygen species and that the oxidative damage to DNA did not accumulate by consecutive exercise, although it was sustained as long as exercise was repeated.

Sumida et al. (1997) investigated the effect of a single bout of intensive exercise on the excretion of 8-OHdG in the 24h-urine from healthy non-smokers. They conducted three exercise tests: experiment one comprised incremental exercise to exhaustion on a treadmill in 11 male long distance runners; experiment two consisted of incremental exercise until reaching exhaustion on a bicycle ergometer in six male untrained subjects; and experiment three involved 20 km run by 11 male long distance runners. After each respective exercise regimen, no differences were observed in the urinary 8-OHdG excretions from days one to three. Nonetheless except for results from experiment two, significant increase in the plasma CK activity at 24h or 48h after exercise was observed. These results suggested that the oxidative stress during a single bout of intensive exercise did not result in an accumulation of oxidative DNA damage.

Hartmann et al. (1998) conducted a study to determine the effects of a short-distance triathlon on the induction of DNA effects in peripheral leukocytes, on urinary excretion of oxidized

DNA bases, and on the frequency of micronuclei in lymphocytes of human volunteers. Using the alkaline comet assay, the induction of DNA effects was measured as increased DNA migration in leukocytes of all individuals at different time points after exercise and the results revealed a biphasic pattern. The elevated DNA migration was found 24 h-post-exercise whereas lower values were detected 48h after exercise. However the maximum increase in DNA migration was found 72h-post-exercise. They also used a modified protocol of the comet assay for the detection of oxidized DNA bases but it revealed no differences in leukocytes, before and directly after the triathlon. The urinary excretion of 8-OHdG remained unaltered during five conse-cutive days. In addition, no differences were found in the micronucleus frequency in lymphocytes before or 48h and 96h after exercise. They suggested that DNA effects detected with the comet assay in leukocytes of humans after exercise are secondary effects that do not originate from oxidized DNA bases and do not result in chromosome damage.

Mars et al. (1998) conducted a study to find out a possible link between exercise-induced DNA damage in leukocytes and apoptosis because post-exercise lymphocytopenia has been well documented and is attributable to the egress of lymphocytes from the vascular compartment. Venous blood samples were taken before, immediately post-exercise, 24h and 48h after exercise from 11 subjects who underwent a ramp treadmill test to exhaustion hours after exercise. Single stranded DNA damage in 10% of lymphocytes immediately after exercise, revealed by SCGE and three patterns of DNA distribution, similar to those seen in apoptosis were observed through fluorescent microscopy. Lymphocytes were prepared for flow cytometry to determine apoptosis using the TUNEL method. Lympho-cyte apoptosis in 63% of lymphocytes was revealed immediately after exercise and 86.2%, 24 hrs after exercise in three subjects who underwent the same exercise protocol. This was the first study, which documented lymphocyte apoptosis after exercise because of exercise-induced lymphocytopenia and reduced immunity.

Niess et al. (1998) investigated whether intensive endurance exercise was capable of inducing comparable effects since it was believed that highly intensive anaerobic exercise induced DNA damage in leukocytes. In their study, 12 men (aged 27.3±4.1 years) who undertook regular training to different extents (running volume 45±25 km/wk) volunteered and they completed a half marathon (HM) of 21.1 km in 93.0±10.4 min. Blood samples were taken at rest, one-, and 24h after HM for determination of CK, neutrophils (PMN) as well as lymphocyte and monocyte counts. DNA damage in leukocytes at rest and 24 h after HM was quantified using the SCGE assay. PMN increased from 2.81±0.69 to 13.13±2.91 1h after HM (p<0.01) and returned to 3.26±0.47 10 (9) cells by 24h recovery. DNA migration (image length, IL) reflected the extent of DNA damage and was significantly elevated in ten to 12 subjects one day after HM i.e. IL rose from 32.7±2.2 to 40.7±3.9 microns (p<0.01). Correlation analysis revealed a relationship between DNA migration 24h after HM and PMN count 1h post-exercise (r=0.67, p<0.05). The results confirmed the hypothesis that DNA damage in leukocytes occurs after intensive endurance exercise. It was suggested that observation of exercise-induced DNA damage in leukocytes is affected by reactive oxygen species which are released from PMN but it still remained unclear whether DNA damage in leukocytes was causal or was involved in exercise-induced modification of the immune system.

Umegaki et al. (1998) undertook a study to analyse the exercise-induced chromosomal damage in trained and untrained subjects, who performed treadmill running (85% VO_2max) for 30 min. Blood samples were taken before, imme-diately after, and 30 min after the running test for analysis of chromosomal damage evaluated by the micronucleus assay. To examine the modification of exercise-induced chromosomal damage by a secondarily induced oxidative stress, the blood samples were subjected to X-ray irradiation *in vitro*. There was no increase in spontaneous chromosomal damage in lympho-cytes until 30 min after running, both in trained and untrained subjects. However the X-ray induced chromosomal damage was significantly increased at 30 min after running in untrained group but not in the trained group. Furthermore the ratio of X-ray-induced/spontaneous chromo-some damage tended to increase after running in the untrained group only. These results demons-trated that intensive exercise induced very slight chromosomal damage and that too only in the untrained group, which could further be

intensified by the secondary-induced oxidative stress.

Asami et al. (1998) conducted a study to investigate the effect of physical exercise on the level of 8-OHGua and its repair activity in human peripheral leukocytes. For the study, whole blood samples were collected by venipuncture from 21 healthy male volunteers (10 trained athletes and 13 untrained men), aged 19-50 years, both before and after physical exercise. Trained athletes showed a lower level of 8-OHGua (2.4±0.5/10(6) Gua, P=0.0032) before exercise when compared to that of untrained men (6.2±3.5). After exercise, the mean levels of 8-OHGua of untrained subjects decreased significantly (p=0.0057) from 6.2±3.5/10(6) Gua (mean ±SD/10(6) to 3.3±1.4/10(6) Gua. The mean levels of repair activity of untrained subjects however significantly increased after exercise (p=0.0093) from 0.037±0.024 (mean DNA cleavage ratio±SD) to 0.056±0.036. The 8-OHGua levels and its repair activity were not changed before and after the exercise in the trained athle-tes. Apart from this, inter-individual differences in 8-OHGua levels and its repair activities were observed. These observations suggested that physical exercise caused both, rapid and long-range reduction of oxidative DNA damage in human leukocytes but with individually different efficiencies.

Radak et al. (1999a) investigated the effect of exercise-induced muscle soreness on maximal force generation, tissue nitric oxide (NO) and 8-OHdG content in human skeletal muscle. For the study, six female volunteers were assigned to control(C) and muscle soreness (MS) groups. To induce muscle soreness, MS group performed 200 eccentric muscle actions of the   rectus femoris. Maximal force generation was measured 24h before and after exercise in both groups. Needle biopsy samples were assayed for the NO content with electron spin resonance spectroscopy after *ex vivo* spin trapping, and using an ELISA, the 8-OHdG contents were measured. It was observed that the maximal force decreased by 11±5.4% (p<0.05) 24h after exercise in MS group. However muscle soreness increased NO and 8-OHdG contents from the controls values of 0.39±0.08 arbitrary units and 0.035±0.004 p mol/micromol DNA to 0.96±0.05 (p<0.05) arbitrary units and 0.44±0.05 (p>0.05) p mol/micromol DNA, respectively. This was the first demonstration that muscle soreness induced a decrease in maximal force generation

due to an increase in muscular NO content and was found associated with enhanced formation of 8-OHdG in human skeletal muscle.

Leaf et al. (1999) conducted a study to evaluate the 'exercise paradox' i.e. although physical exercise training is highly recommended, physical exercise causes oxidative stress, which is poten-tially injurious. The effect of physical exercise on exercise-induced lipid peroxidation was evaluated. Measurement of lipid peroxidation (i.e. expired ethane and pentane and plasma MDA) taken during cardiopulmonary exercise stress testing was compared between a group of ten cardiac patients who underwent physical exercise training in a cardiac rehabilitation setting and a group of ten non-exercising cardiac patients. They concluded that physical exercise training could reduce potential chronic health effects associated with daily activities by contributing to an overall reduction in exercise-induced free radical production because physical exercise-trained people could perform more intense physical work with less oxidative stress.

Radak et al. (1999b) investigated the effect of long-term swimming training on the oxidative status of phospholipids, proteins and DNA using two age-groups of rats, young (four-wk) and middle aged (14mon). They observed that the concentration of TBARS and 4-hydroxynonenal protein adducts did not differ in the gastro-cnemius muscle between exercised and non-exercised animals in the two age groups. However the extent of carbonylation in a protein (molecular weight~29kda) and the amount of 8-hydroxydeoxyguanosine in nuclear DNA were lesser (p<0.05) in the exercised rats than in the sedentary animals. Moreover, activities of DT-diaphorase (C1:29.3±1.9; C2:36.1±2.6; E1: 272±1.3; E2:334±2.9 n mol/mg protein) and proteasome, a major proteolyltic enzyme for oxidatively modified proteins, were significantly higher in the exercised animals of both age-groups (p<0.05). The authors concluded that the adaptive response against oxidative stress induced by moderate endurance exercise constituted a beneficial effect of the exercise.

Poulsen et al. (1999) suggested methods to detect DNA damage by free radicals in relation to exercise. Methods based on HPLC, GC-mass spectrometry and liquid chromatography-tandem mass spectrometry were used to estimate tissue levels, i.e. a local concentration of oxidized DNA, or to estimate the rate of body DNA

oxidation by the urinary output of repair products. The authors reported that acute or prolonged moderate exercise did not produce signs of oxidative DNA damage but long duration and intense exercise led to increase in oxidative DNA modification.

Radak et al. (2000) conducted a study to examine the urinary 8-OHdG levels of five well trained supra-marathon runners during a four-day race with the daily running distances of the four-day race being 93km, 120 km, 56 km and 59 km, respectively. The urine samples (pre-race and post-race) were collected on each day and were analyzed by a monoclonal antibody technique. They observed that the urinary 8-OHdG content increased significantly on the first day, tended to decrease from the third day and by the fourth day, the 8-OHdG content was significantly lowered than measured on the first three days. The serum CK activity changed in a similar fashion, showing a greater increase ($p<0.001$) up to the third day when it decreased significantly from the peak value ($p<0.05$). The authors concluded that extreme physical exercise caused oxidative DNA damage to well-trained athletes; repeated extreme exercise-induced oxidative stress did propagate an increase of urinary 8-OHdG, and rather caused an adaptation leading to normalization of oxidative DNA damage.

Liu et al. (2000) investigated the responses to oxidative stress induced by chronic exercise (eight-wk treadmill running) or acute exercise (treadmill running to exhaustion) in the brain, liver, heart, kidney and muscles of rats. In addition, various biomarkers of oxidative stress, lipid peroxidation (MDA), protein oxidation (protein carbonyl levels and glutamine synthetase activity), oxidative DNA damage (8-OHdG), and endogenous antioxidants (ascorbic acid, alpha-tocopherol, glutathione, ubiquinone, ubiquinol, and cysteine) were measured. The predominant changes were observed in MDA, ascorbic acid, glutathione, cysteine and cystine and the mitochondrial fractions of brain and liver showed oxidative changes as assayed by MDA similar to those of the tissue homogenate. They reported that the responses of the brain to oxidative stress by acute or chronic exercise are quite different from those in the liver, heart, muscle, and that oxidative stress by acute or chronic exercise elicits different responses depending on the organ, tissue type and its endogenous antioxidant levels.

Tsai et al. (2001) using SCGE demonstrated a significant degree of unpaired DNA base oxidation in peripheral immuno- competent cells, despite a concurrent increase in the urinary excretion of 8-OHdG by using a 42 km marathon race as a model of massive exercise. The basis was the belief that reactive species produced during vigorous exercise permeated into cell nuclei and induced oxidative DNA damage. SCGE, with the incorporation of lesion-specific endonucleases further revealed that oxidized pyrimidines (endonuclease III-sensitive sites) contributed to most of the post-exercise nucleotide oxidation. These results suggested that the oxidative DNA damage correlated significantly with plasma levels of CK and lipid peroxidation metabolites and lasted for more than one week following the race and that this phenomenon is one of the mechanisms responsible for the immune dysfunctions after exhaustive exercise.

Kasai et al. (2001) measured 8-OHdG with high resolution by automated HPLC system coupled to an electrochemical detector. The urine samples were taken from 318 healthy men aged 18-58yr. The mean 8-OHdG level (mg/g creatinine) was 4.12 ± 1.73 and a 11-fold inter individual variation was observed. It was found by univariate analysis that moderate physical exercise ($p=0.0023$) and high BMI ($p=0.0032$) reduced the 8-OHdG level, while physical labour ($p=0.0097$), smoking ($p=0.032$), and low meat intake (less than once a week) ($p=0.041$) increased its level. The moderate physical exercise ($p=0.0039$), high BMI ($p=0.0099$) and age ($p=0.021$) showed significant reducing effects on the 8-OHdG level, while low meat intake ($p=0.010$), smoking ($p=0.013$), and daynight shift work ($p=0.044$) increased its level, based on a multiregression analysis of the log-transformed values. Their results suggested that many types of lifestyle factors that generate or scavenge oxygen radicals, affect the level of oxidative DNA damage in each individual.

Moller et al. (2001) conducted a study to test the effect of a single bout of exhaustive exercise on the generation of DNA strand breaks and oxidative DNA damage under normal conditions and at high-altitude hypoxia (4559m for three days). Lymphocytes were isolated for analysis of DNA strand breaks and oxidatively altered nucleotides, detected by endonuclease III and FPG enzymes from 12 healthy subjects who

performed a maximal bicycle exercise test. Urine samples were also collected for 24h periods for the analysis of 8-OHdG. At high altitude, urinary excretion of 8-oxodG increased during the first day and there were more endonuclease III-sensitive sites on day three. The subjects also had more DNA strand breaks in altitude hypoxia while exercise-induced generation of DNA strands breaks were not seen at sea level. The level of FPG and endonuclease III sensitive sites however remained unchanged immediately after exercise in both environments. Their results led to the conclusion that reactive oxygen species generated by leakage of the mitochondrial respiration or during a hypoxia-induced inflammation is the probable cause of DNA strand breaks and oxidative DNA damage and the presence of DNA strand breaks play an important role in maintaining hypoxia-induced inflammation processes. It was also concluded that hypoxia depleted the antioxidant system of its capacity to withstand oxidative stress produced by exhaustive exercise.

Radak et al. (2001) reported that exercise increased the generation of reactive oxygen and nitrogen species (RONS) and through adaptation, there was a decrease in the incident of RONS-associated diseases. However depending upon intensity and duration, a single bout of exercise caused an increase in antioxidant enzyme activity, decreased the levels of thiols and antioxidant vitamins and resulted in oxidative damage which is a sign of incomplete adaptation. Increased levels of RONS and oxidative damage initiated specific adaptive responses, such as the stimulation of the activation of antioxidant enzymes, thiols and enhanced oxidative damage repair. The authors suggested that compensation to oxidative stress developed by regular exercises resulted in overcompensation against the increased level of RONS production and oxidative damage. Also an adaptation of the antioxidant and repair systems by regular exercises resulted in a decreased base level of oxidative damage.

Hsu et al. (2002) conducted a study to examine the effects of different intensities of aerobic exercise (AE) on leukocyte Mitochondrial Transmembrane Potential (MTP) and the tendency of apoptosis because it has been reported that exercise is associated with intensity-dependent immune disturbances and leukocyte mitochondrial alterations and that apoptosis contributes to this phenomenon. For the study,

blood samples were collected from 12 subjects who performed AE for three consecutive days (35% VO_2 max) and leukocyte MTP and apoptosis were measured by flow cytometry. In addition to this, the subjects also performed two additional sessions of AE of higher intensities (60% and 85% VO_2 max) with an intervening four-wk washout period and measurements were repeated during each session. They observed that leukocyte MTP declined during daily, repetitive AE by an intensity of 60% and 85% VO_2 max but similar changes were not found during a more moderate AE (35% VO_2 max). Moreover, leukocytes increased their propensity for apoptosis over a period (three to five days) after the start of the AE. They concluded that high intensity AE had cumulative effects on the mitochondrial energization status and on the vitality of peripheral blood leukocytes and that the leukocyte MTP was also a potentially applicable indicator for monitoring immune distress due to overtraining.

Selman et al. (2002) reported the effect of voluntary exercise on antioxidant enzyme activities (catalase, glutathione peroxidase, superoxide dismutase) in skeletal muscle (hind- and fore-limb) and on the heart of a model small mammalian species: short-tailed field vole, *Microtus agrestis*. Using the comet assay, DNA oxidation was determined in lymphocytes and hepatocytes and by measurement of TBARS, and lipid peroxidation was estimated in hind limb muscle. For the study, voles (approximately six-wk old), were exposed to a 16L: 8D photoperiod (lights on 0500h), and ran almost continuously during darkness. The effects of voluntary running over one or seven days duration, with or without an eight hour rest period, on various biomarkers of oxidative stress compared to those of in non-running controls, were studied. No differences were observed for antioxidant enzyme activities, except for heart total superoxide dismutase activity (p=0.037), with the lowest levels in one- and seven-day runners at 0500h. In addition, DNA oxidative damage in lymphocytes or hepatocytes, and lipid peroxidation did not differ between groups. There was also no evidence of any significant increase in any oxidative stress parameter in running individuals despite having significantly elevated energy expenditures compared to those in sedentary controls.

Boffi et al. (2002) conducted a study to examine training- induced apoptosis in skeletal

muscles because the common inducers of apoptosis (Ca^{2+} and oxygen free radicals/oxidative stress) are implicated in the pathogenesis of exercise-induced myopathies. For the study, thoroughbred horses were subjected to a three-month training programme on a treadmill. The venous blood samples were taken for CK assay and muscle biopsy samples were obtained for a membrane lipid peroxidation measurement by MDA assay for apoptosis detection at the end of the training programme. Apoptosis was studied by visualizing the apoptotic myocytes on paraffin sections by the modified TUNEL method and DNA laddering was evaluated by subjecting the DNA obtained from the biopsies to 1.5% agarose gel electrophoresis. They observed a significant increase ($p<0.05$) of protein–bound MDA, and a non-significant trend ($p=0.14$) for the control group to have higher levels of CK as compared to the trained group. The percentage of the TUNEL positive cells was higher ($p<0.001$) in the training group as observed under light microscopy. This result was similar to the findings from DNA fragmentation by gel electrophoresis, which showed higher ladders of DNA band at the same group. The authors concluded that there is training-induced apoptosis in skeletal muscle and that apoptosis allowed the work/recovery/ rebound/ super-compensation cycle; the unaccustomed muscle cells activated programmed cell death and were replaced by new and stronger cells in the mechanism for training-induced increase in fitness.

Radak et al. (2002) conducted a study to investigate whether regular physical exercise retards a number of age-associated disorders, in spite of the paradox that free exercise generation is significantly enhanced with exercise. In their study, eight-wk of treadmill running resulted in nearly a 40% increase in VO_2max in both middle-aged (20-mon-old) and aged (30-m on-old) rats. They observed that the age-associated increase in 8-OHdG content was significantly attenuated in gastrochemius muscle by exercise and also that 8-OHdG repair was increased in muscle of exercising animals as measured by the excision of ^{32}P-labelled damaged oligonucleotides. The reactive carbonyl derivatives (RCD) of protein did not increase with ageing but when the muscle homogenate was exposed to a mixture of 1mM iron sulphate and 50mM ascorbic acid, the muscle of old control animals accumulated more RCD than that of the trained or adult group. The

chymotrypsin-like activity of proteasome complex also increased in muscle of old trained rats. The authors suggested that regular exercise-induced adaptation attenuated the age-associated increase in 8-OHdG levels, and increased the activity of DNA repair and resistance against oxidative stress in proteins.

In another study, Radak et al. (2003) investigated the effect of a single bout of physical exercise on the activity of repair enzymes. However the same could also induce oxidative DNA damage. They reported that the activity of a functional homolog of FPG, human 8-oxoG DNA glycosylase (hoGG1), increased significantly as measured by the excision of ^{32}P-labeled damaged oligonucleotide, in human skeletal muscle after a marathon race. The AP site repair enzyme however did not change significantly. In their study (despite the large individual differences measured among the six subjects) the data suggested that a single-bout of aerobic exercise increased the activity of hoGG1 which is responsible for the excision of 8-OHdG and that the up-regulation of DNA repair enzymes was also an important part of the regular exercise-induced adaptation process.

Sato et al. (2003) examined the effects of exercise on oxidative DNA damage and measured the levels of 8-OHdG and mRNA of its sanitization enzyme, human Mut T homologue (hMTH1) after mild exercise in healthy male subjects. The basal 8-OHdG levels of physically active subjects were lower than those of sedentary subjects and the 8-OHdG levels of the sedentary subjects significantly decreased after mild exercise for 30 min. In contrast to the change in 8-OHdG levels, the hMTH1 mRNA levels of the physically active subjects were higher than those in the sedentary subjects and the levels of hMTH1 mRNA of sedentary subjects increased after mild exercise. They suggested that a mild exercise has a beneficial effect on the maintenance of low levels of 8-OHdG by keeping the sanitization system at higher levels.

Palazzetti et al. (2003) conducted a study to investigate whether the overloaded training (OT) in triathlon induced oxidative stress and damage on muscle and DNA. For the study, nine male triathletes and six male sedentary subjects participated and triathletes exercised for a dualthlon, before and after a four-wk OT. Before and after OT in pre-and post-exercise, blood ratio of reduced   vs. oxidized glutathione (GSH/

GSSG), plasma TBARS, leukocyte DNA damage, CK, erythrocyte superoxide dismutase (SOD) activity, erythrocyte and plasma glutathione peroxidase (GSH-Px) activities, and plasma total antioxidant status (TAS) were measured. They observed that triathletes were overloaded in response to OT. In the rest conditions, OT-induced plasma GSH-Px activity increased and plasma TAS decreased (both p<0.05) whereas in exercise conditions, OT resulted in higher exercise-induced variations of blood GSH/GSSG ratio, TBARS level (both p<0.05), and decreased TAS response (p<0.05). It was suggested that increased exercise induced oxidative stress and increased the susceptibility to cellular damage.

Ding et al. (2003) carried out a study to identify the effects of over training on human sperm chromatin structure. For the study, molecular epidemiological investigation of 249 men from different groups (training and non-training) was carried out by using flow cytometer to detect the integrity and damage in *in situ* DNA of sperm nucleus, and the sperm chromatin structure assay was performed. They observed that the average COMP alpha (t) in training group was 11.02% while that in control group was 5.90% (p<0.01). COMP alpha (t) was significantly correlated with sperm activity (r=0.41, p<0.05). They concluded that over training induced sperm DNA injury and affected sperm activity, thus decreasing the potentiality for reproduction.

Mastaloudis et al. (2004) carried out a study to find out whether endurance exercise resulted in DNA damage and to determine if six wk of supplementation with antioxidants could alleviate exercise-induced DNA damage in 21 runners during a 50 km ultramarathon. The subjects were randomly categorised as placebos (PL) or those taking antioxidants (AO; 1000 mg vitamin C and 400 IU RR-alpha-tocopheryl acetate). The DNA damage in circulating leukocytes at selected time points: pre-, mid-, and 2h post-race and daily for six days' post-race, was assessed by the comet assay. All subjects completed the race: run time 7.1±0.1h, energy expenditure 5008±80 kcal for women (n=10) and 6932±206 kcal for men (n=11). The found that the percentage DNA damage increased at mid-race (p<0.02), but returned to baseline by 2h post-race, indicating that the exercise bout induced non-persistent DNA damage. Moreover, women taking AO had 62% less DNA damage than PL women (p<0.08). In contrast, there were no differences between the two treatment groups of men at any time point. Their findings suggested that endurance exercise resulted in DNA damage as shown by the comet assay and also that AO enhanced recovery in women but not in men.

Siu et al. (2004) conducted a study to investigate the effect of regular moderate physical activity (i.e. exercise training) on the extent of apoptosis in rat post- mitotic tissues viz. skeletal and cardiac muscles. For the study, adult Sprague Dawley rats were trained (TR) five days weekly for eight-wk on treadmill and sedentary rats served as controls (CON). The mono-and oligo-nucleosome fragmentation (an indicator of apoptosis) was detected by ELISA and *bcl*-2, *bax*, *apaf*-1, *aif* cleaved PARP, cleaved caspase-3, cleaved/active caspase-9 and heat shock protein (HSP) 70, Cu/Zn-SOD, Mn-SOD protein levels were determined by western analysis. RT-PCR was used to estimate *bcl*-2 and *bax* transcripts and a spectrofluorometric assay was used to determine caspase-3 activity. The authors observed that DNA fragmentation in ventricles of the TR group decreased by 15% whereas that in soleus of the TR group tended to decrease (p=0.058) when compared with CON group. The protein contents of *Bcl*-2, HSP70, and Mn-SOD also increased in both, soleus and ventricle muscles of TR animals when compared to the values in CON animals. However, apaf-1 protein content in the soleus of TR animals was lower than that in CON animals. *Bcl*-2 mRNA levels increased in both ventricle and soleus muscles of TR animals but *bax* mRNA levels decreased in the soleus of TR animals when compared with CON animals. In addition to this, HSP70 protein content was negatively correlated to *bax* mRNA content and was positively correlated to *bcl*-2 protein and mRNA contents. Similarly, Mn-SOD protein content was negatively correlated to the apoptotic index and caspase-3 activity and was positively correlated to *bcl*-2 transcript content and HSP70 protein content. They suggested that exercise training attenuated the extent of apoptosis in cardiac and skeletal muscles.

Lim et al. (2004) investigated the effect of muscle exercise in Duchenne muscular dystrophy (DMD) control (n=15) and 15 *mdx* mice (an animal model of DMD) were divided into free-living (n=6), exercise (n=6), and immobilization (n=3) groups and were further sub-divided into

steroid-treated and sham-treated groups to evaluate the effect of steroid administration. *In situ* DNA nick-end labeling, DNA fragmentation assay, and western blotting for *bcl*-2 and *bax* measured the apoptotic changes. Apoptosis was not promi-nent in the sham-treated exercise group, and it was significantly reduced in the steroid-treated exercise group but the steroid-treated free-living group showed higher rate of apoptotic change than the sham-treated free-living group. In addition to this, apoptosis was minimized in the free-living condition, whereas exercise loading and immobilization caused apoptotic change in this muscular dystrophy animal model. Steroid administration induced apoptosis in muscles of free-living mice, but alleviated the apoptotic damage caused by exercise loading in *mdx* mice. These findings suggested that steroid adminis-tration prevented a post-exercise deterioration of skeletal muscles in animal models of DMD.

Hoffman-Goetz and Duerrstenin (2004) determined the effect of chronic (wheel running, WR) and acute (treadmill, TREAD) exercise on *in vivo* apoptosis of thymocytes using an animal model of menopause with supplementation from dietary Genistein (GEN). Genistein, a soy isoflavone with estrogen-like properties, is believed to induce lymphocyte apoptosis *in vitro*. At menopause, many women consume phytoestrogens instead of beginning hormone replacement therapy and many start exercise programs for health benefits. Aerobic exercise is also believed to induce apoptosis in lymphoid cells. For the study, 99 ovariectomized B6D2F1 mice were fed 250 (GEN) or O(C) ppm Genistein and given concurrent exercise (voluntary wheel running-WR; or WR followed by a bout of high intensity treadmill running - WR+TREAD) or remained sedentary (SED). The mice were sacrificed after 21 days for measurements of body weights, tissue weights, thymocyte apoptosis and necrosis by annexin-V FITC and propidium iodide staining and flow cytometry, DNA fragmentation by ELISA, and plasma estrogen concentrations by RIA. The results showed that WR+TREAD mice had lower percentages of apoptotic and necrotic cells from thymus com-pared with SED or WR conditions ($p<0.001$). WR resulted in greater DNA fragmentation in thymus cell lysates than observed in samples from SED mice ($p<0.005$). There were however no differences in thymocyte apoptosis or DNA fragmentation between GEN and C mice, either independently or interactively with exercise. Apart from this, GEN mice tended to have greater wheel running activity than C mice ($0.05<p, <0.09$, depending on day). Their results suggested that intense aerobic exercise increased the percentage of early apoptotic cells in the thymus and also that dietary Genistein was associated with a small increase in voluntary wheel running activity in ovariectomized mice.

Orhan et al. (2004) carried out a study on 18 moderately trained males (mean age 24.6 ± 0.7yr) who exercised 60 min at 70% of maximal O_2 uptake on a cycle ergometer and their urine fractions for 12h were collected one day before, and for three consecutive days after exercise. The eight aldehydes (i.e. propanal, butanal, pentanal, hexanal, heptanal, octanal, nonanal and MDA) as biomarkers of lipid peroxidation and acetone were analyzed in urines by Gas Chromatography with electron capture detection (GC-ECD). In a similar manner, a biomarker of protein oxidation, O, O'-dityrosine and a biomarker of oxidative DNA damage, urinary excretion of 8-OHdG was analyzed in urine samples by isotope dilution HPLC-atmospheric pressure chemical ionization (APCI)-tandem-mass spectrometry (HPLC-APCI-MS/MS) methodology and ELISA method, respectively. On the day of exercise, significant increase in urinary excretions of acetone ($p<0.025$, n-18) and butanal ($p<0.01$, n=19) were observed in the 12h daytime fractions compared to the daytime fractions before exercise. The urinary acetone excretion was significantly ($p<0.05$) increased on the first day after exercise but octanal and nonanal were increased in the daytime urine fraction on the second day after exercise. The urinary O,O'-dityrosine amounts were significantly elevated in day time fraction on the day of exercise ($p<0.025$) and on the first day after exercise ($p=0.07$) compared to the before-exercise day time fraction. Similarly, the excretion of urinary 8-OHdG was significantly increased in the day time fractions on the day of exercise ($p=0.07$) and on the first day after exercise ($p<0.025$) compared to before-exercise day time fraction. Though the study was originally conducted to evaluate a multi-parameter, non-invasive biomarker set for damage to three main cellular targets of ROS, yet it showed that one hour of exercise induced oxidative damage in moderately trained individuals and that the chosen urinary

biomarkers were sensitive enough to monitor such damage.

Singh et al. (2005) reported that physical exercise by Dalton lymphoma (DL)-bearing, mice, on a treadmill on a daily basis for various time durations for ten days, increased the life span along with an inhibition of tumor growth. The ascitic growth of a transplantable T-cell lymphoma of spontaneous origin (designated as DL, is associated with a concomitant immuno-suppression and progressive *in vivo* growth of DL) resulted in an inhibition of macrophage functions. There was significant decrease in the volume of ascitic fluid and number of cells in the tumor in mice, which underwent exercise. DL cells obtained from exercised groups showed a decreased proliferation *in vitro*. However, an augmentation in the percent of cells showing apoptotic morphology and percent specific DNA fragmentation was observed, suggesting that physical exercise increased the incidence of apoptosis in tumor cells. The macrophages obtained from tumor-bearing mice that underwent exercise training also showed augmented tumoricidal molecules like interleukin-1 (IL-1), tumor necrosis factor (TNF) and nitric oxide (NO). It was concluded that the regression of tumor growth consequent to physical exercise training of tumor-bearing host was due to an exercise-dependent augmentation of macrophage tumoricidal functions.

Ogonovszky et al. (2005a) conducted a study to test the hypothesis that training with moderate–(MT), strenuous-(ST), or over–(OT) load can cause alterations in memory, lipid peroxidation, protein oxidation, DNA damage, activity of OGG1 and brain-derived neurotrophic factor (BDNF), in rat brain. For this study, rat memory was assessed by a passive avoidance test and the ST and OT group demonstrated improved memory. They observed that the content of BDNF was increased only in the OT group and the oxidative damage of lipids and DNA (as measured by TBARS and 8-OHdG) did not change significantly with exercise. Similarly, the activity of DNA repair enzyme, OGG1, was not altered with exercise training. However, the content of RCDs decreased in all groups, as did the activity of the proteasome complex.

Sim et al. (2005) conducted a study to investigate the effect of long-term treadmill exercise on short-term memory and apoptotic neuronal cell death in the hippocampus following transient global ischemia in gerbils. For this study, TUNEL assay, and immunohistochemistry for caspase-3 were carried out. Ischemia was induced by occlusion of both; the common carotid arteries of gerbils for 5 min and gerbils in the exercise groups were forced to run on a treadmill for 30 min once a day for four consecutive weeks. Their results revealed that treadmill exercise for four weeks improved short-term memory by suppressing the ischemia-induced apoptotic neuronal cell death in the hippocampus. Their results showed that long-term treadmill exercise for four weeks overcame the ischemia-induced apoptotic neuronal cell death and thus facilitated the recovery of impaired short-term memory inducted by ischemic cerebral injury.

Ogonovszky et al. (2005b) also carried out a study to find out whether training with moderate (MT), strenuous (ST), or over (OT) load could cause alterations in the activities of antioxidant enzymes, lipid peroxidation, protein oxidation, DNA damage, or activity of OGG1 in rat liver because it was believed that physical exercise above a certain load, causes oxidative stress. They observed that levels of corticosterone decreased in all exercising groups but the differences were not significant though adrenocorticotrophhin hormone (ACTH) levels decreased in MT and OT as compared to control. The activity levels of antioxidant enzymes also did not change significantly in the liver but the levels of RCD content decreased in the liver of exercising animals. These changes in the levels of lipids peroxidation (LIPOX) were not significant but were lower in the exercised groups. It was further observed that the 8-OHdG levels increased in the OT group, as did the activity of OGG1 measured from crude cell extracts in MT and ST. These resulted suggested that over training induced oxidative damage to nuclear DNA but not to liver lipids and proteins.

Nakatani et al. (2005) conducted a study to investigate whether habitual exercise (HE) modulates levels of oxidative DNA damage and the responsiveness to oxidative stress induced by renal carcinogen, Fe-nitrilotriacetic acid (Fe-NTA). Their study consisted of two groups of rats, which either remained sedentary or underwent swimming for 15-60 min per day, five days per week during a ten-week period. The rats were injected with Fe-NTA and were sacrificed 1h after the injection. They determined the

activity of superoxide dismutase (SOD) in the diaphragm and kidney, evaluated the levels of 8-OHdG, catalase, and glutathione peroxidase, and assayed the OGG1 protein levels in kidneys. The authors reported that SOD activity in the diaphragm and kidney was increased in HE rats. The HE group had no effect on the level of 8-OHdG but it significantly suppressed the induction of 8-OHdG by Fe-NTA. These results suggested that HE induced resistance to oxidative stress, and at least at the initiation stage, inhibited carcinogenesis.

Venous blood samples (n = 14) taken immediately before (PRE), immediately after (IPE) and 3 h after (3PE) 2.5 h of treadmill running at 75% of VO_2 max from eight well-trained male endurance athletes (age 34.2 ± 2.44 years) were analysed for cellular content and serum cortisol concentra-tions. Apoptotic and necrotic cell were detected by flow cytometry using Annexin V-FITC and propidium iodide uptake. DNA strand breaks were measured by single-cell gel electrophoresis. Despite a significant ($P < 0.001$) exercise-induced increase in mean serum cortisol concentrations and reduction in lymphocyte counts, the mean % Annexin-V positive cells were not significantly different at the three time-points ($P > 0.05$). Mean DNA strand breaks in the lymphocytes also did not change significantly ($P > 0.05$). Peters et al.(2006) concluded that exercise-induced changes in total blood lymphocyte counts and cortisol concentrations did not result in a significant change in % apoptotic lymphocytes or DNA strand breaks in the endurance-trained athletes during this prolonged, submaximal exercise.

Vincent et al.(2006) reported that antioxidant supplementation attenuated post-exercise oxidative stress and contributors to oxidative stress (inflammation, blood lipids) in overweight young adults.

The effects of a maximal test in 18 young subjects with different training levels (six professional athletes and 12 non-agonists (NA)) were examined for redox homeostasis (total antioxidant activity (TAS), vitamin C and glutathione (GSH)), oxidative damage (diene conjugation and hemolysis), lymphocyte cell death and repair systems (apoptosis, micronuclei and Hsp70 expression). Agonistic training led to a chronic oxidative insult (high baseline values of oxidized glutathione (GSSG), micronuclei and hemolysis). NA with the lowest level of training

frequency, showed a well balanced profile at rest, but were more susceptible to exercise-induced variations (GSSG/GSH and diene increased values) with respect to the NA with an higher level of training. Almost all the parameters employed in this study showed inter-individual variations (Pittaluga et al. 2006).

Effects of treadmill exercise test (TET) on oxidative/antioxidative parameters and DNA damage were investigated on 113 untrained healthy. Blood samples were obtained before and after TET. Total peroxide, TAC, vitamin C and DNA damage were measured. (Demirba et al. 2006).TET increased oxidants, decreased TAC and vitamin C namely, the balance shift towards oxidative side, but the stress was not enough to produce DNA damage in the SCGE assay.

DNA strand breaks [as determined by the conventional and formamidopyrimidine glycosylase (FPG)-modified Comet assay] and antioxidant defense status [as indicated by superoxide dismutase (SOD) activity and reduced glutathione (GSH) concentration] were evaluated in healthy adult chub (*Leuciscus cephalus*) after exhaustive exercise [swimming to their critical swimming speed (U_{crit}), twice in succession with a 40 min rest period between] vs. confined (unexercised) control fish. The conventional Comet assay revealed significantly higher DNA strand breaks in all the tissues (blood, liver, and gill), with the highest increase in the epithelial gill cells of swum fish compared to the controls. The FPG-modified Comet assay revealed specific oxidative lesions, the gill cells of exercised fish sustained the highest level of oxidative DNA damage in comparison to the control. These observations suggest that fish living in fast flowing and polluted rivers are at increased risk of DNA damage (Aniagu et al. 2006).

The effects of spirulina (*Spirulina platensis)* supplementation on preventing skeletal muscle damage on untrained 16 students were examined after the Bruce incremental treadmill exercise before and after treatment. The results showed that plasma concentrations of malondialdehyde (MDA) were significantly decreased, superoxide dismutase (SOD) was significantly raised after supplementation with spirulina or soy protein and both glutathione peroxidaes (GP$_x$) and lactate dehydrogenase (LDH) levels were significantly different between spirulina and soy protein supplementation by an ANCOVA analysis ($P <$

0.05). In addition, the lactate (LA) concentration was higher and the time to exhaustion (TE) was significantly extended in the spirulina trail after supple-mentation with spirulina ($P < 0.05$). These results suggested that ingestion of *S. platensis* showed preventive effect of the skeletal muscle damage and that probably led to postponement of the time of exhaustion during the all-out exercise (Lu et al. 2006).

Free plasma DNA, C-reactive protein (CRP), creatine kinase (CK), and uric acid (UA) were measured in 17 recreationally trained men participating in a 12-week resistance training regimen (8 resistance multi-joint exercises selected to stress the entire musculature: bench press, squat, leg press, snatch, hang clean, dead lifts, barbell arm curls and rowing), consisting of 4 training periods (t1, t2, t3, and t4). This study demonstrated that plasma DNA concentrations increased in proportion to training load, suggesting it to be a sensitive marker for over training- induced inflammation (Fatouros et al. 2006).

The capillary blood *in vivo* micronucleus test in lymphocytes of body builders and wrestlers revealed elevated frequencies indicating the clastogenic / aneugenic consequences of continuous strenuous exercising (Gandhi and Mahajan; Gandhi and Kumar, unpublished). Percent cell damage and DNA damage in the SCGE assay were significantly increased in individuals maintaining a physical fitness regimen by workouts in gymnasiums (Gandhi and Gunjan, unpublished).

Influence of Physical Activity on The Incidence of Cancer

The induction of DNA damage due to inflammatory reactions is of profound interest because of an elevated risk of cancer development in a number of inflammatory diseases. Some epidemiological studies have investigated the influence of physical activity on the induction of cancer (Table 2). On the other hand, a 40 –50% reduction in colon carcinoma incidence in active compared with sedentary individuals and a 30 – 40% reduction in breast carcinoma incidence among women engaging in three or more hours per week of regular vigorous activity have been documented while inconsistent data have associated prostate and lung carcinoma

Table 2: Physical Activity And Incidence Of Cancer: Epidemiological Data

Reference	Physical Activity	Subjects	Results
Poldnak, 1976	Competitive sports	8393 males and females	Higher incidence of cancer in active individuals.
Severson et al., 1989	Occupational and recreational	2321 women	No protective effect on breast cancer risk, increased risk in more active women (marginally significant).
Le Marchand et al. ,1991	Lifetime occupational physical activity	452 prostate cancer cases identified through the population-based Hawaii Tumor Registry and 899 population controls	Physical activity positively associated with prostate cancer Risk (weak and indirect).
Dorgan et al., 1994	Moderate-to-heavy leisure and occupational activities	2,307 women	117 Breast cancer cases diagnosed.
Zeegers et al., 1995	Non-occupational physical activity, sports participation, occupational physical activity	58,279 men	Increased risk of prostate cancer for men who were physically active.
Mink et al., 1996	Physical Activity	31,396 postmenopausal women	Two fold increase in risk of ovarian cancer among the most active women.
Cerhan et al., 1997	Physical Activity	3,673 persons	Positive cancer association for physical activity.

with inactivity (The Scientific Program Committee 2002). Beneficial effects of physical activity on quality of life during and after therapy in cancer patients have also been demonstrated. Reason-able data in literature underline the preliminary positive physiological and psychological benefits from exercise when undertaken during or after traditional cancer treatment (Galvao and Newton 2004). In contrast to these, a few epidemiological studies have focused on the incidence of cancer in highly trained athletes, former athletes or those who are physically active or have been / are indulging in moderate-to-heavy leisure and occupational activities (Table 2). Since over training was reported to induce sperm DNA injury and affected sperm activity (Ding et al. 2003), strenuous exercise may also decrease the potentiality for reproduction.

Though physical activity promotes fitness and a healthy disposition (it has an inverse effect on coronary artery diseases and delays all cause mortality) yet its influence on cancer is unclear-probably because cancer is a matrix of diseases differing in etiology, site, symptoms, timings and course (Paffenbarger et al. 1987). Free radicals induce signal transduction, proliferation and activation of certain cells and cytokines, and DNA damage. Since DNA damage and repair occur continuously and there exists the potential for misrepair leading to DNA restructuring, mutage-nesis, and carcinogenesis and physical activity may affect this balance (The Scientific Program Committee 2002). Exercising results in an increase in free radicals. Since cell disruption (commonly observed after strenuous exercise) increases free radical reactions (Gutteridge and Halliwell 1990), and free radical damage increases cellular deterioration by replacing cellular regeneration with cellular degeneration, which ultimately accelerates the aging process. Accelerated aging is associated with cancer, diabetes and coronary artery disease. Endurance exercise also may suppress the immune system and increase the risk for malignancy (Ambros-Rudolph et al.2006).

The results of a study by Lu et al. (2006) have shown that voluntary running wheel exercise enhanced UVB-induced apoptosis in the epidermis as well as apoptosis in UVB-induced tumors in tumor-bearing mice and that decreasing tissue fat by surgical removal of the parametrial fat pads (partial lipectomy) enhanced UVB-induced apoptosis. The results suggested that substances secreted by fat cells promote tumori-genesis by having an anti-apoptotic effect in DNA-damaged cells and in tumors.

DISCUSSION

The mechanism (s) behind the induction of DNA effects after exercise still remains unclear. The initial contraction-induced (mechanical) injury can be followed by secondary or delayed onset tissue damage as part of the reparative process (Zerba et al. 1990). Various physiological changes detected after mechanical muscle injury resemble those seen in acute inflammation, like increased body temperature, increased number of circulating granulocytes, increased concen-tration of serum factors like interleukin – 1 and acute phase proteins (Smith et al. 1990; Smith,1991). The initial injury is mechanical in origin and results in activation of polymor-phonuclear (PMN) neutrophils (Benoni et al. 1995) and these PMN cells infiltrate sites of muscle damage and release substantial amounts of oxygen radicals and other reactive agents as part of the phagocygotic process, which may further lead to secondary tissue damage (Ebbeling and Clarkson 1989). The subsequent macrophage infiltration observed at the site of injury rises steadily and is maintained through several days post-exercise (Smith 1991).

It is also likely that DNA effects in leukocytes after exercise are induced due to a secondary release of reactive products by activated infla-mmatory cells like neutrophils and macrophages (Wiseman and Hallwell 1996). Furthermore, activated neutrophils induce prolonged DNA damage in co-cultivated cells (Shacter et al. 1995) and play a causative role in inducing elevated DNA migration in the comet assay (Betancourt et al. 1995).

Other mechanisms by which exhaustive exercise can induce DNA damage include mitochondrial electron transport chain, ischemia-reperfusion mechanism, xanthine oxidase catalyzed reaction, inflammatory mechanism and the production of catecholamine (Hartmann and Niess 1999). Exercise-increased mitochondrial ROS production is based on the fact that tissue and whole-body O_2 consumption increases dramatically during strenuous exercise. Whole body oxygen consumption (VO_2) increases up to 20-fold, whereas VO_2 at the muscle fiber level

is elevated by as much as 100-fold during maximal exercise (Ames et al. 1995). The percentage of O_2, which becomes $O_2^{\cdot-}$ remains the same but ROS production is increased proportionally. Mito-chondrial oxidative damage supports this hypothesis that mitochondria are the primary site of ROS generation. After exhaustive exercise, respiration increases in the mitochondria of muscle and liver (Grollman and Moriya 1993) and of heart (Ji et al. 1994), indicating a possible inner membrane leakage. Mitochondrial lipid peroxi-dation is also enhanced after exercise and is accompanied by loss of thiol protein content and the inactivation of oxidative enzymes (Li et al. 1988).

Hartmann et al. (1995) had suggested that DNA damaging effects can occur as a conse-quence of DNA base oxidation or DNA repair processes induced by oxidized DNA bases after their analysis of urinary excretion of 8-OhdG. This base modification was found to be significantly induced by ROS and has been reported to be directly involved in the process of carcinogenesis due to its mispairing properties (Floyd 1990). The repair of 8-OHdG in DNA involves the pathways of base-excision yielding 8-OHGua and nucleotide excision repair, yielding 8-OhdG which are excreted in urine (Shigenaga and Ames 1991; Suzuki et al. 1995; Germadinik et al. 1997; Pilger et al. 1997). A relationship was observed between oxygen consumption and urinary excretion of this base modification and a concomitantly elevated level of 8-OHdG in the urine of athletes after strenuous exercise (Loft et al. 1994, 1995) though not after distance running (Okamura et al. 1997), long-distance training of athletes (Pilger et al. 1997) or in triathletes 0-96 h after a competitive race (Hartmann et al. 1998). Rather the extent of exercise, urinary collection periods, and creatine concentrations have to be taken into account for using 8-OHdG as a parameter. Also though the majority of urinary 8-OHdG originates from nuclear DNA, yet this base modification is also derived from oxidation of mitochondrial DNA and the nucleotide pool as well as from cell turnover (Halliwell and Auroma 1994).

Moreover there is evidence that programmed cell death or apoptosis besides necrotic cell death, occurs with exercise in lymphocytes and skeletal muscle since physiological reactions (increases in glucocorticoid secretion, intracellular calcium concentrations, and reactive oxygen species production) which have a potential to activate apoptosis occur during strenuous exercise (Davies et al.1982; Carraro and Franceschi 1997; Bejma and Li 1999; Leeuwenburgh et al. 1999). Phaneuf and Leeuwenburgh (2001) have speculated that exercise-induced apoptosis is a normal regulatory process that removes damaged cells without a pronounced inflammatory response so that body functions remain normal. However, failure to activate this genetically regulated cell death may result in cancer and certain viral infections. Lu et al. (2006) have suggested from their studies in mice that substances secreted by fat cells have an anti-apoptotic effect during tumorigenesis and that decrease in cellular fat (as after exercise) may inhibit carcinogenesis and tumour growth by selectively enhancing apoptosis in DNA-damaged cells and in tumours but not in normal cells. The DNA damaging effects of strenuous exercise need to be further researched in order to elucidate whether disruption / alteration in the genetically programmed cell death or some other mechanism(s) is responsible for the presence of genetic damage in individuals who are continuously exercising and in those where physical activity poses an elevated risk for cancer. This is an area for future research. Antioxidant supplements (vitamin C, vitamin E, and beta-carotene) have been reported for enhancing exercise performance and for preventing certain diseases and exercise-induced oxidative stress. Routine antioxidant supplementation from a diet that contains five to seven servings of fruits and vegetables daily may be recommended (Adams and Best 2002) as probably the most beneficial prescription for attenuating post-exercise tissue damage.

REFERENCES

Adams AK, Best TM 2002. The role of antioxidants in exercise and disease prevention.*The Physician and Sports Medicine,* 30: 37-44.

Ambros-Rudolph, C M , Hofmann-Wellenhof R, Richtig E, Muller-Furstner M, Soyer P H, Kerl, H 2006. Malignant Melanoma in Marathon Runners. *Arch Dermato,* 142: 1471-1474.

Ames B N, Shigenaga MK, Hagen TM 1995. Mitochondrial decay in aging. *Biochem Biophys Acta,* 1271:165–170.

Aniagu S O, Day N,Chipman J K, Taylor E W, Butler P J, Winter M J 2006. Does exhaustive exercise result in oxidative stress and associated DNA damage in the chub (*Leuciscus cephalus*). *Environ Mol Mutagen,* 47: 616-623.

Asami S, Hirano T, Yamaguchi R, Itoh H, Kasai H 1998. Reduction of 8-hydroxyguanine in human leukocyte DNA by physical exercise. *Free Radic Res,* 29: 581-584.

Bejma J, Ji LL 1999. Aging and acute exercise enhance free radical generation in rat skeletal muscle. *J Appl Physiol,* 87: 465– 470.

Benoni G, Bellavite P, Adami A, Chirumbolo S, Lippi G, Cuzzolin L B 1995. Effect of acute exercise on some haematological parameters and neutrophil functions in active and inactive subjects. *Eur J Appl Physiol Occup Physiol,* 70: 187 –191.

Bergh V 1987. The influence of body mass in cross-country skiing. *Med Sci Sports Exerc,* 19: 324-331.

Betancourt M, Ortiz R, Gonzalez C, Perez P, Cortes L, Rodriguez L, Villasenor L 1995. Assessment of DNA damage in leukocytes from infected and malnourished children by single cell gel electro-phoresis/comet assay. *Mutat. Res,* 331: 65-77.

Blomqvist, CG, Lewis SF, Taylor WF, Graham RM 1981. Similarity of the hemodynamic responses to static and dynamic exercise of small muscle groups. *Circ Res,* 48 *Suppl*I: I87-I92.

Boffi FM, Cittar J, Balskus G, Muriel M, Desmaras E 2002. Training-induced apoptosis in skeletal muscle. *Equine Vet J Supp,* 34: 275-278.

Bompa TO, Di Pasquale MG, Cornacchia L 2003. *Serious Strength Training,* 2nd Edition, USA:Champaign IL: Human Kinetics, pp. 87-102.

Bouchard C, Perusse L 1994. Heredity, activity level, fitness and health. In : C.Bouchard R J Shephard, T Stephens (Eds.): *Physical Activity, Fitness and Health.* Champaign IL: Human Kinetics pp 106-118.

Bouchard C, Tremblay A, Després JP, Nadeau A, Lupien P J, Thériault G 1994. The response to exercise with constant energy intake in identical twins. *Obes Res,* 2: 400–410.

Burdon RH 1999. *Genes and the Environment.* Pennsylvania: Taylor and Francis Inc.

Camus G, Deby-Dupont G, Duchateau J, Deby C, Pincemail J, Lamy M 1994. Are similar inflammatory factors involved in strenuous exercise and sepsis? *Int Care Med,* 20: 602-610.

Carraro U, Franceschi C 1997. Apoptosis of skeletal and cardiac muscles and physical exercise. *Aging Clin Exp Res,* 9: 19–34.

Caspersen CJ, Powell KE, Christensen GM 1985. Physical activity, exercise, and physical fitness: definitions and distinctions for health-related research. *Public Health Reports,* 100: 126–131.

Cerhan JR, Torner JC, Lynch CF, Rubenstein LM, Lemke JH, Cohen MB, Lubaroff DM, Wallace RB 1997. Association of smoking, body mass, and physical activity with risk of prostate cancer in the Iowa 65+ Rural Health Study (United States). *Cancer Causes Control,* 8: 229–238.

Davies K J A, Quintanilha A T, Brooks G A, Packer L 1982.Free radicals and tissue damage produced by exercise. *Biochem Biophys Res Comm,* 107: 1198–1205.

Demirbag R, Yilmaz R, Guzel S, Celik H, Kocyigit A, Ozcan E 2006. Effects of treadmill exercise test on oxidative/ antioxidative parameters and DNA damage. *Anadolu Kardiyol Derg,* 6: 135-140.

Dennog C, Speit G 1996. Induction of DNA damage by hyperbaric oxygen therapy. *Mutagenesis,* 11: 605-609.

Devasagayam TPA, Kamat JP 2000. Free radicals, antioxidants and human disease. *EMSI Newsletter,* 23: 3-13.

Ding XP, Yan SW, Zhang N, Zhang L, Tang J, Lu HO 2003. The effect of overtraining on human sperm chromatin structure. *Zhonghua Lao Dong Wei Sheng Zhi Ye Bing Za Zhi,* Chinese 21: 260-262.

Dorgan JF, Brown C, Barrett M, Splansky GL, Kreger BE, D'Agostino RB, Albanes D, Schatzkin S 1994. Physical activity and risk of breast cancer in the Framingham Heart Study. *Am J Epidemiol,* 139: 662–669.

Downey JM 1990. Free radicals and their involvement during long term myocardial ischemia-reperfusion. *Ann Rev Physiol,* 52: 487-504.

Ebbeling CB, Clarkson PM 1989. Exercise-induced muscle damage and adaptation. *Sports Med,* 7: 207-234.

Fatouros IG, Destouni A, Margonis K, Jamurtas AZ, Vrettou C, Kouretas D, Mastorakos G, Mitrakou A, Taxildaris K, Kanavakis E, Papassotiriou I 2006. Cell-free plasma DNA as a novel marker of aseptic inflammation severity related to exercise overtrain-ing. *Clin Chem,* 52: 1820-1824.

Floyd RA 1990. The role of 8-hydroxyguanine in carcinogenesis. *Carcinogenesis,* 11: 1447-1450.

Galvao DA, Newton R U 2005. Review of Exercise Intervention Studies in Cancer Patients. *Journal of Clinical Oncology,* 23: 899-909.

Germadnik D, Pilger A, Rudiger HW 1997. Assay for the determination of urinary 8-hydroxy-2-dexygua-nosine by high performance liquid chromatography with electrochemical detection. *J Chromatogr B*, 689: 399-403.

Grollman AP, Moriya M 1993. Mutagenesis by 8-oxoguanine: An enemy within. *Trends Genet,* 9: 246-249.

Gutteridge JMC, Halliwell B 1990. The measurement and mechanism of lipid peroxidation in biological systems. *Trends Biochem Sci,* 15: 129-135.

Hack V, Storbel G, Rau JP, Weicker H 1992. The effect of maximal exercise on the activity of neutrophil granulocytes in highly trained athletes in moderate training period. *Eur J Appl Physiol,* 65: 520-524.

Halliwell B, Auroma OI 1994. DNA damage by oxygen-derived species: Its mechanism and measurement in mammalian systems. *FEBS Lett,* 281: 9-19.

Hartmann A, Niess AM 1999. Oxidative DNA damage in exercise. In: C K Sen, L Packer, O Hänninen (Eds.): *Exercise and Oxygen Toxicity: A Handbook.* Elsevier Science: B.V. North Holland/New York, pp.195-217.

Hartmann A, Niess AM, Grunert-Fuchs M, Poch B, Speit G 1995. Vitamin E prevents exercise-induced DNA damage. *Mutat Res,* 346(4): 195-202.

Hartmann A, Pfuhler S, Dennog C, Germadnik D, Pilger A, Speit G 1998. Exercise-induced DNA effects in human leukocytes are not accompanied by increased formation of 8-hydroxy-2'-deoxyguanosine or induction of micronuclei. *Free Rad Biol and Med,* 24: 245-251.

Hartmann A, Plappert U, Raddatz K, Grunert-Fuchs M, Speit G 1994. Does physical activity induce DNA damage? *Mutagenesis,* 9: 269-272.

Helbock HJ, Beckman KB, Ames BN 1999. 8-Hydroxyguanosine as biomarker of oxidative DNA damage. *Methods Enzymol,* 300:156-165.

Hellsten-Westing Y, Balsom PD, Normon B, Sjodin B 1993.

The effect of high intensity training on purine metabolism in man. *Acta Physiol Scand*, 149: 405-412.

Hellsten Y 1994. Xanthine dehydrogenase and purine metabolism in man : with special reference to exercise. *Acta Physiol Scand,* 621: 1-73.

Hoffman-Goetz L, Duerrstein L 2004. The effect of chronic and acute exercise on thymocyte apoptosis and necrosis in ovariectomized mice given dietary genistein. *J Sports Med Phys Fitness,* 44: 281-287.

Hsu TG, Hsu KM, Kong CW, Lu FJ, Cheng H, Tsai K 2002. Leukocyte mitochondria alterations after aerobic exercise in trained human subjects. *Med Sci Sports Exerc*, 34: 438-442.

Inoue T, Mu Z, Sumikawa K, Adachi K, Okochi T 1993. Effect of physical exercise on the content of 8-hydroxydeoxyguanosine in nuclear DNA prepared from human lymphocytes. *Jpn J Cancer Res,* 84: 720-725.

Ji LL 1999. Antioxidants and oxidative Stress in exercise. *Exp Bio and Med,* 222: 283-292.

Kasai H, Iwamoto-Tanaka N, Miyamoto T, Kawanami K, Kawanami S, Kido R, Ikeda M 2001. Life style and urinary 8-hydroxydeoxyguanosine, a marker of oxidative DNA damages: effects of exercise, working conditions, meat intake, body mass index, and smoking. *Jpn J Cancer Res,* 92: 9-15.

Kelly S, Havrilla C M, Brady T C Abramo K H, Levin E D 1998. Oxidative Stress in Toxicology: Established Mammalian and Emerging Piscine Model Systems. *Environ Health Perspect***,** 106: 375-384**.**

Kuppasamy P , Zweier JL 1989. Characterization of free radical generation by xanthine oxidase : Evidence for hydroxyl radical generation. *J Biol Chem,* 264: 9880-9884.

Leaf DA, Kleinman MT, Hamilton, M, Deitrick RW 1999. The exercise-induced oxidative stress paradox: the effects of physical exercise training. *Am J Med Sci,* 317: 295-300.

Leeuwenburgh C,. Hansen, P A, Holloszy J O, Heinecke J W 1999. Hydroxyl radical generation during exercise increases mitochondrial protein oxidation and levels of urinary dityrosine. *Free Rad Bio Med,* 27: 186–192.

Le Marchand L, Kolonel LN, Yoshizawa CN 1991. Lifetime occupational physical activity and prostate cancer risk. *Am J Epidemiol,* 133**:** 103–111.

Lewis SF, Taylor WF, Graham RM, Pettinger WA, Schutte JE and Blomqvist CG 1983. Cardiovascular responses to exercise as functions of absolute and relative work load. *J Appl Physiol,* 54: 1314-1323.

Ji LL, Mitchell EW 1994. Effects of adriamycin on heart mitochondrial function in rested and exercised rats. *Biochem Pharmacol,* 47: 877–885.

Ji LL, Stratman FW, Lardy HA 1988. Enzymatic down regulation with exercise in rat skeletal muscle. *Arch Biochem Biophys,* 263: 137–149.

Lim JH, Kim DY, Bang MS 2004. Effects of exercise and steroid on skeletal muscle apoptosis in the mdx mouse. *Muscle Nerve,* 30: 456-462.

Liu J, Yen HC, Overvik-Douki E, Hagen T, Doniger SJ, Chyu DW, Brooks GA, Ames BN 2000. Chronically and acutely exercised rats: biomarkers of oxidative stress and endogenous antioxidants. *J Appl Physiol,* 89: 21-28.

Loft S, Astrup A, Buemann B, Poulsen HE 1994. Oxidative DNA damage correlates with oxygen consumption in humans. *FASEB J,* 8: 534-537.

Loft S, Wierik EJMV, van der Berg H, Poulsen HE 1995. Energy restriction and oxidative DNA damage. *Cancer Epiderm Biomark Prev,* 4: 515-519.

Lu, Y-P, Lou, Y-R, Nolan, B., Peng, Q.-Y, Xie, J-G, Wagner G C and Conney, AH 2006. Stimulatory effect of voluntary exercise or fat removal (partial lipectomy) on apoptosis in the skin of UVB light-irradiated mice. *Proc Natl Acad Sci USA,* 103: 16301-16306.

Mars M, Govender S, Weston A, Naicker V, Chuturgoon A 1998. High intensity exercise: a cause of lymphocyte apoptosis? *Biochem Biophys Res Commun,* 249: 366-370.

Mastaloudis A, Yu TW, O'Donnell RP, Frei B, Dashwood RH, Traber MG 2004. Endurance exercise results in DNA damage as detected by the comet assay. *Free Radic Biol Med,* 36: 966-975.

McArdle WD 2002. *Exercise Physiology*. Baltimore USA: Lippincott Williams and Wilkins, pp. 223-248.

Meydani M, Evans W, Handelman G, Fielding RA, Meydani SN, Fiatarone MA, Blumberg JB, Cannon JG 1992. Antioxidant response to exercise-induced oxidative stress and protection by vitamin E. *Ann NY Acad Sci,* 669: 363-364.

Mink PJ, Folsom AR, Sellars TA, Kushi LH 1996. Physical activity, waist-to-hip ratio, and other risk factors for ovarian cancer: A follow-up study of older women. *Epidemiology,* 7: 38–45.

Moller P, Loft S, Lundby C, Olsen NV 2001. Acute hypoxia and hypoxic exercise induce DNA strand breaks and oxidative DNA damage in human. *FASEB J,* 15: 1181-1186.

Nakatani K, Komatsu M, Kato T, Yamanaka T, Takekura H, Wagatsuma A, Aoyama K, Xu B, Hirano T, Kasai H, Ando S, Takeuchi T 2005. Habitual exercise induced resistance to oxidative stress. *Free Radic Res,* 39: 905-911.

Niess AM, Baumann M, Roecker K, Hartmann T, Mayer F, and Dickhuth HH 1998. Effects of intensive endurance exercise on DNA damage in leukocytes. *J Sports Med Phys Fitness,* 38: 111-115.

Niess AM, Hartmann A, Gurnert-Fuchs M, Poch B, Speit G 1996. DNA damage after exhaustive treadmill running in trained and untrained men. *Int J Sports Med,* 17: 397-403.

Norman B, Sovell A, Kaijser L, Jansson E 1987. ATP breakdown products in human muscle during prolonged exercise to exhaustion. *Clin Physiol,* 7: 503-510.

Ogonovszky H, Berkes I, Kumagai S, Kaneko T, Tahara S, Goto S, Radak Z 2005a. The effects of moderate-strenuous-and over-training on oxidative stress markers, DNA repair, and memory in rat brain. *Neurochem Int,* 46: 635-640.

Ogonovszky H, Sarsvari M, Dosek A, Berkes I, Kaneko T, Tahara S, Nakamoto H, Goto S, Radak Z 2005b. The effects of moderate, strenuous, and overtraining on oxidative stress markers and DNA repair in rat liver. *Can J Appl Physiol,* 30: 186-195.

Okamura K, Doi T, Hamada K, Sakurai M, Yoshioka Y, Mitsuzono R, Migita T, Sumida S, Sugawa Katayama Y 1997. Effect of repeated exercise on urinary 8-hydroxy-deoxyguanosine excretion in humans. *Free Radic Res,* 26: 507-514.

Orhan H, van Holland B, Krab B, Moeken J, Vermeulen NP, Hollander P, Meerman JH 2004. Evaluation of a multi-parameter biomarker set for oxidative damage in man:

increased urinary excretion of lipid, protein and DNA oxidation products after one hour of exercise. *Free Radic Res,* 38: 1269-1279.

Paffenbarger RSJ, Hyde RT, Wing AL 1987. Physical activity and incidence of cancer in diverse popu-lations: a preliminary report. *Am J Clin Nutr*, 45 (Suppl.): 312–317.

Park RJ 1989. Measurement of physical fitness: a historical perspective. Office of Disease Prevention and Health Promotion Monograph Series. Washington, DC: U S Department of Health and Human Services, Public Health Service, pp. 1–35

Pate RR 1983. A new definition of youth fitness. *Physician and Sports Medicine,* 11: 77–83.

Pérusse L, Leblanc C, Bouchard C 1988. Inter-generation transmission of physical fitness in the Canadian population. *Can J Sport Sci,* 13: 8-14.

Pérusse L, Tremblay A, Leblanc C, Bouchard C 1989. Genetic and familial environmental influences in physical activity level. *Am J Epidemiol,* 129: 1012-1022.

Peters EM, Van Eden M, Tyler N, Ramautar A, Chuturgoon AA 2006. Prolonged exercise does not cause lymphocyte DNA damage or increased apoptosis in well-trained endurance athletes. *Eur J Appl Physiol*, 98: 124-131.

Phaneuf S, Leeuwenburgh C 2001. Apoptosis and exercise. *Med Sci Sports Exerc,* 33: 393–396.

Pilger A, Germadnik D, Formanek D, Zwick H, Winkler N, Rudiger HW 1997. Habitual long-distance running does not enhance urinary excretion of 8-hydroxydeoxy-guanosine. *Eur J Appl Physiol Occup Physiol,* 75: 467-469.

Pittaluga M, Parisi P, Sabatini S, Ceci R, Caporossi D, Valeria C, Savini M, Avigliano I L I 2006. Cellular and biochemical parameters of exercise-induced oxidative stress: Relationship with training levels. *Free Rad Res,* 40: 607-614.

Polednak A P 1976. College athletics, body size and cancer mortality. *Cancer (Phila.),* 38: 382-387.

Poulsen HE, Loft S, Vistisen K 1996. Extreme exercise and oxidative DNA modification. *J Sports Sci,* 14: 343-346.

Poulsen HE, Weimann A, Loft S 1999. Methods to detect DNA damage by free radicals: relation to exercise. *Proc Nutr Soc,* 58: 1007-1114.

Radak Z, Apor P, Pucsok J, Berkes I, Ogonovszky H, Pavlik G, Nakamoto H, Goto S 2003. Marathon running alters the DNA base excision repair in human skeletal muscle. *Life Sci,* 72: 1627-1630.

Radak Z, Kaneko T, Tahara S, Nakamoto H, Ohno H, Sasvari M, Nyakas C, Goto S 1999b. The effect of exercise training on oxidative damage of lipids, proteins and DNA in rat skeletal muscle: evidence for beneficial outcomes. *Free Radic Biol Med,* 27: 69-74.

Radak Z, Naito H, Kaneko T, Tahara S, Nakamoto H, Takahashi R, Cardozo-Pelaez F, Goto S 2002. Exercise training decreases DNA damage and increases DNA repair and resistance against oxidative stress of proteins in aged rat skeletal muscle. *Pflugers Arch,* 445: 273-278.

Radak Z, Pucsok K, Boros S, Josfai L, Taylor AW 2000. Changes in urine 8-hydroxydeoxyguanosine levels of super-marathon runners during a four-day race period. *Life Sci,* 66: 1763-1767.

Radak Z, Pucsok J, Meeseki S, Csont T, Ferdinandy P 1999a. Muscle soreness-induced reduction in force generation

is accompanied by increased nitric oxide content and DNA damage in human skeletal muscle. *Free Radic Biol Med,* 26: 1059-1063.

Radak Z, Taylor AW, Ohno H, Goto S 2001. Adaptation to exercise-induced oxidative stress: from muscle of brain. *Exerc Immunol Rev,* 7: 90-107.

Remmen HV, Hamilton ML, Richardson A 2003. Oxidative damage to DNA and aging. *Exerc Sport Sci,* 31: 149-153.

Sahlin K, Ekberg K, Cizinsky S 1991. Changes in plasma hypoxanthine and free radical markers during exercise in man. *Acta Physiol Scand,* 142: 273-281.

Sandri M, Carraro U, Podhorska-Okolov M, Rizzi C, Arslan P, Monti D, Franceschi C 1995. Apoptosis, DNA damage and ubiquitin expression in normal and mdx muscle fibers and exercise. *FEBS Lett,* 373: 291-295.

Sandri M, Podhorska-Okolow M, Geromel V, Rizzi C, Arslan P, Franceschi C, Carraro U 1997. Exercise induces myonuclear ubiquitination and apoptosis in dystrophin-deficient muscle of mice. *J Neuropathol Exp Neurol,* 56: 45-57.

Sato Y, Nanri H, Otha M, Kasai H, Ikeda M 2003. Increase of human MTH1 and decrease of 8-hydro-xydeoxyguanosine in leukocyte DNA by acute and chronic exercise in healthy male subjects. *Biochem Biophys Res Commun,* 305: 333-338.

Schiffl, C, Ziers C, Zankl H 1997. Exhaustive physical exercise increases frequency of micronuclei. *Mutat Res,* 389: 243-246.

Selman C, McLaren JS, Collins AR, Duthie GG, Speakman JR 2002. Antioxidant enzyme activities, lipid peroxidation and DNA oxidative damage: the effects of short-term voluntary wheel running. *Arch Biochem Biphys,* 40 :255-261.

Sen CK, Rankinen T, Vaisanen S, Rauramaa R 1994. Oxidative stress after human exercise: Effect of N-acetylcysteine supplementation. *J Appl Physiol,* 76: 2570-2577.

Severson RK, Nomura AMY, Grove JS, Stemmermann GN 1989. A prospective analysis of physical activity and cancer. *Am J Epidemiology,* 130: 522–529.

Shacter E, Beecham EJ, Covey JM, Kohn EW, Potter M 1988. Activated neutrophils induce prolonged DNA damage in neighboring cells *Carcinogenesis,* 9: 2297-2304.

Shingenaga MK, Ames BN 1991. Assays for 8-hydroxy-2'de-oxyguanosine: A biomarker of *in vivo* oxidative DNA damage. *Free Radic Bio. Med,* 10: 211-216.

Sim YJ, Kim H, Kim JY, Yoon SJ, Kim SS, Chang HK, Lee TH, Lee HH, Shin MC, Shin MS, Kim CJ 2005. Long-term treadmill exercise overcomes ischemia-induced apoptotic neuronal cell death in gerbils. *Physiol Behav,* 84: 733-738.

Singh NP, Singh G, Singh SM 2005. Role of host's antitumor immunity in exercise-dependent regression of murine T-cell lymphoma. *Comp Immunol Microbiol Infect Dis,* 28: 231-248.

Siu PM, Bryner RW, Martyn JK, Alway SE 2004. Apoptotic adaptations from exercise training in skeletal and cardiac muscles. *FASEB J,* 18: 1150-1152.

Smith JA, Telford RD, Mason IB, Weidemann MJ 1990. Exercise, training and neutrophil microbicidal activity. *Int J Sports Med,* 11: 179-187.

Smith LL 1991. Acute inflammation: The underlying mechanism in delayed onset muscle soreness? *Med Sci Sports Exerc,* 23: 542-551.

Sumida S, Okamura K, Doi T, Sakurai M, Yoshioka Y, Sugawa-Katayama Y 1997. No influence of a single bout of exercise on urinary excretion of 8-hydroxy-deoxyguanosine in humans. *Biochem Mol Biol Int*, 42: 601-609.

Suresh C, Tiwary RS 1999. Free radicals in health and diseases. *Everyman's Science* 33: 149-153.

Suzuki J, Inoue Y, Suzuki S 1995. Changes in the urinary excretion level of 8-hydroxyguanine by exposure to reactive oxygen-generating substances. *Free Radic Biol Med,* 18: 431-436.

The Scientific Program Committee 2002. Physical Activity across the Cancer Continuum: Report of a Workshop Review of Existing Knowledge and Innovative Designs for Future Research-This article is a US Government work and, as such, is in the public domain in the United States of America- Published by the American Cancer Society DOI 10.1002/cncr.10771.

Tice RR, Andrews PW, Singh NP 1990. The single cell gel assay: a sensitive technique for evaluating intercellular difference in DNA damage and repair. *Basic Life Sci,* 53: 291-301.

Tsai K, Hsu TG, Hsu KM, Cheng H, Liu TY, Hsu, CF, Kong CW 2001. Oxidative DNA damage in human peripheral leukocytes induced by massive aerobic exercise. *Free Radic Biol Med*, 31: 1465-1472.

Umegaki K, Higuchi M, Inoue K, Esashi T 1998. Influence of one bout of intensive running on lymphocyte micronucleus frequencies in endurance-trained and untrained men. *Int J Sports Med,* 19 : 581-585.

U S Department   of   Health and Human Services 1996. *Physical Activity and Health: A Report of the Surgeon General*. Atlanta, GA: U.S. Department of Health and Human Services, Centers for Disease Control and Prevention, National Center for Chronic Disease Prevention and Health Promotion http://www.cdc. gov/HealthyYouth/physicalactivity/publications. htm

Vincent HK, Bourguignon CM, Vincent KR, Weltman, AL, Bryant M, Taylor AG 2006. Antioxidant supplementation lowers exercise-induced oxidative stress in young overweight adults. *Obesity (Silver Spring),* 14: 2224- 2235.

Wiseman H, Halliwell B 1996. Damage to DNA by reactive oxygen and nitrogen species: Role in inflammatory disease and progression to cancer. *Biochem J*, 313: 17-29.

Woodson RD 1984. Hemoglobin concentration and exercise capacity. *Am Rev Respir Dis,* 129 (Suppl): 72.

Wyndham CH, Hegns AJA 1969. Determinants of oxygen consumption and maximum oxygen intake of Caucasians and Bantu males. *Int Z Angew Physical,* 27: 51-57.

Zeegers MPA, Dirx MJM, van den Brandt PA 2005. Physical Activity and the Risk of Prostate Cancer in The Netherlands Cohort Study, Results after 9.3 Years of Follow-up. *Cancer Epid Biomark & Preven*, 14: 1490-1495.

Zerba E, Komorowski T, Faulkner JA 1990. Free radical injury of skeletal muscles of young, adult, and old mice. *Am J Physiol,* 258: 429-435.