Review of Literature
Stress and the effect on health

The word ‘Stress’ generally has an indefinite meaning and symbolizes different things to people of different disciplines. Psychologists as well as non-professionals usually employ the term stress to denote emotional tension emanating from psychological demands. Biological stress was first defined by Hans Selye (1936) as the non-specific response of the body to any demand. Selye found a triphasic response to a wide variety of stressors. The initial response he termed as the alarm reaction, the enhanced state of recovery as the stage of resistance and the stage of exhaustion when resistance fails he labeled this trial as ‘General Adaptation Syndrome’ (GAS).

The conditions, which lead to disturbance in their normal physiological and mental equilibrium, are called ‘stressors’. The very process of living is a continual interplay between the individual and his environment, often taking the form of a struggle resulting in injury or disease. Sustained stress can have numerous pathological effects. Among the molecules that mediate such effects are the adrenal steroid hormones, including the human glucocorticoid hydrocortisone. Along with epinephrine (adrenaline) and nor-epinephrine, glucocorticoids are essential for surviving acute physical stress. When neuro-hormonal response mechanisms become overloaded with demands from stressors, functional hormonal imbalances may develop. The subsiding of stress may alleviate the imbalances and can progress to greater severity and become persistent. This long-term hormonal disorder leads to tissue damage (Ramsey 1982). Later in 1975 Fraser et al defined Stress a defence response of an animal to adverse physical or emotional stimuli.
CRH ACTH-cortisol sequence: The stress pathway involves the hypothalamus through neural pathways. The hypothalamus releases CRH which stimulates the anterior pituitary gland to secrete ACTH. ACTH then stimulates the adrenal cortex to secrete cortisol. Cortisol acts on target cells to reduce responsiveness to CRH. Not shown is that vasopressin and other messengers released both by hypothalamic neurons and by peripheral tissues also act on the anterior pituitary to stimulate ACTH secretion. The common denominator of many of the effects of cortisol is to maintain plasma glucose concentration and to facilitate a person's responses to stress.
Cold water swimming stress

Exposure to cold-water swimming may evoke several kinds of reflex responses. These reflex responses are mediated through the vegetative nervous system and represent a part of the reaction pattern that has commonly been named the stress reaction. This response generally reflects primitive defence responses of the body. If the exposure is temporary, the system usually returns to a normal or pre-exposure state within minute. If the cold-water swimming stimulation is sustained or consistently repeated, it has been postulated that persistent changes may develop in the neurosensory, circulatory, endocrine and digestive systems.

Swimming in small laboratory animals has been widely used for studying the physiological changes and the capacity of the organism in response to stress (Tan et al 1992; Greenen et al 1988). Swimming has got a number of advantages over other types of exercise such as treadmill running. The amount of work done during swimming exercise is far greater than that during the treadmill running of identical time duration. Swimming is not always a simple exercise stress, because emotional factors are difficult to be eliminated (Kramer et al 1993). Water temperature is another important factor in the swimming test. By varying the water temperature, Richter (1957) found that rats could survive as long as 80 hours in lukewarm water (36°C). Increase or decrease in water temperature above/below this point influences the overall behaviour of the animal with the involvement of glucocorticoids (Abel 1991).

Stress on Hypothalamo – Pituitary – Adrenal (HPA) Axis

Acute cold stress is known to stimulate sympathetic activity as well as the hypothalamo-pituitary-adrenal (HPA) axis, produces a significant increase in adrenomedullin (ADM) levels in the pituitary gland (Yuksel et al 2002). They also
studied the effect of ADM in addition to cold stress and suggested that ADM acts via receptors on different end organs, and involved in the regulation of metabolism. Djordjevic et al (2003) reported that all the stressors (fasting, crowding, cold and heat) activate HPA system. The measurement of blood ACTH, corticosterone and cholesterol content in the adrenal revealed that heat seems to be the strongest stressor and fasting seems to be the weakest stressor, as it causes the smallest increase in blood ACTH and corticosterone concentrations. However Kioukia-Fouzia et al (2002) on comparing different stressors model and their effects on the HPA axis, reported that a distinct effect on HPA axis occurs only on the 14-day and no effect was observed in the 1-day after cold swimming stress.

Gesing et al (2002) investigated whether acute stressors regulate functional properties of the hippocampal mineralocorticoid receptor (MR), which inhibits the hypothalamic-pituitary-adrenocortical activity. Exposure of rats to forced swimming evoked a significant rise in density of MR in all hippocampal sub fields after 24 hr, whereas exposure to a cold environment was ineffective. They also pretreated with the CRH receptor antagonist and were able to block the effect of forced swimming on hippocampal MR levels. This study identifies CRH as an important regulator of MR.

Endocrine System

After brain perception of a specific stressor, the crucial event in neuroendocrine activation of stress responses is the triggering of CRH release from the paraventricular nucleus of the hypothalamus. Activation of the HPA axis with secretion of glucocorticoids (GC), mainly cortisol, 18 - hydroxycorticosterone, aldosterone, and dehydrops, androsterone, is the end – point of the neuroendocrine cascade of events generated by hypothalamic CRH release (Gaillard and
Glucocorticoids, in turn, may deeply affect brain cells by inducing neuronal and glial synthesis of proteins useful in generating adaptive responses (McEwen 1992). Besides its main role in controlling ACTH and GC secretion, CRH may be considered the central regulator of a series of other stress induced body functions, including activation of the sympathoadrenomedullary axis, behavioral inhibition, appetite suppression, and reproductive quiescence. (Herbert 1992; Melia and Duman 1991). In fact, apart from the paraventricular nucleus, CRH is also present in central nervous systems areas, such as limbic areas, median raphe nuclei, locus coeruleus, and cortical interneuron circuits.

Under chronic abnormal conditions, impairment of the circadian rhythmic pattern of HPA hormone release estimated at a single point appears to be an indicator of chronic stress syndrome. Circulating ACTH is the key regulator of glucocorticoid secretion by the adrenal cortex, but other hormones, some of them originating in the adrenal medulla, may also participate (Hinson 1990). Glucocorticoids are the final effectors of the organism’s response to stress. They also play a key regulatory role on the basal activity of the HPA axis and on the termination of the stress response by exerting negative feedback at the CNS components of the stress system (DeKloet 1991).

Psychological factors always alter the normal pituitary adrenal activity, which result in increased amounts of ACTH and cortisol secretion (Liddle 1969; Ganong et al 1974). Rats exposed to -20° C showed an increase in plasma catecholamines (Chin et al 1973; Takahashi et al 1984). Following acute cold swim stress, hypothalamic epinephrine concentration was markedly lowered and remained decreased for 24 hours, while norepinephrine concentration was decreased but returned to baseline within 14 hours (Roth et al 1982). Armario et al (1986)
subjected adult male rats to different acute stressors and found an increased corticosterone and glucose. This increases is also related to the intensity of stressors.

Sakellaris et al (Sakellaris and Vernikos – Danellis 1975; Danies – Severs et al 1973) hypothesized that adaptation involves an increased responsiveness of the entire pituitary – adrenocortical system. Selye (1976) felt that the process represented exhaustion instead of adaptation. The apparent discrepancies exist as the change depends on the type of stress, the length of the experiment, and the coping resources available.

Elevated (Growth Hormone) GH and prolactin plasma levels have been reported after various stressors, such as physical exercise, surgery, and also involve the activation of numerous monoaminergic pathways (Ader et al 1991; Rose 1984). Circulating thyroid hormone (TH) levels are important regulators of cellular metabolism and energy expenditure; in this context, the hypothalamus – pituitary – thyroid (HPT) axis is strongly involved in the stress – adaptive response, with reduction in thyroid hormone levels after prolonged exposure to stress, likely due to the increased peripheral thyroid hormone turnover (Rose 1984).

The elevation of plasma corticosterone levels in other forms of acute stress, like cold stress and noise were also reported (Panaetto and Vickery 1970; Prabhakaran et al 1988; Sembulingam et al 1994). Degenerative alterations of the aging brain may be a useful example of disruption of the balance in the individual neuroendocrine responses to adequately counteract the perturbative event represented by the stressful stimulus, with impaired ability to cope with stress (Sapolsky et al 1986). Acute cold exposure causes a rapid rise in the concentration of plasma glucagons and hepatic cAMP in man and rat (Seitz et al 1981). Habara et al (1987) have shown the enhanced formation of cAMP after cold acclimatization in rats.
Stress responses are relevant to successful adaptation of an organism by increasing readily available energy substrates to sustain brain and heart activity. Chronic and prolonged exposure of individuals to stress may induce excessive exposure to the central and peripheral effects of GC, causing detrimental effects to the organism itself, so-called hormonal detrimental effects (Munck et al 1984; McEwen 1992). This causes alterations in the complex neuroendocrine network underlying the stress adaptive response as well as damage to neural tissue (Sapolsky et al 1986).

**Stress and Analgesic systems**

Girardot and Holloway (1984) suggested that continuous cold-water swimming induces analgesia, which is mediated by non-opioid systems, while intermittent cold-water swimming mediates through the morphine analgesia. Benedek and Szikszay (1985) suggested that analgesic and thermoregulatory effects of morphine were simultaneously enhanced, as the pathways mediating opiate analgesia and thermoregulatory effects converge.

Hanada et al (1985) showed that various stressor like the electric foot-shock, immobilized-water immersion, and cold-water swimming, produced analgesia. Hence it prolonged the effect induced by pentobarbital, hypnosis, morphine and clonidine. Stress induced hypoalgesia was blocked by pretreatment with the opioid antagonist naltrexone. Naltrexone eliminated the hypnosis prolonging effect of morphine on foot-shock, but failed to reverse the effect of clonidine on immobilized-water immersion and cold-water swimming. Differences in the analgesic and hypnosis prolonging effects and also the respective naloxone sensitivity of each drug and stress suggest the diversity of the underlying mechanisms. Foo and Helmsetter (2000) showed that the opioid receptors in the
rostroventral medulla are critically involved in mediating expression of hypoalgesia following noise stress.

Sensitivity to mutagens was studied in mouse lines selectively bred for high analgesia (HA) and for low analgesia (LA) induced by 3-min swimming in 20° C water. HA mice appeared more susceptible to the mutagenic effect of whole-body gamma-radiation and mitomycin-C injection and more over higher frequencies of chromosomal aberrations and micronucleus in bone marrow cells in the HA than in the LA line (Sacharczuk et al 2003).

**Effect on Enzymes**

In male Sprague-Dawley rats pineal -N-acetyltransferase (NAT) was statistically significantly depressed by physical immobilization stress for 2 hours, swimming (15 min in 10° C), exposure for 2 hours to cold (5° C), heat (40° C), noise (90 db) for 2 hours and hunger for 17 hours. (Welker and Vollrath 1984). This indicates that the different stressor can cause different effect on body systems.

Kaushik and Kaur’s studied (2003) antioxidant enzymes, in brain, heart, kidney, liver and small intestine. Chronic cold exposure resulted in a significant increase in LPO (Lipid Peroxidation) in all the tissues studied while XOD (Xanthine Oxidase) was increased in the brain and intestine. Total SOD (Superoxide dismutase) activity was significantly decreased in all the tissues and CAT (Catalase) activity was significantly increased in the kidney and decreased in heart, liver and intestine in the animals exposed to cold. GPx activity was increased only in the brain and intestine of stressed rats. Chronic cold exposure resulted in significant decrease in), GR (Glutathione Reductase) activity in heart, liver and intestine. GST activity was increased (except heart) and GSH was significantly decreased in all the tissues in treated rats.
Effect on Immune Response

Individuals, who experience stressful events in life, appear more susceptible to a variety of illnesses. Shu et al. (1993) studied the cell-mediated immune responses. After cold water stress rat responses to Con A, LPS (lipopolysaccharide), IL-2 production, and CD4+ and CD8+ percentages of blood and spleen lymphocytes were decreased by the 1-day and increased by the 5th day. Corticosterone levels were increased in 1-day and also in 5-day cold-water stress. According to him the cold water stress is a natural stressor, might act to induce a unique pattern of neuroendocrine changes. Joasoo and McKenzie (1976) found that different stressors affect on the antibody production in animals differently and this depends on the sex. Blecha and Kelley (1981) showed that exposure of pigs to cold increased the serum antibody levels and this increase was due to their capacity to synthesize antibody.

Mastropaolo et al (1992) have studied the effect of swimming in cold and its effect on the antagonizing property of flurazepam. He reported that stress-induced reduction in flurazepam's antiseizure efficacy indicates that the therapeutic efficacy of benzodiazepines may be altered after a severe stress. Kubera et al (1998) observed that mice exposed to acute swimming stress showed a reduction in spleen weight and in the ability of splenocytes to produce IFN - gamma. Restraint stress in mice induced an increase in the plasma interleukin -6 levels (Song et al 1998).

Stress and Behaviour

Increased open field behaviour, shortened motor latencies and decreased defecation scores were observed in rats exposed to acute noise. Katz and Manik (1984). Armario et al (1985) observed that male rats exposed to chronic noise showed reduction in exploratory activity without any change in defecation rate.
Acute noise stress produced a behavioural activating effect, whereas chronic noise stress caused a state of depression. (Lai 1987).

Khaliulin et al (1993) studied the periodical cold exposure in rats (3 hours daily, 6 times a week within 2 months at the air temperature of 1°-2° C) and to the exercise training (swimming 1 hour daily, 6 times a week within 2 months at the water temperature of 32° - 33° C) and reported a limitation of motional and vegetative manifestations of the stress reaction in the "open field" test.

**Cardiovascular system**

Chronic exposure to noise caused development of duodenal ulcer in rats and increased gastrointestinal motility (Selye 1976). Muza et al (1988) have studied, the effects of cold acclimatization on the cardiorespiratory responses to cold air and water stress tests and reported that BP increased significantly during the first cold water exposure, but not during the last cold water immersion. So he concluded that the thermoregulatory adjustments associated with cold acclimatization altered the control of blood pressure during acute cold stress. Chaswal et al (1999) results showed that hypertensives on placebo had higher rise of heart rate and systolic blood pressure as compared to normotensive controls after cold pressure test.

**Reproductive system**

Bidzinska et al (1993) showed that the chronic stress induced a twofold decrease in plasma testosterone levels. Suppression of gonadal function has been demonstrated in highly trained runners of both sexes (Dom and Chrousos 1993; Benttins1986) and also in ballet dancers (Brooks and Warren 1985). In male athletes have low luteinizing hormone and testosterone levels, whereas females have amenorrhea (Benttins1986; Brooks and Warren 1985).
Suppression of gonadal function caused by chronic HPA activation has been demonstrated in highly trained runners of both sexes (Beitins 1986; Dom and Chrousos 1993), and ballet dancers (Brooks – Gunn and Warren 1985). These subjects have increased evening plasma cortisol and ACTH levels, increased urinary free cortisol excretion, and blunted ACTH responses to exogenous CRH. (Beitins 1986; Broo’s – Gunn and Warren 1985). Tommaselli et al (1994) founded that abnormalities in gonadotropin levels in women affected by stress – induced amenorrhea and anorexia nervosa.

Stress and Gastrointestinal system

Plourde (1999) reviewed that chronic stress induces alteration of gastric emptying in response to various stressors. He reported acceleration in gastric emptying in cold stress, Further he suggested that it might be through the secretion of thyroglobulin-hormone, acceleration of intestinal transit, and stimulation of colonic transit and fecal output. However in humans, the cold-water immersion test has been associated with an inhibition of gastric emptying.

Stress induced Free Radicals

A reactive molecule contains one or more unpaired electrons, the molecule is termed a free radical. Because of the relative instability of free radicals and high reactivity leads to damage cells and tissues. In human subjects who swim regularly in ice-cold water during the winter (winter swimming), were evaluated before and after this short-term whole body exposure (Siems et al 1994) and observed a drastic decrease in plasma uric acid concentration following the exposure to the cold stimulus. They hypothesized that the uric acid decrease could be caused by the formation of oxygen radicals. In addition they observed, the level of oxidized glutathione and the ratio of oxidized glutathione/total glutathione in erythrocytes,
which also increased following cold exposure. Furthermore, increase in reduced glutathione and the decrease in concentration of oxidized glutathione in winter swimmers when compared to those of non-winter swimmers support their hypothesis.

According to the experiments of Aravind et al (1998) white noise exposure (100 dB, 30 min, 30 min) in male Wistar rats, increased lipid peroxidation and superoxide dismutase and glutathione peroxidase activity in the brain while total reduced glutathione level was reduced. Gumuslu et al (2002) investigated the influences of different stress models on the antioxidant status and lipid peroxidation (LPO) in rats exposed to both cold and immobilization stresses for 15 days. LPO and SOD activities were increased after cold and immobilization stresses, whereas CAT, GSH-Px activities and GSH levels were decreased. From these findings, it can be suggested that these stress models can cause oxidative stress.

**Stress and Lipids**

Even slightly raised serum levels of cholesterol and triglycerides (Carlson and Ericsson 1975) are considered to be risk factors for the development of coronary heart diseases and atherosclerosis in general (Kannel et al 1979). Studies concerning the distribution of cholesterol between different lipoprotein fractions have shown that although elevated low and very low density lipoprotein (LDL, VLDL) cholesterol were associated with increased risk, high density lipoprotein (HDL) cholesterol had a negative correlation with coronary heart diseases (Gordon et al 1977).

A decreased cholesterol turnover rate in liver was noticed in normal as well as in diabetic rats, when subjected to continuous cold environment (Kato 1985). Total plasma free fatty acids increased by 92 % after cold exposure to rats (Ferguson and Shultz 1975).
In hamsters, a reduced caloric intake at 25°C was found to cause a loss of weight involving proportionately less fat than during cold exposure (Kodama and Pace 1964). Watanbe (1959) reported that exposure of guinea pigs for 48 hours to -20°C increased plasma total lipids and total fatty acid (both free and esterified). Plasma triglycerides were increased in dogs on acute exposure to cold (Salvador et al 1964). Dell’Erba (1954) found that acute exposure to cold caused plasma cholesterol to fall in guinea pigs, whereas in the same species Watanbe (1959) reported that acute cold exposure did not affect plasma cholesterol, ester cholesterol and however slightly increased free cholesterol levels. Exposure of guinea pigs for 48 hours to -20°C increased plasma phospholipid levels, whereas exposure of rats to 2-3°C for 7 days had no effect on plasma phospholipids.

Radowshi and Wood (1965) found that although cold exposure of rats for 10 days did not affect serum cholesterol and phospholipids, there was a marked fall in plasma triglycerides levels and in low-density lipoproteins. Certain pathological changes involving lipids have been related to cold exposure. Acute exposure to white noise (100dB) resulted in serum elevations of free fatty acids, triglycerides, cholesterol and glucose in laboratory animals. The release of lipids from the adipose tissue and the increased blood glucose level observed during stress is due to the action of epinephrine. In human beings noise elevated the metabolic rate (Ramsey 1982).

Reports on a Triglycerides values are contradictory (Taggart et al 1972, 1973), while a significant decrease has been found in rats after strenuous swimming (Papadopoulos et al 1969; Wolinsky et al 1979). Rats exposed to stress have shown decreased levels of serum total lipids after 3 months (Unley and Friedman 1959), while cold stress in rabbits has decreased their levels after 9 weeks (Barter et al
Sangeeta Singhal et al (1997) showed that 1, 3, and 7 days immobilization stress result in a serum HDL level decreased, serum cholesterol increased and the HDL/Cholesterol ratio declined significantly.

Nagaraja and Jeganathan (1999) found that the forced swimming stress had decreased the body weight, leucocyte, eosnophil, absolute neutrophil count, blood sugar and total cholesterol. But the body weight had been increased after exposure to 15 days. They also observed heart, kidney and adrenal gland weight was significantly increased. The experiments of Vij and Satija (1993) on healthy males who were exposed to noise stress of 100 dB for 30 min showed higher cholesterol, triglycerides and cortisol levels in the plasma however the use of ear defenders prevented the increase in cholesterol and cortisol levels indicating the effect to be of auditory origin. Prabhakaran et al., (1987) found that acute auditory stress (>97 dB, 1000Hz, 30 min) caused a significant increase in the total cholesterol, SGOT and SGPT while a marked reduction was observed in serum triglycerides level in Wistar albino rats. Cold swimming stress in rats was associated with significant decline in HDL – Cholesterol, Free Fatty Acid and Total cholesterol levels in continuous exposure of 5°C for 10 days (Tasopanakis and Tesserommatis 1991).

Brown and Goldstein (1984) and their colleagues discovered the underlying cellular mechanism controlling cholesterol metabolism. They observed that cells contain specific receptors for LDL, and demonstrated a correlation between LDL binding and control of the rate – limiting enzyme for cholesterol biosynthesis – HMG CoA (3 – hydroxy – 3 – methylglutaryl coenzyme A) reductase – and thus a mechanism for maintenance of cholesterol homeostasis. They proposed that the number of LDL receptors displayed by a cell varies with the need of the cell for cholesterol. This protects the cell against excess cholesterol however, when the number of LDL receptors decreases, this also leads to a decrease in removal of LDL.
from the circulation and an increase in plasma LDL (Goldstein and Brown 1977; Goldstein et al. 1983; Brown and Goldstein 1984).

Daniel Steinberg (1988) hypothesized that blocking oxidative modification of LDL should decrease the rate at which native LDL is converted to the oxidized form and therefore slow the rate at which it is taken up and degraded by resident arterial macrophages on their way to becoming foam cells. It should be noted that oxidative modification most likely takes place within the arterial wall.

Oxidized LDL could promote atherosclerosis in several ways: 1) by its cytotoxicity, 2) its chemotactic effect on monocytes, 3) its inhibitory effect on macrophage motility and 4) its uptake by the macrophage scavenger receptor mechanism, the latter leads to stimulation of the cholesterol esterification and foam cell formation (Morel et al. 1983; Quinn et al. 1987; Quinn et al. 1985; Henriksen et al. 1983).

**Lipid Peroxidation**

Kovacs et al (1996) studied that the influence of 30 min cold-immobilization stress in rats LPO on their study showed the increase of LPO in the heart, stomach and liver. In addition, Kaushik and Kaur (2003) reported the chronic cold exposure causes a significant increase in LPO. Gumuslu et al (2002) investigate in the influences of different stress models on the lipid peroxidation (LPO) swiss-Albino female rats (3 months old) and found that the LPO was increased after cold and immobilization stresses.

Schmidt et al (2002) studied in Forty physically active male volunteers (aged 18-40) and they were randomly assigned to a treatment (antioxidant) group or a control (placebo) group. Both groups exhibited increased levels of oxidative stress
after 24 days of field training, as indicated by an increased LPO, pentane, and 8-hydroxy deoxyguanosine. There was no significant difference between the treatment and placebo groups at day 24, which indicates antioxidant are not effective within this duration.

**Stress Proteins**

When a cell is subjected to an environmental stress, such as an increase above physiological temperatures, the subsequent destabilization of protein quaternary, structure may lead to the exposure of interactive surfaces. The cell responds to this danger of protein aggregation and loss of function by increasing the amount of molecular chaperones, which are able to bind the exposed surfaces and 'protect' the damaged proteins from aggregation.

In thermal shock (Lindquist 1986; Ang et al 1991), ischemia (Zimmerman et al 1991; Knowlton et al 1991), infections (Garry et al 1983), hemodynamic overload (Delcayre et al 1983), exposure to oxygen radicals or cytokines (Fincauto et al 1991) the proteins get irreversible denaturation and associated with the synthesis of Heat Shock Proteins (HSPs) as readily as a cellular defenses. Depending upon the organism and upon the type of cell within the same organism the expression of heat shock genes can be regulated at the transcriptional or the translational levels (Lindquist 1986). Activation of gene transcription is mediated by heat shock transcription factors (HSFs) (Lis and Wu 1993); HSF, inactive as a monomer, trimerizes into an active form which binds to a specific DNA recognition sequence, the heat shock element (HSE) (Morimoto 1993; Westwood and Wu 1993; Sargek et al 1993). Members of the HSF family have different functions and their DNA - binding properties are activated by specific factors.
Role of LDL on HSP

LDL molecules are having chemical composition (weight %) is 22.3 % phospholipids, 5.9 % triglycerides, 9.6 % free cholesterol, 22.0 % protein and total cholesterol content is 34.7 %. Oxidation of LDL is free radicals mediated through and result in lipid peroxidation. Oxidized LDL is cytotoxic to different types of cells (Hessler et al 1983; Kosugi et al 1987). Zhu et al (1994) has demonstrated that incubation of human endothelial cells and of human and rabbit smooth muscle cells with oxidized LDL and it triggers the expression of HSP70 (Zhu et al 1994; 1995).

Frostergard et al (1996) found that relatively low concentrations of chemically oxidized LDL (5 to 20 mg/ml) induced the synthesis of HSP in U937 and BL60 monocytic cell lines. The observation that oxidized LDL activate a stress response in vitro suggested that, in vivo, expression of HSP 70 may be induced at sites of LDL oxidation and/or oxidized LDL accumulation, which also correspond to sites of lesion. To date the components of oxidized LDL responsible for the induction have not been identified. Cajone et al (1989) demonstrated that HSP 70 synthesis is stimulated, either directly or indirectly, via a transcriptional mechanism. Another product of lipid oxidation 12 – HETE, which is also a product of membrane lipid oxidation, induces the expression of heat shock proteins in human leukocytes (Konig et al 1992; Koller et al 1993).

Burton et al (1988) showed that heat shock protein synthesis could be induced during recovery from cold treatment of Drosophila melanogaster larvae. The conditions that induce tolerance to cold are similar to those, which confer tolerance to heat. Because of this and the universality of the response, it has been suggested that heat shock proteins play a role in protecting cells from damage caused by these stresses. The precise functions of the heat shock proteins as well
their role in stress protection are still uncertain. Heat shock proteins are synthesized during recovery from prolonged exposure to cold and also prevent death from exposure to cold.

**Antioxidant**

Antioxidants offer protection against the free radicals. There are enzymes and small – molecular weight molecules with antioxidant capabilities to protect against the adverse effects of free radical reactions. A critical balance between free radical generation and antioxidant defences. Many of the protective antioxidants are essential nutrients. In the field of nutrition, the term essential is given to those nutrients (like the vitamins) that must be consumed because the body cannot synthesize these compounds.

Endogenous source of free radicals include those that are generated and act intracellularly as well as those that are formed within the cell and are released into the surrounding area. Intracellular free radicals are generated from the autoxidation and consequent inactivation of small molecules such as reduced flavins and thiols, and from the activity of certain oxidases, the cytosol. Molecular species include, hydroxyl, peroxy, hypochlorite, superoxide, and alkoxy radicals, and reactive molecules such as hydrogen peroxide and singlet oxygen (Freeman and Crapo 1982).

Exogenous sources of free radicals include tobacco smoke, certain pollutants and organic solvents, anesthetics, hyperoxic environments, and pesticides. Some of these compounds as well as certain medications are metabolized to free radical intermediate products that have been shown to cause oxidative damage to the target tissues. Exposure to radiation results in the formation of free radicals within the exposed tissues (Sies 1985; Halliwell and Gutteridge 1985; Taylor et al 1986).
Prime targets for free radical reactions are the unsaturated bonds in membrane lipids. Consequent peroxidation results in a loss of membrane fluidity and receptor alignment and potentially in cellular lysis. Free radical damage to sulfur – containing enzymes and other proteins culminates in inactivation, cross – linking, and denaturation. Nucleic acid can be attacked. Subsequent damage to the DNA can cause mutations that may be carcinogenic. Oxidative damage to carbohydrates can alter any of the cellular receptor functions including those associated with hormonal and neurotransmitter responses (Sies 1985; Halliwell and Gutteridge 1985; Taylor et al 1986).

Free radicals such as peroxy radicals, the superoxide anion, and the hydroxyl radical are responsible for many of the damaging reactions. In addition, certain aldehydes such as malondialdehyde and hydroxynonenal, arising from the free radical degradation of polyunsaturated fatty acids, can cause cross – linking in lipids, proteins, and nucleic acids (Flohe et al 1985; Slater 1987). The highly reactive breakdown products interact with apolipoprotein B on the surface of LDL and cause changes in receptor recognition, which result in LDL being recognized and taken up by macrophage scavenger receptors (Parthasarathy et al 1987; Sparrow et al 1989), leading to the production of lipid – laden foam cells. LDL particles contain many natural antioxidants able to trap free radicals that can prevent or limit the extent of the chain reaction.

There are three essential nutrients can directly scavenge free radicals. Vitamin E (\(\alpha\) – tocopherol), the major lipid soluble antioxidant presents in all cellular membranes and protects against lipid peroxidation (Machlin 1980). Vitamin E can act directly with a variety of oxy radicals, including the peroxy radical (ROO\(-\)), CCl\(_3\), and HO (McCay 1985; Burton et al 1985), as well as with the superoxide radical (O\(_2\)\(^-\)) (Fukuzawa and Gebicki 1983; Ozawa et al 1983).
Tocopherol can also react directly with singlet oxygen (Fahrenholtz et al 1974; Littarru et al 1984). Vitamin C (ascorbic acid) is water soluble and, along with Vitamin E, can quench free radicals as well as singlet oxygen. Ascorbic acid has been shown to react directly with superoxide (Hemila et al 1985; Nishikimi 1975), hydroxyl radicals (Bielski 1982), and singlet oxygen (Bodannes and Chan 1979). Ascorbic acid can also regenerate the reduced, antioxidant form of vitamin E, in the presence of transition metals.

Some work has shown that β – carotene, a pigment found in all plants, is the most efficient quencher of singlet oxygen known in nature and can also function as an antioxidant (Burton and Ingold 1984). β – Carotene is the major carotenoid precursor of vitamin A. Vitamin A, however, cannot quench singlet oxygen and has a very small capacity to scavenge free radicals (Urbach et al 1951; Mathews 1986). β – Carotene has been found in cellular membranes, including those of lysosomes (Mayne and Parker 1986). These three nutrients are also present in relatively high concentrations in the serum. The adrenal and pituitary glands, the brain, white blood cells, platelets, and the lens of the eye concentrate one or more of these nutrients at levels up to 20 – fold of that found in the serum (Machlin 1987).

α – tocopherol can compete for peroxyl radicals much faster than can polyunsaturated fatty acids. A small amount of α – tocopherol is able to protect a large amount of polyunsaturated fat. Concentrations of α – tocopherol in biological membranes are approximately one part per 1000 lipid molecules (Burton et al 1983). Vitamin E is consumed eventually during protection against peroxidation. The tocopheroxyl radical need regeneration by vitamin C (Doba et al 1985; Niki et al 1985; Packer et al 1979), vitamin E will need to be replenished either directly through the diet or from reserves elsewhere. Inhibition of peroxidation is obtained when both Vitamin E and Vitamin C are present. Vitamin C by itself is a good
antioxidant when peroxyl radicals are generated in the aqueous phase, but it is very much less effective when radicals are initially generated within a membrane (Doba et al. 1985; Niki et al. 1985). Presumably vitamin C cannot penetrate the membrane sufficiently to interact with a peroxyl radical as vitamin C is water soluble.

The importance of vitamin E for protecting the integrity of lipid structures (especially membranes) in vivo is giving importance by the finding that it is the only major lipid – soluble, chain breaking antioxidant that has been found in plasma, red cells, and tissues (Burton et al. 1982; 1983; Cheeseman et al. 1984; 1988). This finding has been reported in children with chronic severe vitamin E deficiency (Ingold et al. 1987). Although β – carotene has chain – breaking antioxidant activity also, it is less efficient than vitamin E and is expected to be important only in regions of very low oxygen partial pressure (Burton and Ingold 1984).

Demonstrations of the effectiveness of vitamin E in lessening the effects of lipid peroxidation in living systems are rare and difficult to obtain. However, Lemoyne et al. (1987; 1988) have shown that pentane, a minor product released during the peroxidation of polyunsaturated fat is reduced in the breath of humans supplemented with vitamin E. This strongly suggests that vitamin E prevents the peroxidation of polyunsaturated fat in vivo. (Tocopherol occurs in food as the free nonesterified form, whereas supplemental Vitamin E is usually provided as tocopheryl acetate). Extensive studies (Martin and Hurley 1977) showed that the acute and chronic toxicities of oral intakes of vitamin E in animals are low. In several species the oral LD – 50 was found to be $\geq 2$ g/kg body wt. Frogs, rabbits, cats, dogs, and monkeys can tolerate 200 mg vitamin E/kg body wt without apparent toxic signs (FASEB 1975). In general, deleterious effects have been observed in animals only when daily doses were $\geq 1$ g/kg body wt (Machlin 1984).
To test the effect of vitamin E on exercise – induced lipid peroxidation, Dilard et al (1978) administered 1200 IU of tocopherol/d to subjects for 2 weeks and observed a significant reduction in expired pentane at rest and during exercise. Francis and Hoobler (1986) reported that muscle soreness was not reduced by vitamin E supplementation (600 IU/d) 2 days before and 2 days after damage – inducing eccentric exercise.

Morel and Chisolm (1989) demonstrated that treatment of diabetic rats with vitamin E (probucol) inhibits both the in vivo oxidation and in vitro cytotoxicity of LDL and VLDL. Several studies have also shown that vitamin E supplementation affects lipoproteins metabolism by reducing serum triacylglycerols (Oriani et al 1997) and total cholesterol, and increasing HDL – cholesterol levels. In Streptozotocin – diabetic rats vitamin E supplementation prevented accumulation of lipid peroxides and maintained normal triacylglycerol levels (Karasu et al 1997). In rabbits administration of cholesterol with vitamin E protected against oxidative damage but had no effect on the lipid levels (Prasad and Kalra 1993).