

LETTER TO THE EDITOR

EFFECT OF STARVATION STRESS ON LIPID PEROXIDATION AND LIPID PROFILE IN RABBITS

Sir,

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Considerable progress has been made in the identification of risk factors which predispose an individual to major. Cardiovascular diseases, cancer, diabetes mellitus etc. Recently, attention has been focussed on the significance of psychosocial factors and life style in the aetiology of a number of diseases, especially in coronary artery disease (CAD) (1).

Stress is a universal feature in human life. Formation of excessive free radicals due to stressful conditions is a major internal threat to cellular homeostasis of aerobic organisms (2). Free radicals are formed in human body both in physiological and pathological conditions in most of the cellular organelle and plasma membranes (3). These free radicals are extremely reactive and unstable and react with most of the intracellular molecules (4). They enhance the process of lipid peroxidation (5). The products of lipid peroxidation are themselves reactive species and lead to extensive membrane, organellar and cellular damage (6). Many studies have implicated oxygen free radicals and lipid peroxidation in ageing and various diseases. Malondialdehyde (MDA) is one of the end products of lipid peroxidation and extent of lipid peroxidation is measured by estimating MDA levels most frequently. Increased serum level of MDA has been reported in cardiovascular, neurological and other diseases (7, 8).

Hence, the present work was undertaken to study the effect of starvation stress on lipid profile and lipid peroxidation in rabbits.

The study was conducted in 25 albino rabbits of either sex weighing between 1-2 kilograms. The rabbits were housed under standard laboratory conditions at ambient temperature with 12 hour day-night cycle. Starvation was done for 36 hours. Food was removed from the cages of all the animals. Water was given ad libitum. The study was approved by institutional ethics committee.

Blood samples of 5 millilitres (ml) from each rabbit were collected from marginal ear vein in a plain test tube under aseptic conditions using disposable needle. Blood samples during starvation were collected at 0 h (control), after 24 h and 36 h of starvation. Blood samples were allowed to clot at room temperature and serum samples were separated by centrifugation at 3000 rpm.

Estimation of MDA was done by Thiobarbituric acid (TBA) assay method using Elico spectrophotometer (9). Serum total cholesterol, HDL cholesterol, LDL cholesterol, VLDL cholesterol and triglycerides were analysed on multi-channel autoanalyser (Hitachi-911) using kits from Boehringer Mannheim Ltd. All the results

were analysed using student's paired t-test. Association between lipid peroxidation (MDA) and lipid profile was analysed using correlation co-efficient (r). $P < 0.05$ was considered as statistically significant.

Table I shows effect of starvation stress on lipid peroxidation and lipid profile. The findings of this study reveal significant increase in serum MDA levels (an index of lipid peroxidation) after starvation stress in rabbits. Level of MDA is also reported to be increased in other stress models of rabbits (10). Stress induced lipid peroxidation has also shown effect on lipid profile. Total serum cholesterol concentration is also significantly increased after starvation in this study and the increase is duration dependant. The results of the present study are in agreement with previous studies (11, 12). In another study, Swaner et al (13) observed an increase in total plasma cholesterol by 400% after starvation of 26–32 days. These results support the hypothesis that cholesterol stored in the lipid droplets of the adipose tissue cells is released into plasma and is the chief source of the hypercholesterolemia observed during complete starvation. Hepatic and intestinal cholesterol synthesis during fasting has been found to be markedly decreased. Another

cause for hypercholesterolemia during starvation may be continued biosynthesis concomitant with decreased or completely absent intestinal excretion (13).

Starvation stress in rabbits has significantly increased LDL levels. Serum levels of LDL almost doubled after 36 h of starvation as compared to control value. Increased level of LDL is considered to be a major risk factor for development of atherosclerosis (14). Bijlani et al (15) has reported increased concentration of total cholesterol and LDL in male medical students near exams (mental stress) as compared to pre exam levels. Recent studies indicate that antibodies formed against peroxidatively modified LDL (oLab) contribute to atherosclerotic process and may have some function in other disorders (16). Immunocytochemical analysis has revealed the presence of protein modified by MDA, which convert LDL, the major carrier of plasma cholesterol, to an abnormal form. Receptor-mediated clearance of this altered LDL, produces cholesteryl ester deposition in macrophage-derived foam cells of atheromas. These findings provide direct evidence for the existence of protein modified by a physiological product of lipid peroxidation within arterial lesions (17).

TABLE I: Serum MDA levels and lipid profile before (control) and after starvation of given duration (n=25).

Duration	MDA $\mu\text{mol/L}$	Total cholesterol	LDL	HDL (Mean \pm SEM; mg/100ml)	VLDL	TGs
0 h (control)	2.45 \pm 0.65	43.16 \pm 3.57	15.44 \pm 1.67	15.52 \pm 1.53	11.8 \pm 0.90	127.2 \pm 14.06
24 h	3.56 \pm 1.11*	53.28 \pm 4.31*	24.16 \pm 2.57*	14.88 \pm 1.35	14.48 \pm 1.26*	160.88 \pm 20.20
36 h	4.05 \pm 1.16*#	64.36 \pm 5.93*#	31.48 \pm 3.74*#	15.12 \pm 1.29	16.40 \pm 1.75*	132.40 \pm 16.31

* $P < 0.001$ vs 0 h; # $P < 0.01$ vs 24 h

Plasma samples from hypercholesterolemic rabbits and from several human subjects have been found to contain antibodies that were found to react with peroxidised LDL, suggesting that peroxidation does indeed take place *in vivo* (17, 18). Oxidation products of cholesterol have also been reported to be atherogenic (13).

Increased level of HDL is considered a protective factor in atherosclerosis (14). In the present study it is observed that HDL levels are almost constant with stress. Previous studies have reported either no change or decrease in HDL levels (15). This may be due to different experimental preparation and individual differences in susceptibility to stress. There is significant increase in VLDL levels. The increase is duration dependant. VLDL is reported to be increased in different hyperlipoproteinemias (type II, III, IV and V) and may be a contributing factor in atherosclerosis (19). No previous study has reported effect of starvation stress on VLDL concentration.

There was no significant alteration in triglycerides concentration after starvation of given duration. Swaner et al (13) has reported decreased TG levels in starvation model of rabbits where starvation was done for 7 days. Another study has shown significant increase in TG levels from 108 to 178 mg/100 ml after fasting for six days in rabbits (20).

The lipoprotein fractions are more predictive of developing coronary artery disease instead of total cholesterol. LDL is well recognised as a risk factor and HDL as a protective factor against atherosclerosis (21). In addition, it has been shown that HDL/total cholesterol ratio is one of the most powerful predictors of risk of developing CHD (14). HDL/total cholesterol ratio has decreased in this study. These changes predict increased susceptibility of the animals to atherosclerosis as a result of starvation stress.

Statistically significant positive correlation of MDA with total cholesterol, LDL and VLDL has also been obtained in this study. Hence increased lipid peroxidation leads to increased cholesterol and LDL, the known major risk factors for atherosclerosis and other lipid peroxidation induced diseases. Increased VLDL concentration may also have some role in atherogenesis (19). Negative correlation of MDA with HDL suggests that increased lipid peroxidation decreases HDL concentration. Decreased HDL levels may be a contributing factor in atherogenesis.

These findings suggest that there is accelerated stress induced lipid peroxidation (increased MDA), which has mainly affected serum total cholesterol, LDL and VLDL. These are significant and consistent changes in lipid peroxidation and lipid profile associated with starvation and its duration as well.

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