39. Ramesh, N., De, A. and Nagaraja, V., *Prot. Expr. Purif.*, 1994, **5**, 379–384.
40. Sabara, M., Parker, M., Aha, P., Cosco, C., Gibbons, E., Parsons, S. and Babiuk, L. A., *J. Virol.*, 1991, **65**, 6994–6997.
41. Aijaz, S., Gowda, K., Jagannath, H. V., Reddy, R. R., Maiya, P. P., Ward, R. L., Greenberg, H. B., Raju, M., Babu, A. and Rao, C. D., *Arch. Virol.*, 1996, **141**, 715–726.
42. Das, M., Dunn, S. J., Woode, G. N., Greenberg, H. B. and Rao, C. D., *Virology*, 1993, **194**, 374–379.
43. Gentsch, J. R., Das, B. K., Jiang, B., Bhan, M. K. and Glass, R. I., *Virology*, 1993, **194**, 424–430.
44. Tosser, G., Labbe, M., Bremont, M. and Cohen, J., *J. Virol.*, 1992, **66**, 5825–5831.
45. Crawford, S. E., Labbe, M., Cohen, J., Burroughs, M. H., Zhou, Y. J. and Estes, M. K., *J. Virol.*, 1994, **68**, 5945–5952

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Undernutrition and aging: Effects on DNA repair in human peripheral lymphocytes

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Subjects of Indian population belonging to 3 age groups – young (8–14 yrs), adult (20–35 yrs) and old (≥ 55 yrs) were divided into 'normal' and 'undernourished' groups based on Body Mass Index (BMI) and history of diet consumption. DNA repair markers like unscheduled DNA synthesis (UDS), activities of DNA polymerase β and two endodeoxyribonucleases, (UV- and AP-DNases) were studied in the lymphocytes of these subjects under different conditions. The 'undernourished' group showed higher activities of these enzymes and also a reduced decline in age-related DNA repair capacity. These results provide evidence for beneficial effects of reduced calorie consumption in humans as well.

THE integrity of DNA, maintained by a number of DNA repair systems, is essential for the survival of cells and organisms[1,2]. Numerous physical and chemical factors damage DNA *in vivo* with their origin being both endogenous and exogenous. The resultant DNA damage has been associated with various biological end points, including cancer, mutation, birth defects, aging and other age-associated diseases[3]. A positive correlation has been claimed between maximum life span and the capacity to repair UV induced DNA-damage, both across species[4,5], as well as within and among closely related species[6–9].

In a different direction, dietary restriction (DR) is the only environmental paradigm that has been demonstrated to increase maximum achievable life span in a variety of species[10–13]. DR has also been reported to modulate the rate or eliminate the occurrence of almost all age-associated degenerative diseases. Furthermore, it is well documented that DR reduces the incidence of both naturally occurring and induced tumours[14–19].

It is possible that the mechanism behind the beneficial effects of dietary restriction is positive affectation of DNA repair potential by that regime. Reports, from this laboratory as well as from elsewhere, have indicated that DR does lead to improved DNA repair capacity in experimental animals[20–22]. However, such effects are yet to be demonstrated in humans. We have therefore, taken up a study on healthy subjects (both sexes) of Indian population living in their natural conditions, with no familial history of organic defects and premature deaths, and belonging to 3 age groups, young (8–14 yrs), adult (20–35 yrs), and old (≥ 55 yrs). At each age, the subjects were divided into two groups of 20 numbers each: one group referred to as normal (NBMI) in which the individuals with a Body Mass Index (BMI)[23] of around 20 or more are included. The other group consisted of individuals with a low BMI of 18 or less (LBMI). Care is taken to see that the range is 16–18 with only a few subjects (5 out of 60) showing marginally lesser than 16.

Table 1 *a*. Average BMI of experimental subjects

	Average BMI	
Age	N	UN
Y	22.5 ± 1.3	16.5 ± 0.5
A	21.5 ± 1.8	17.1 ± 0.7
O	23.0 ± 2.2	17.0 ± 0.7

Subjects, based on their BMI, were categorized as 'normal' (BMI ≥ 20) and 'undernourished' (BMI ≤ 18). The average BMI of the subjects (20 nos) in the various age groups are shown, where N = Normal (NBMI) and UN = Undernourished (LBMI). Y – subjects grouped as 'young' (8–14 yrs); A – subjects grouped as 'adult' (20–35 yrs); O – subjects grouped as 'old' (≥ 55 yrs).

Table 1 *b*. Clinical parameters studied in experimental subjects

		Serum cholesterol (md/dl)	Blood haemoglobin (g%)	Random blood sugar (mg/dl)	Serum total protein (g/dl)	Serum albumin (g/dl)	Serum globulin (g/dl)
Y	N	155.2 ± 25.2	12.0 ± 1.1	83.0 ± 10.9	6.9 ± 0.53	4.3 ± 0.62	2.6 ± 0.43
Y	UN	145.8 ± 22.5	11.8 ± 0.4	83.0 ± 12.7	6.9 ± 0.48	4.1 ± 0.51	2.7 ± 0.50
A	N	180.0 ± 27.2	14.5 ± 1.3	76.0 ± 11.5	6.7 ± 0.79	4.6 ± 0.91	2.5 ± 0.45
A	UN	150.2 ± 26.2	13.6 ± 0.8	81.0 ± 27.0	6.5 ± 0.77	4.3 ± 0.93	2.3 ± 0.64
O	N	171.5 ± 26.8	13.8 ± 0.4	79.0 ± 14.0	6.5 ± 0.57	4.0 ± 0.43	2.4 ± 0.50
O	UN	163.0 ± 33.8	13.0 ± 1.4	82.0 ± 31.4	6.7 ± 0.98	4.2 ± 1.10	2.4 ± 0.60

Clinical parameters studied in experimental subjects were Hematology: Total leukocyte count, differential leukocyte count, total erythrocyte count, packed cell volume, blood hemoglobin; Biochemistry: Random blood sugar, serum cholesterol, serum total proteins, serum albumin, serum globulin; Others: Hepatitis B surface antigen detection, chest X-ray, electro-cardiograph. The mean values of clinical examinations of 20 individuals in each group are given. The differences, if any, between any two groups were not found to be statistically significant, where Y = young, A = adult, O = old, N = normal (NBMI), UN = undernourished (LBMI).

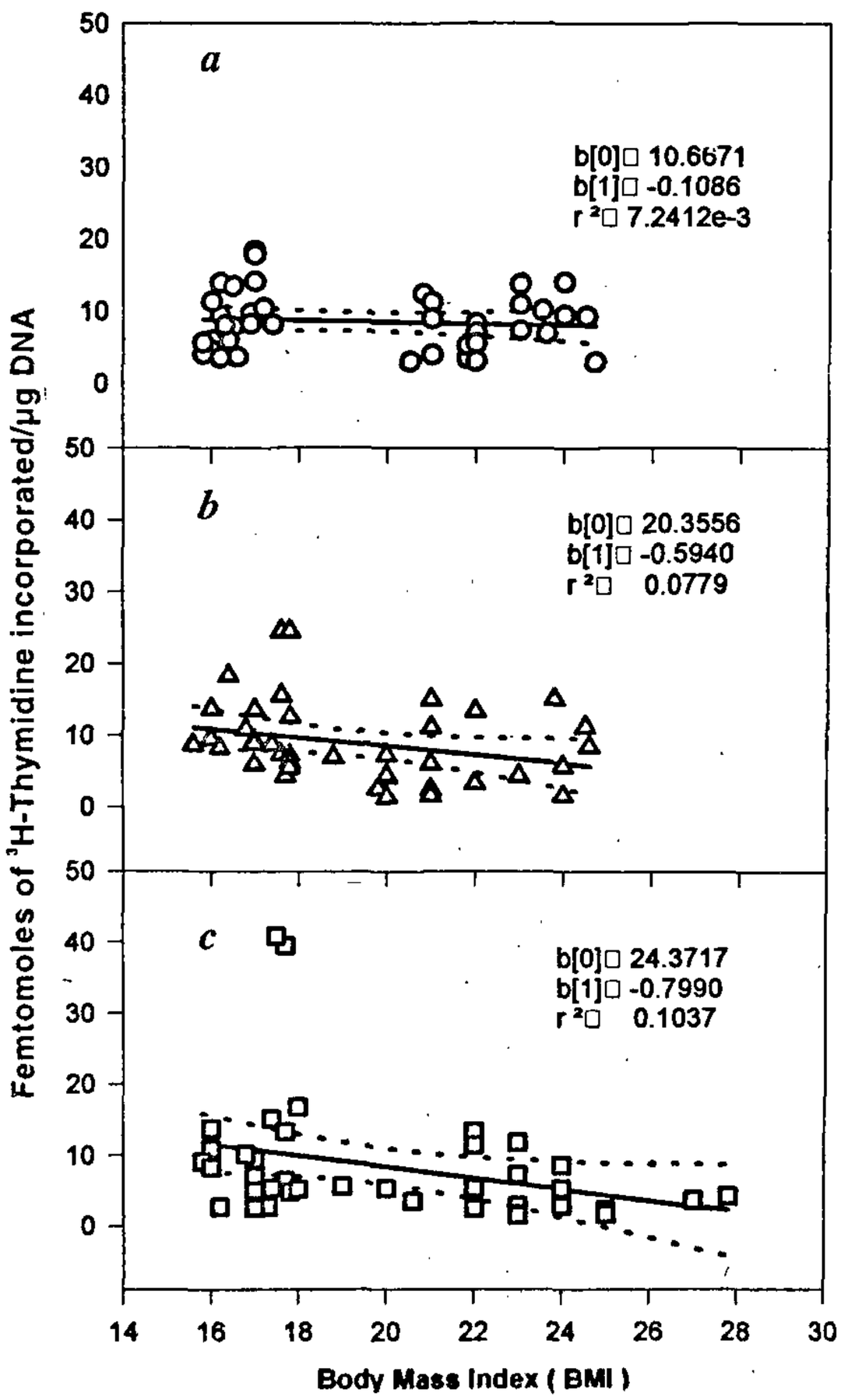

Figure 1. Basal DNA repair in peripheral lymphocytes of human subjects as a function of Body Mass Index at young (*a*), adult (*b*) and old (*c*) ages. Linear regression analyses are shown inside plot-box. $b(o)$ = intercept on *y*-axis; $b(i)$ = slope of the regression curve; r^2 = regression coefficient. At each age, 40 independent values are plotted.

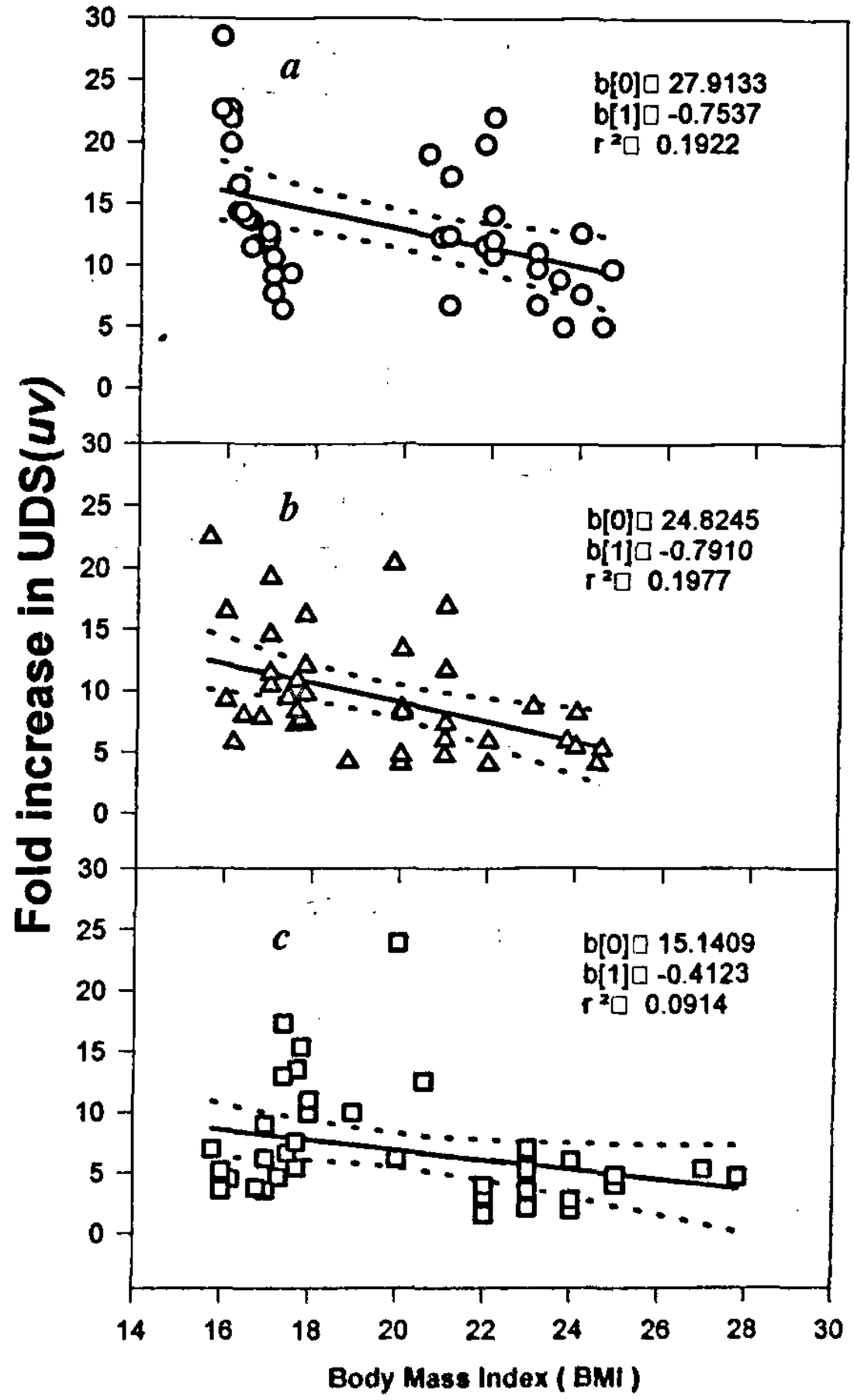

Figure 2. Ultraviolet light (UV) induced fold increase in DNA repair in the peripheral lymphocytes of human subjects as a function of Body Mass Index at young (*a*), adult (*b*) and old (*c*) ages. Linear regression analyses are shown. Other details are the same as in Figure 1.

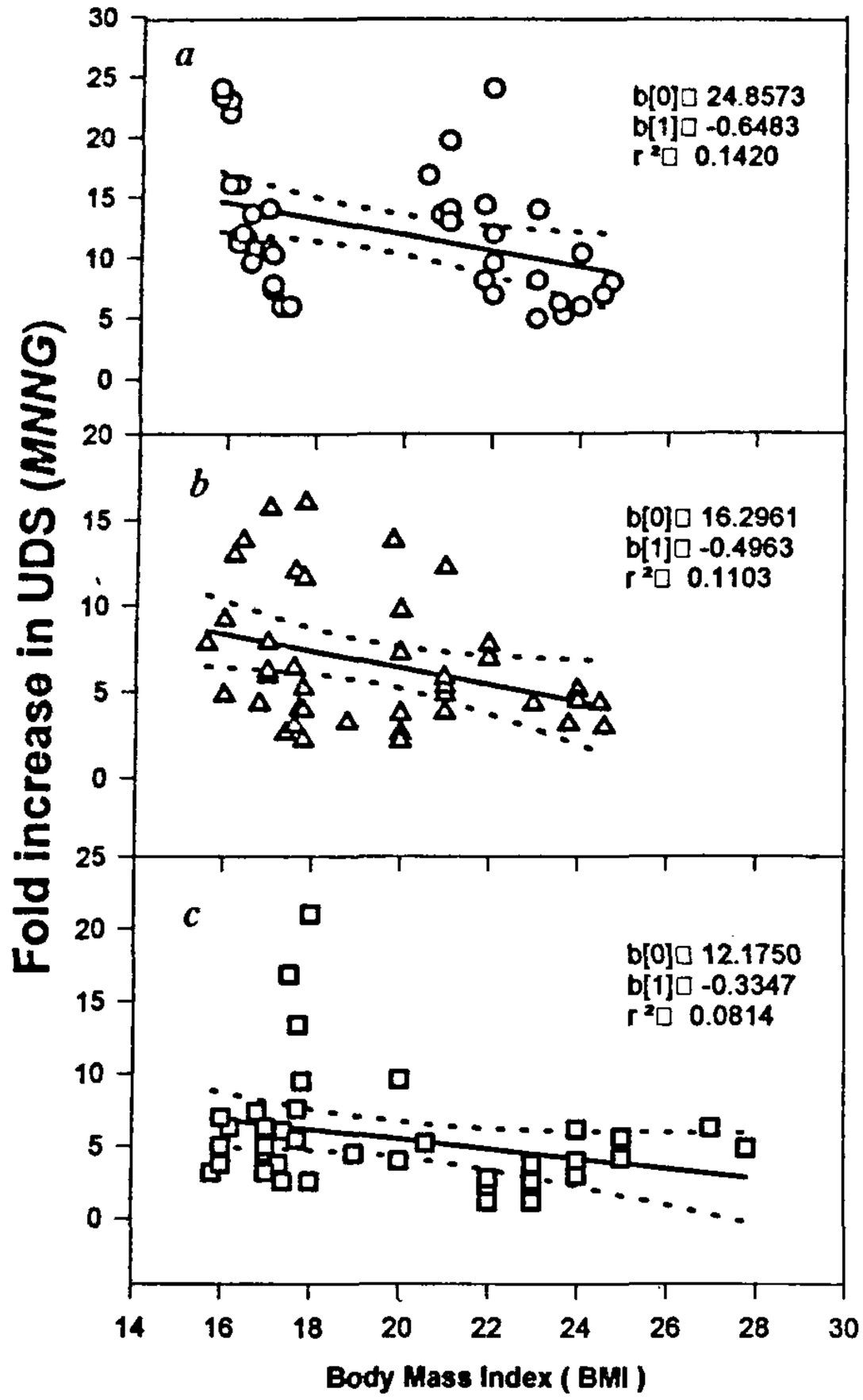

Figure 3. MNNG-induced fold increase in DNA repair in peripheral lymphocytes of human subjects as a function of Body Mass Index at young (*a*), adult (*b*) and old (*c*) ages. Linear regression analyses are shown. Other details are the same as Figure 1.

The average BMI of the subjects in the various age groups are shown in Table 1 *a*. Since the subjects were selected after careful examination of their familial and dietary history (most of these subjects belong to lower to upper middle class society and largely belong to either this university community or old age homes with a steady income behind them) and extensive clinical examination (data shown in Table 1 *b*), the low BMI value is taken to indicate a chronic but natural intake of low calories. Thus this LBMI group is assumed to be similar to an experimentally diet-restricted animal and therefore considered as 'undernourished' without any apparent malnutrition. Lymphocytes from these subjects were examined for various DNA repair parameters under different conditions.

Table 2. Basal and induced UDS in peripheral lymphocytes of human subjects with normal BMI (normal) and low BMI (undernourished) at different ages

Status	Basal repair	Induced repair	
		UV	MNNG
Young:			
Normal BMI	7.3 ± 3.7	83.4 ± 36.2	70.6 ± 33.6
Low BMI	9.2 ± 4.3	123.2 ± 49.9*	106.7 ± 46.0*
Adult:			
Normal BMI	6.5 ± 4.5	43.0 ± 29.9	35.3 ± 22.6
Low BMI	11.2 ± 5.8*	117.4 ± 50.1*	110.6 ± 62.0*
Old:			
Normal BMI	5.3 ± 4.5†	27.6 ± 27.2	21.4 ± 17.6
Low BMI	11.4 ± 10.7*	87.3 ± 78.8*	78.6 ± 76.0*

Values are expressed as femtomoles of [^{3}H]-thymidine incorporated per µg DNA and represent average from 20 individual experiments. Unscheduled DNA synthesis (UDS) is measured as follows: Lymphocytes at a concentration of 10^6/ml in RPMI-1640 medium with 2 mM glutamine, 100 IU/l penicillin, 100 µg/l streptomycin and 10% fetal calf serum, were incubated with and without 5 mM hydroxyurea (HU) and 5 µCi/ml [^{3}H]-thymidine (specific activity – 17Ci/mmol) for 2 h in a CO_2 incubator at 37°C with 90% humidity. The radioactivity incorporated into DNA was measured by standard procedure[44]. The radioactivity in the cells incubated with HU is taken as the measure of UDS (DNA repair). *These values are significantly different from those of corresponding age matched NBMI group at a ρ value of ≤ 0.05. †This value is significantly different from that of NBMI group of 'young' age at a P value of ≤ 0.05.

Lymphocytes from anticoagulated blood of the subjects in the above groups were isolated by Ficoll-paque density gradient centrifugation[24,25] and the unscheduled DNA synthesis (UDS), which is a measure of DNA repair, was examined in the peripheral lymphocytes. For the statistical analysis the Student's t test (paired) was used and the results are shown in Table 2. The basal DNA repair in NBMI subjects is decreased by 27% in old age as compared to 'young'. The difference among the young and adult groups was found to be not significant. In the LBMI group, however, no significant difference was noticed in the basal repair capacity among the three age groups and also the values at adult and old ages were higher than the corresponding age matched normals ($\rho \leq 0.05$). When the lymphocytes were challenged with a mutagenic treatment of exposure to either ultraviolet (UV 254 nm, 20 J/m^2) irradiation or to 50 µM of *N*-methyl-*N'*-nitro-*N*-nitroso-guanidine (MNNG), there was a response in the groups by way of increased UDS. The increased UDS due to this mutagenic treatment is also shown in Table 2. In normal individuals, there is a decrease in this response with age. However in the case of LBMI individuals, the response was always higher as compared to the normal group and this trend was similar in all the three age groups studied. This is to be expected because the LBMI groups already

had a higher level of basal repair and when this is coupled with the better response to mutagenic challenge, the result is a distinctly improved DNA repair capacity in LBMI subjects.

The DNA repair capacity as revealed by UDS is also examined as a function of BMI in each of the age groups by generating computerized regression curves (Sigma plot program, version 2.01), where BMI values are plotted against DNA repair. Figure 1 *a*, *b* and *c* show the regression analyses for 'young', 'adult' and 'old' age groups along with 95% confidence limits. As can be seen, BMI, therefore the nutritional status, has practically no correlation with DNA repair level in young, weak correlation in adult, but significant correlation in old. In line with this, the differences between the average values of low BMI group and normal BMI group at different ages were statistically significant at $P \leq 0.05$ level (Table 2). Similar regression analysis curves are shown for the UV (Figure 2) and MNNG (Figure 3) induced fold increase in DNA repair as a function of BMI at all the three ages. It can be seen that there is always an inverse correlation, albeit to varying degrees, depending upon the age and the mutagen employed, between the BMI and the fold increase in DNA repair. In all these analyses, only the basal DNA repair in 'young' has failed to show any relation with BMI.

These results with human subjects are in good agreement with the findings of earlier workers on experimental animals when dietary restriction was shown to improve the unscheduled DNA synthesis in lymphocytes, hepatocytes and kidney cells in rats[26–28].

DNA repair in mammalian cells is a complex process involving many gene products[29]. The overall process consists of recognition of the damage, excision of the damaged portion, resynthesis of the excised portion and finally ligation of the last nucleotide gap. Several endonucleases/protein factors were identified for the recognition of the damage and incision at the damaged site[29]. During the past several years, we have identified two major endodeoxyribonucleases, one with an acidic pH optimum and the other with alkaline pH optimum, in rat brain. Detailed studies on the properties of these enzymes suggested a role in DNA repair at the initial incision step. The enzyme with acidic pH optimum was able to attack UV irradiated DNA while the second enzyme with alkaline pH optimum could attack a variety of damaged DNAs including apurinic/apyrimidinic DNA (AP-DNA)[30,31]. An endonuclease acting on UV irradiated DNA and an AP endonuclease have been shown in mammalian tissues/cells including brain[32,33]. Similarly DNA-polymerase β, generally considered to be a repair enzyme especially in base excision repair and also shown to be induced against damage to cellular DNA[34,35] is found to be the major polymerase in adult brain[36,37]. We have recently shown that the activity of this enzyme decreases in the aging rat brain possibly due to post-translational modification of the enzyme molecules[38]. In view of this information, we have measured the activities of DNA polymerase β, UV DNase and AP DNase as markers of DNA repair potential in the lymphocytes of the subjects under study.

Table 3. Basal (BA) and UV induced (IA) DNA-polymerase activity in peripheral lymphocytes of subjects with normal BMI and low BMI at different ages

	DNA polymerase activity	
Status	BA	IA
Young:		
Normal BMI	395 ± 167	732 ± 350
Low BMI	424 ± 158	911 ± 386
Adult:		
Normal BMI	204 ± 51	361 ± 109
Low BMI	249 ± 63*	421 ± 113
Old:		
Normal BMI	136 ± 69	240 ± 160
Low BMI	204 ± 69*	362 ± 171*

Values are expressed as picomoles [^{3}H]-TMP incorporated per mg of protein per hour. DNA polymerase assay: The preparation of cell extracts and assay was carried out according to the procedure of Nagasaka and Yoshida[45], which is optimally suited for measuring the DNA-polymerase β activity. Other details are as in Table 2. *These values are significantly higher than those of corresponding NBMI group at a P value of ≤ 0.05.

The activities of DNA polymerase β in lymphocytes with and without UV challenge are shown in Table 3. There is a decrease in the basal activities in both the normal and LBMI subjects, as age progresses, but the enzyme levels are significantly higher in the LBMI when compared to normals in adult and old age groups. Even under induced conditions, the pattern remained the same in that while the age dependent decrease is there in both groups, the values were always higher in LBMI group. However, statistical analysis showed that only in the old age the differences between NBMI and LBMI groups were significant. It can be seen that at old age the values in LBMI group are 50% higher than those in NBMI group. The activity profile observed in cells stimulated with phytohemagglutinin (PHA) and with and without UV challenge was also seen to follow a similar pattern (data not shown). The observed increase in basal DNA repair (Table 2) in the LBMI group at adult and old ages may be attributed to these enhanced activities of β polymerase. It is interesting that Srivastava and Busbee[39] have shown that both α and β polymerases of calorie restricted aged mice exhibit a higher level of fidelity than polymerases of *ad libitum* fed aged mice.

The activities of two DNases, UV DNase and AP DNase, in the lymphocytes of the subjects are shown in Table 4. An-age associated decline in basal levels of UV DNase is seen among the normal subjects. The LBMI

Table 4. Basal (BA) and UV induced (IA) activities of UV DNase and AP DNase in peripheral lymphocytes of subjects with normal BMI and low BMI at different ages

	UV DNase activity/mg DNA		AP DNase activity/mg DNA	
Status	BA	IA	BA	IA
Young:				
Normal BMI	199 ± 37	341 ± 64	246 ± 70	363 ± 70
Low BMI	210 ± 45	368 ± 56	287 ± 66	375 ± 54
Adult:				
Normal BMI	140 ± 35	236 ± 66	208 ± 39	355 ± 115
Low BMI	258 ± 123*	363 ± 159*	253 ± 117	365 ± 167
Old:				
Normal BMI	126 ± 57	160 ± 71	133 ± 65	176 ± 102
Low BMI	222 ± 68*	260 ± 64*	237 ± 55*	276 ± 72*

Values are expressed as μg of DNA-P liberated per mg DNA and are the averages of 20 individual experiments. DNases assay: UV and AP DNases were assayed essentially according to the procedure of Rao and Rao[46]. *Substrate for UV* DNase: UV DNA prepared by UV irradiation of highly polymerized calf thymus DNA (2 mg/ml) at a dose of 2×10^4 J/m^2 using Phillips TUV 8 15 W germicidal lamp. Substrate for AP DNase: Depurinated DNA prepared essentially as described earlier[47]. Native calf thymus DNA (2 mg/ml) mixed with equal volume of depurination buffer (40 mM sodium citrate, 40 mM NaCl, 40 mM potassium phosphate, pH 5.0 incubated at 70°C for 15 min). Other details are as in Table 2. *These values are significantly higher than those of the corresponding NBMI group at a P value of ≤ 0.05.

group at young age showed marginally higher values which were not statistically significant. However, at both adult and old ages, the values were significantly higher in LBMI group as compared to normal. Also the age-dependent decline seen in normal individuals is not evident in the LBMI individuals. A similar pattern of changes is seen even after the lymphocytes are exposed to UV except that the values are higher.

In the case of AP DNase, the pattern of changes in basal activities is similar to UV DNase. UV exposure of the lymphocytes resulted in comparable induction of this activity in both normal and LBMI groups at all ages, but in old age both the basal as well as induced activities in LBMI groups were significantly higher than those in NBMI group. Thus the overall picture seems to be that either the activities are unaffected or higher in LBMI individuals.

Age-related decline in UV-induced DNA repair has been reported in human peripheral lymphocytes[40,41] and also in tissue from the central nervous system[42]. The present results while confirming the age-dependent decline in the activities of DNA repair enzymes also show that human subjects with low BMI presumably facing chronic low calorie consumption exhibit either unaffected or even improved activities with the age-dependent decline being distinctly slower.

This is the first study of its kind on human subjects to demonstrate the beneficial effects of chronic undernutrition, a phenomenon reported earlier in experimental animals[26–28]. Walford and his group are conducting an elaborate experiment to examine the effects of diet on general physiology of humans including aging[43]. These experiments are still continuing. However the present study is planned and executed with a population that is naturally available in India. The results indicate the possibility of a very intriguing and interesting phenomenon that LBMI (moderate undernutrition with no apparent malnutrition) is actually beneficial in human subjects in maintaining good DNA repair capacity. How undernutrition is able to achieve this is a matter of speculation. It is logical to expect that in LBMI individuals the cellular metabolism would be at a low ebb. However, even against this reduced calorie availability, the cell might choose to maintain certain essential pathways at normal rates and DNA repair pathway could be one such pathway in view of its cardinal role in protecting the genomic integrity. This in itself may be a major contributing factor for improved longevity in such human subjects.

1. Tice, R. R. and Setlow, R. B., in *Handbook of the Biology of Aging* (eds Finch, C. E. and Schneider, E. L.), Academic Press, New York, 1986, 2nd ed.
2. Rao, K. S., *Mol. Neurobiol.*, 1993, **7**, 23–48.
3. Bohr, V. A., Evans, M. K. and Albert, J. F. Jr., *Lab. Invest.*, 1989, **61**, 143–161.
4. Hart, R. W. and Setlow, R. B., *Proc. Natl. Acad. Sci. USA*, 1974, **71**, 2169–2173.
5. Hall, K. Y., Hart, R. W., Birnirschke, A. K. and Walford, R. L., *Mech. Ageing Dev.*, 1984, **24**, 163–174.

6. Hart, R. W., Sacher, G. A. and Hoskins, T. L., *J. Gerontol.*, 1976, **34**, 808–817.
7. Paffenholz, V., *Mech. Ageing Dev.*, 1978, **7**, 131–150.
8. Sacher, G. A. and Hart, R. W., *Birth Defects: Original Article Series*, 1978, **14**, 71–96.
9. Licastro, F. and Walford, R. L., *Mech. Ageing Dev.*, 1985, **31**, 171–186.
10. McCay, C. M., Crowell, M. F. and Maynard, L. A., *J. Nutr.*, 1935, **10**, 63–79.
11. Ross, M. H., *J. Nutr.*, 1961, **75**, 197–210.
12. Fernandes, G., Yunis, E. J. and Good, R. A., *Proc. Natl. Acad. Sci. USA*, 1976, **73**, 1279–1283.
13. Masoro, E., in *Molecular Biology of Aging* (eds Blackett, A. D. and Hollander, A.), Plenum Press, 1984.
14. Sarkar, N. H., Fernandes, G., Telag, N. T., Koorides, I. A. and Good, R. A., *Proc. Natl. Acad. Sci. USA*, 1982, **79**, 7758–7762.
15. Pollard, M. and Lucker, P. H., *J. Natl. Cancer Inst.*, 1985, **74**, 1347–1349.
16. Rogers, A. E., Nields, H. M. and Newberne, P. M., *Arch. Toxicol.*, 1987, **10**, 231–242.
17. Ruggeri, B. A., Klurfeld, D. M. and Kritchevsky, D., *Biochim. Biophys. Acta*, 1987, **929**, 239–246.
18. Weindruch, R. and Walford, R., in *The Retardation of Aging and Disease by Dietary Restriction*, Thomas, Springfield, Illinois, 1988.
19. Wei, Q., Matanoski, G. M., Farmer, R. M., Hedayati, M. A. and Grossman, L., *Proc. Natl. Acad. Sci. USA*, 1993, **90**, 1614–1618.
20. Licastro, F., Weindruch, R., Davis, L. J. and Walford, R. L., *Age*, 1988, **11**, 48–52.
21. Weerarchakul, N. and Richardson, A., Meeting Abstract No. 5309, *FASEB J.*, 1988, **2**, 1209.
22. Rao, K. S., Surekha, A., Raji, N. S. and Prapurna, R., *Proc. Nutr. Soc. India*, 1996, vol. 43, in press.
23. *Nutrition News*, National Institute of Nutrition, 1991, **12**, 1–5.
24. Boyum, A., *Scand. J. Immunol.*, 1976, **5**, 9–15.
25. Smith, T. A. D., Neary, D. and Itzakhi, R. F., *Mutat. Res.*, 1987, **184**, 107–112.
26. Licastro, F. and Walford, R. L., *Mech. Ageing Dev.*, 1985, **31**, 171–186.
27. Weraarchakul, N., Strong, R., Gibson Wood, W. and Richardson, A., *Exp. Cell. Res.*, 1989, **181**, 197–204.
28. Lipman, J. M., Turturro, A. and Hart, R. W., *Mech. Ageing Dev.*, 1989, **48**, 135–143.
29. Thompson, L. H., *Environ. Mol. Mutagen.*, 1989, **14**, 264–281.
30. Suvarchala, E. and Rao, K. S., *Mol. Cell. Biochem.*, 1994, **137**, 109–116.
31. Venugopal, J. and Rao, K. S., *Biochem. Arch.*, 1993, **9**, 341–348.
32. Bacchetti, S. and Beene, R., *Biochim. Biophys. Acta*, 1975, **390**, 285–297.
33. Ivanov, V. A., *Biokhimiya*, 1987, **52**, 1133–1137.
34. Singhal, R. K., Prasad, R. and Wilson, R. H., *J. Biol. Chem.*, 1995, **270**, 949–957.
35. Jinno *et al.*, *Mech. Ageing Dev.*, 1995, **85**, 95–107.
36. Waser, J., Hübscher, U., Kuenzle, C. C. and Spadari, S., *Eur. J. Biochem.*, 1979, **97**, 361–368.
37. Subrahmanyam, K. and Rao, K. S., *Biochem. Int.*, 1988, **16**, 1111–1118.
38. Rao, K. S., Vinay Kumar, D., Bhaskar, M. S. and Sripad, G., *Biochem. Mol. Biol. Int.*, 1994, **32**, 287–294.
39. Srivastava, K. and Busbee, D. L., *Biochem. Biophys. Res. Commun.*, 1992, **182**, 712–721.
40. Roth, M., Emmons, L. R., Häner, M., Müller, Hj. and Boyle, J. M., *Exp. Cell Res.*, 1989, **180**, 171–177.
41. Grossman, L. and Wei, Q., in *DNA Repair Mechanisms: Impact on Human Diseases and Cancer* (ed. Jean-Michel, Vos. H.), R. G. Landes Company, 1994, pp. 327–345.
42. Gibson, R. E. and D'Ambrosio, S. M., *Photochem. Photobiol.*, 1982, **35**, 181–185.
43. Walford, R. L., Harris, S. B. and Gunion, M. W., *Proc. Natl. Acad. Sci. USA*, 1994, **89**, 11533–11537.
44. Subrahmanyam, K. and Rao, K. S., *Mech. Aging Dev.*, 1991, **57**, 283–291.
45. Nagasaka, A. and Yoshida, S., *Endocrinology*, 1984, **115**, 1110–1115.
46. Rao, K. V. S. and Rao, K. S., *Biochem. Arch.*, 1982, **2**, 45–52.
47. Sharper, N. L. and Grossman, L., *Methods Enzymol.*, 1980, **65**, 216–224.

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A simple technique to expose tree seedlings to elevated CO_2 for increased initial growth rates

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Initial growth rates of most tree species that are used in afforestation programmes are very low. Therefore, polybag planted seedlings have to be maintained in the nurseries for a long period of time. Growing plants in an elevated CO_2 atmosphere increases the growth rates as well as biomass production in many annual crop and tree species. Higher temperature and relative humidity in association with elevated CO_2 concentration helps to boost the biomass and leaf area production. We demonstrate here an easy and cost-effective method for obtaining elevated CO_2 concentrations for better growth of tree seedlings in the nursery.

APART from maintaining a balanced ecosystem, forests are major sinks of CO_2. Deforestation and burning of fossil fuel (due to population pressure) are the two major reasons for accumulation of CO_2 in the atmosphere leading to global climate change. Currently the CO_2 concentration in the atmosphere is around 360 ppm and it is increasing at the rate of 1.8 ppm per year[1]. Hence afforestation is a practically feasible way to address the global climate change, especially in tropical countries where forest felling is occurring at a faster rate.