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Dietary regulation of adenosine deaminase activity in stomach, small intestine and spleen of mice

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Activity of adenosine deaminase (ADA) and its regulation by dietary restriction were studied in the stomach, small intestine and spleen of mice. ADA activity (U/mg protein) was highest in the stomach, followed by small intestine and spleen of mice on normal diet. The activity decreased significantly in the stomach (41%) and small intestine (45%) of 24 hr fasted mice, when compared to mice fed *ad-libitum*. However, ADA activity in spleen did not show any change by dietary intervention. Refeeding of fasted mice for 24 hr restored the activity of ADA in tissues. In addition, dietary restriction (alternate days of feeding for three months) had a cumulative effect, whereby ADA activity decreased significantly in the stomach (53% on the day of feeding and 60% on the day of fasting) and small intestine (50% and 54% on the day of feeding and fasting, respectively) without any change in activity in spleen. These findings indicate that dietary restriction reduces ADA activity in a tissue-specific manner. Long-term dietary restriction leads to a cumulative adaptation in lowering the ADA activity of GIT, but not in spleen.

Adenosine deaminase (ADA; EC 3.5.4.4) catalyses the irreversible hydrolytic deamination of adenosine and 2'-deoxyadenosine to inosine and 2′-deoxyinosine, respectively[1]. The physiological function of ADA is critical in controlling the effects of these metabolites on immunological[2], neurological and vascular[3] systems. ADA is also involved in the development of B and T lymphocytes as is evident from the fact that ADA-deficient animals suffer from B and T lymphopenia[4-6]. In ADA-deficient animals, high intracellular concentrations of dATP and S-adenosylhomocysteine inhibit ribonucleotide reductase activity and thereby block DNA synthesis. Rapidly proliferating cell types such as lymphocytes are particularly susceptible to diminished DNA synthesis[4]. ADA is distributed in various tissues although its level is particularly high in the gastrointestinal tract (GIT)[1,7]; however, its precise role in these tissues is not yet clear. In adult mice, the highest level of ADA is present in the keratinized squamous epithelium that lines the alimentary canal, where ADA accounts for 20% of all soluble proteins[1,7]. High level of ADA in GIT has also been correlated with the maturation of B and T lymphocytes in the secondary lymphoid organs like epithelium associated lymphoid tissues in the GIT and spleen[7]. Adenosine, a natural substrate of ADA, is known to stimulate Cl^- secretion in intestinal epithelium that results in movement of isotonic fluid into the lumen, and help to hydrate the mucosal surface[8]. Being the first enzyme in the degradation of adenosine, ADA is subjected to regulation by various modulators. A wide range of physiological, hormonal or dietary manipulations[9,10] may alter enzyme levels in mammalian tissues.

Dietary restriction (DR) has been shown to increase longevity and reduce pathology by modulating the immune responses. DR increases longevity by lowering oxidative stress/damage and modulating specific gene expression to produce the beneficial adaptive responses[11,12]. DR also reduces metabolic load on the experimental animals due to reduced energy input leading to altered energy metabolism[11]. The changes in epithelial cell enzyme activities that result from altering caloric intake have served as a model system for adaptive responses in the GIT[13]. However, very little is known on the influence of DR in regulating ADA activity in mice. The present work describes tissue-specific expression of ADA activity and its regulation by short- and long-term dietary intervention in different tissues of mice.

Swiss albino (Balb/ c strain) male mice of 8-10 weeks, purchased from the Pasteur Institute, Shillong and maintained under normal laboratory conditions at 25±2°C were fed with a standard pellet diet (Amrut Laboratory, Pune). All the chemicals used were of analytical grade, and the biochemicals were purchased from Sigma Chemical Co., USA.

Effect of dietary intervention on the activity of ADA in different tissues of mice was studied in two sets of experiments as follows: i) In the first set, the mice were subjected to a defined period of fasting (24 hr) followed by refeeding (24 hr) to observe the influence of diet on the activity of ADA with reference to *ad-libitum* fed control; ii) In the second experiment, the mice were fed on alternate days for a period of three months[19] and ADA activity in different

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tissues of mice was determined. For these studies, one group of animals was sacrificed on the day of feeding and the other on the day of fasting.

Animals were sacrificed by cervical dislocation at a fixed time of the day (13.00 hr). Stomach, small intestine and spleen were dissected out, washed in normal saline (0.9% NaCl) and blotted dry. Homogenates (20% w/v) of these tissues were prepared in ice-cold 100 m*M* sodium citrate buffer, *p*H 6.0 containing 0.25 *M* sucrose, centrifuged at 27,000×*g* for 60 min at 4°C and the supernatants were used for the assay of ADA and protein estimations.

ADA activity was measured spectrophotometrically in a Hitachi Model U-2000 spectrophotometer by the method of Kalchar[14] and Yoshida[15] with certain modifications of our own[16]. The standard assay was carried out at 25°C in 3.0 ml of 100 m*M* sodium citrate buffer, *p*H 6.0 with 100 μ*M* adenosine and 50 μl of suitably diluted enzyme preparation which gave a linear decrease in absorbance at 265 nm. The protein concentration of the enzyme preparation was determined by the method of Bradford[17], using BSA as standard. The activity of ADA was expressed as units (μmol of adenosine deaminated per min) per mg protein. The data were statistically analysed[18] and the level of significance (*p*-values) between different sets of data was calculated according to student's t-test.

The normal endogenous activity of ADA in different tissues of mice was found to vary significantly, the highest being in the stomach, followed by the small intestine and spleen (Fig. 1).

Fasting for 24 hr decreased ADA activity (U/mg protein) in stomach (41%) and small intestine (45%) without any significant influence on the activity of spleen ADA. The refeeding of fasted mice for 24 hr elevated (+45%) the level of ADA activity in those tissues of mice (Fig. 1).

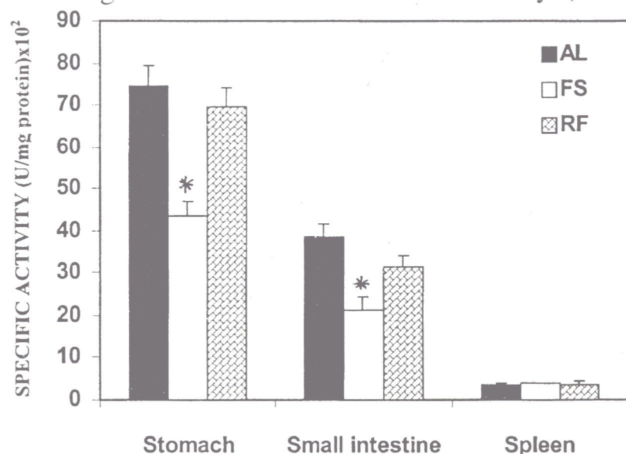

Fig. 1—Effects of fasting and refeeding on ADA activity of mice in different tissues. [AL, *ad libitum*; FS, fasted (24 hr); RF, refed (24 hr). Dietary regimen and other experimental conditions are mentioned in the text. Values are mean of 6-8 mice for each tissue. Bars represent standard deviation. *$p < 0.05$ as compared to *ad libitum* fed mice]

Dietary restriction (DR) by alternate days of feeding for three months also reduced the level of ADA in stomach (53% and 60%) and small intestine (50% and 54%), respectively when mice were sacrificed on the day of feeding and fasting. However, there was no marked change in ADA activity in spleen by the same dietary regimen (Fig. 2). These results suggest that GIT level of ADA is under tonic control of diet while spleen ADA is not under such control. DR led to a cumulative decrease in the level of ADA in GIT, possibly an adaptation to calorie intake in these animals.

Adenosine influences the cardiovascular, central nervous, respiratory, immune systems and the gastrointestinal tract, mediated by cell-surface receptors[20]. Higher level of ADA in the GIT may ensure that dietary sources of adenosine do not exert unwanted physiological effects[1]. Adenosine is known to influence the hydration of GIT mucosal surface by controlling the chloride secretion in intestinal epithelium that results in movement of isotonic fluid into the lumen[8]. The cells in GIT are deficient in the *de novo* synthesis of purines and pyrimidines and thus need to accumulate purines and pyrimidines, which

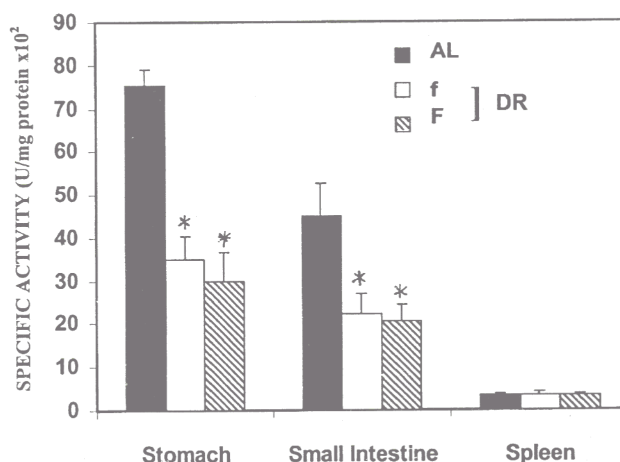

Fig. 2—Effect of dietary restriction (DR) on ADA activity of mice in different tissues. [DR was effected by feeding mice on alternate days for 3 months. AL, *ad libitum* fed; DR, dietary restricted; f, mice sacrificed on the day of feeding; F, mice sacrificed on the day of fasting. Other experimental conditions for tissue preparation and enzyme assay are mentioned in the text. Values are mean obtained from 7-8 mice for each tissue. Bars represent standard deviation. *$p < 0.05$ values as compared to *ad libitum* fed mice]

have either been ingested or produced in other cells. The high ADA of GIT cells metabolizes adenosine by deamination to keep the concentration of adenosine low and thus maintain an inwardly directed concentration gradient[21].

Our data show that fasting for 24 hr decreases ADA activity in stomach and small intestine without having significant influence on the activity of spleen ADA. Interestingly, refeeding of the fasted mice for 24 hr brings back the level of ADA in those tissues. These findings indicate that the GIT ADA is under the control of caloric-intake and corroborate with the biochemical adaptation of GIT enzymes during dietary intervention[13]. In addition, DR (alternate days of feeding for three months) also reduced (50-60%) significantly the level of ADA activity in the same tissues, corroborating results obtained from earlier experiments. Our findings also indicate that long-term dietary restriction leads to a cumulative lowering of ADA activity in the GIT. Lowered activity of ADA in the stomach and small intestine of dietary restricted mice may reflect a biochemical adaptation so that there is no futile synthesis of excess ADA when there is no need for it to metabolise dietary adenosine or alternatively to compensate the changes required for the hydration of GIT during such intervention[8]. Reduced ADA activity may also give rise to excess cellular adenosine, which in turn may be a signal for inadequate ATP levels during low energy situations[22]. Search on the genetic basis of obesity has established a link between ADA gene expression to body fat[23]. Also, the level of ADA is elevated in sumo wrestlers who consume a diet rich in energy[24]. Our findings of lowered ADA activity during dietary restriction corroborate the fact that ADA level could be related to body fat and obesity in mammals. We conclude from the present study that dietary restriction reduces ADA activity selectively in the GIT without affecting the level of ADA activity in spleen, indicating the tissue-specific dietary regulation of ADA in mice.

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