5. SUMMARY

The neuroendocrine system is responsible for preservation and maintenance of the internal homeostasis despite continuous fluctuations in the environment. Age-related neuronal dysfunction causes a decline in cognitive function and other subtle changes within the cortex including alterations in receptors, loss of dendrites, spines and myelin dystrophy, as well as the alterations in synaptic transmission. The effectiveness of homeostatic adjustments by neural and endocrines signals declines with increasing age. Adaptation of an organism to external and/or internal stress depends on control mechanisms orchestrated by the combined interplay of the nervous and endocrine systems. Stressful stimuli activate both the hypothalamo-pituitary-adrenocortical and sympathoadrenal systems which coordinate physiological reactions through a series of neuroendocrine signals which involve various hormones and brain centres. Stress hormones, the glucocorticoids (called corticosterone in animals, and cortisol in humans), and the catecholamines (epinephrine, norepinephrine) in response to a stressor constitute the primary mediators in the chain of hormonal events triggered in response to stress. Aging disrupts the synchrony of these signals, the capacity to restore the original homeostasis is altered and the hypothalamus is unable to undergo the major remodelling of its circuitry necessary for adaptation.

Life exists by maintaining a complex dynamic equilibrium or homeostasis that is constantly challenged by intrinsic or extrinsic adverse forces, the stressors. Certain types of mild stresses such as caloric restriction, may extend lifespan and reduce the risk of diseases, whereas some other types of stresses are clearly detrimental. The term “biomarker of aging” thus implies a parameter that reflects physiologic or functional age; it must show significant age-related changes, be slowed or reversed by treatments that increase longevity (e.g., dietary restriction), and must be measured reliably. Dietary restriction has been shown to improve synaptic functions such as long-term potentiation and learning ability as well as to elongate life span. This is because DR has a neuroprotective property, produces free radical scavenger to counter oxidative stress, an enhanced benefit through hormetic response besides its role in metabolism to prevent the
accumulation of unwanted damaged proteins in the cell by shifting or reprogramming the different physiological processes.

Acetylcholinesterase (AChE, EC 3.1.1.7) is an enzyme working at the neuronal synapse to quench the chemical signal transmitter, acetylcholine (ACh), which is released from the presynaptic nerve terminal in response to an external electrical impulse, called an action potential. AChE has evolved into one of the fastest enzymes because ACh must be degraded rapidly to restore the synapse to the initial phase for receiving the next neural signal transmission. AChE is widely expressed in tissues that receive cholinergic innervations, such as neurons, muscle cells, and cells of the autonomic nervous system and of the immune nervous system. In mammals, AChE is expressed in a variety of tissues including muscle, nerve, white and grey matter of brain, in amniotic fluid, placenta and hematopoietic cells. It is found not only at neuromuscular junctions, but also in cholinergic interneuronal synapses, in some noncholinergic neurons and nonneural cells such as erythrocytes. AChE has been used as a marker for cholinergic function in neural tissue because of its implications in synaptogenesis and its involvement in neurodegeneration in adult tissues.

- In the assessment of endogenous activity level of AChE activity and its protein expression level in different tissues of mice as a function of age, the study revealed a higher AChE activity in the cerebral hemispheres, heart and kidney of 1-month young mice in comparison to the 18-month aged mice. On the contrary, in the spleen a higher level of AChE activity was observed in aged mice than the younger ones. However, in cerebellum and liver no significant changes were observed in AChE activity of young, adult and aged mice.

- Dexamethasone treatment increased AChE activity and its protein expression level in liver, kidney and heart of both young (1-month) and aged (18-month) mice. This increase is dictated in an age-and tissue- specific manner. The significant antagonistic effect of RU486 against the observed effect of dexamethasone in these tissues is also controlled in an age- and tissue- specific manner. However, no significant changes were observed in the cerebral
hemispheres and cerebellum of both the ages studied on either dexamethasone treatment or in a group that received RU486 prior to dexamethasone treatment. These findings suggest that dexamethasone regulates AChE activity and its protein expression level by binding to glucocorticoid receptors and this regulation is based upon the tissue sensitivity and age of the mice.

- Epinephrine treatment increased AChE activity and its protein expression level in liver, spleen and heart of both young (1-month) and aged (18-month) mice. This increase is dictated in an age- and tissue- specific manner. However, no significant changes were observed in cerebral hemispheres, cerebellum and kidney of both the ages studied. The significant antagonistic effect of propranolol (β-adrenergic receptor antagonist) was observed by decreasing the level of AChE activity and protein expression in comparison with the respective age-matched group that received only dexamethasone treatment. In comparison to propranolol, phentolamine (α-adrenergic receptor antagonist) exhibits a lesser and significant antagonistic effect only in the heart. These findings suggest that epinephrine regulates AChE activity and protein expression level in different tissues of mice mostly by binding to its β-adrenergic receptors and the magnitude of this regulation is based on tissue- and age- of the mice.

- Norepinephrine administration to young (1-) and aged (18- month) mice decreased AChE activity in kidney. Similarly, in the heart a decrease in AChE activity was observed in young (1-month) mice and without any change in 18-month aged mice. However, no significant changes were observed in cerebral hemispheres, cerebellum, liver and spleen at both the ages studied upon norepinephrine treatment. A significant increase of AChE in kidney of young and aged mice indicates an antagonistic effect of propranolol in comparison with the age- matched group that received only norepinephrine treatment.

- Our results indicate that dopamine significantly increased the activity of AChE and its protein expression level in liver and kidney of mice and this effect is dictated in an age-specific manner. On the contrary, it was also observed that dopamine produced an inhibitory effect in the spleen of 1-month young mice.
Data indicate that haloperidol antagonized the observed dopamine effect on AChE activity in liver, kidney and spleen of both young (1-) and aged (18- month) mice. The present findings is in support of the following views that dopamine selectively regulates AChE activity by binding to its receptor in mice and the magnitude of this regulation is governed in an age- and tissue-specific manner.

- A decrease in the activity and protein expression level of AChE in the cerebral hemispheres of 1-month-old mice on 24 h fasting and its restoration after 24 h re-feeding suggests that short-term dietary interventions play a role in regulating the activity of this enzyme during maturation process. In the heart of young mice too, a significant decrease was observed on 24 h of fasting which increased upon 24 h of re-feeding. However, no significant changes were observed in cerebellum, liver, kidney and spleen in comparison with the ad libitum fed control either on 24 h fasting and / or 24 h re-feeding. The present study of short-term dietary restriction indicates the level of high synaptic plasticity of neurons present in cerebral hemispheres and heart of young mice and the role that diet plays in the regulation through AChE hydrolysis of ACh.

- The present study reveals that after three months of food restriction, the young (1-month) mice showed a significant decrease in body weight when compared to control ad libitum fed mice. Comparatively, in aged (18- month) mice, irrespective of the day of sacrificing the mice, a lesser degree of body weight reduction is observed with a significant decrease in comparison to the respective age-matched ad libitum control group. This significant decrease in body weight is irrespective of the day of sacrificing the mice i.e. either on the day of feeding and non-feeding day. The present observation of body weight reduction confirms that the animals have a reduced food intake and is of the view that long-term dietary restriction manifested one of its benefits through weight reduction.

- Long-term dietary restriction (3 months of alternate day feeding and non feeding) decreased AChE activity and its protein expression in most tissues studied except cerebellum and spleen. This effect is irrespective of the day of sacrificing the
animals i.e. on the feeding and non-feeding day and is dictated in an age- and tissue- specific manner. These findings indicate that long-term DR has a more pronounced and cumulative effect on AChE, thereby, confirming the benefit of long-term DR as lifestyle AChE inhibitor versus synthetic drugs.

In conclusion, our findings indicate that AChE activity and its protein expression level exhibit a different pattern as a function of age and clearly demonstrate the different neuroendocrine regulations that stress hormones like dexamethasone, epinephrine, norepinephrine and dopamine have on AChE based on tissue- and age- specific manner. Additionally, diet has an important role in controlling the activity of this enzyme and it further support the importance of long-term dietary restriction as potential AChE inhibitors which may pave the way in controlling different AChE- related pathophysiological conditions.