CHAPTER – 9

NEONATAL MELATONIN ANTAGONISM SIGNIFICANTLY DECREASES SERUM LIPID FRACTIONS AND TISSUE LIPIDS BUT INCREASES TISSUE CHOLESTEROL IN YOUNG ADULT RATS.

INTRODUCTION:

Melatonin, a pineal secretary product, not only possess free radical scavenging and antioxidant activities (Reiter, 1991; Reiter *et al.*, 1997; Reiter *et al.*, 1999), but also alters the activities of enzymes that improves the total antioxidative defense capacity of the organism (Pozo *et al.*, 1994; Barlow-Walden *et al.*, 1995; Reiter, 1995; Rodoriguez *et al.*, 1989). Furthermore, melatonin can also reduce the serum levels of triglycerides and cholesterol in mammalian species (Rasmussen *et al.*, 1999; Hoyos *et al.*, 2000; Nishida *et al.*, 2002), and has an inhibitory effect on the uptake of plasma fatty acids for lipogenesis as well as fasting induced lipolysis in the inguinal fat pad perfused *in situ* in normal rats, by a melatonin-receptor mediated mechanism (Sauer *et al.*, 2001). Although melatonin is known to affect body mass, adiposity and energy intake of seasonal mammalian species (Wade and Bartness, 1984; Valtonen *et al.*, 1995; Le Gouic *et al.*, 1996), effects of melatonin on energy metabolism of mammals remain largely unknown.

Ayoama *et al*, (1988) have found that long-term melatonin administration significantly decreased plasma cholesterol and prevented fatty liver in genetically hypercholesterolemic rats. In rats, melatonin prevented hyperlipemia caused by glucocorticoids, administration (Ayoama *et al.*, 1988) or by high cholesterol food feeding (Mori *et al.*, 1989) but did not prevent hypercholesterolemia in old rats (Vaughan *et al.*, 1982).

Pinealectomy in rats has been shown to decrease hepatic and muscle glycogenesis and increase blood pyruvate concentration (Milcu *et al.*, 1971). Some of the recent studies have shown that pinealectomy causes glucose intolerance, insulin resistance and decreased adipose cell responsiveness to insulin (Seraphim *et al.*, 1997; Lima *et al.*, 1998). Biochemically, usage of selective agonist or antagonist is not only important in identifying melatonin receptor subtypes but can also be used as an important tool for identifying specific physiological functions of melatonin, an added advantage over the generalized effects of pinealectomy. Luzindole has been identified as a most specific antagonist for the MT_2 subtype of receptors more than MT_1 (Dawson and Van Den Heuvel, 1998; Zhou *et al.*, 2003). Previous studies from our laboratory on melatonin antagonism using luzindole during the neonatal period decreased tissue lipid contents in the weaning and pubertal period whereas increased the serum lipid fractions at both the ages. It is in this background that the present study has been undertaken to assess the lipid status of adult animals subjected to neonatal hypomelatonemia. To this end rat neonates

have been treated with luzindole from day 1 to day 21 and the serum insulin level and serum lipid fractions along with adipose tissue, muscle and hepatic lipid and cholesterol contents have been assessed on day 60.

MATERIAL AND METHODS: See page numbers 18-38.

RESULTS:

- **Hepatic lipid and cholesterol contents:** The hepatic lipid content decreased marginally while, the hepatic cholesterol content increased significantly in the luzindole treated rats as compared to control rats (Figure and Table; 9.1, 9.2).
- **Muscle lipid and cholesterol contents:** The muscle lipid content decreased significantly whereas, the muscle cholesterol content increased significantly in the experimental animals as compared to control animals (Figure and Table; 9.1, 9.2).
- **Lipid and cholesterol contents in the adipose tissue:** The adipose tissue cholesterol content increased significantly while, the adipose tissue lipid content decreased significantly in the luzindole treated rats as compared to control rats (Figure and Table; 9.3, 9.4).
- **Serum lipid fractions:** The total lipids, triglyceride, cholesterol, and free fatty acid levels decreased significantly in the luzindole treated rats as compared to control rats, while the phospholipid level decreased non significantly (Figure and Table; 9.5).

Figure 9.1: Hepatic and muscle total lipid content in the adult rats on 60th day subjected to neonatal luzindole treatment:

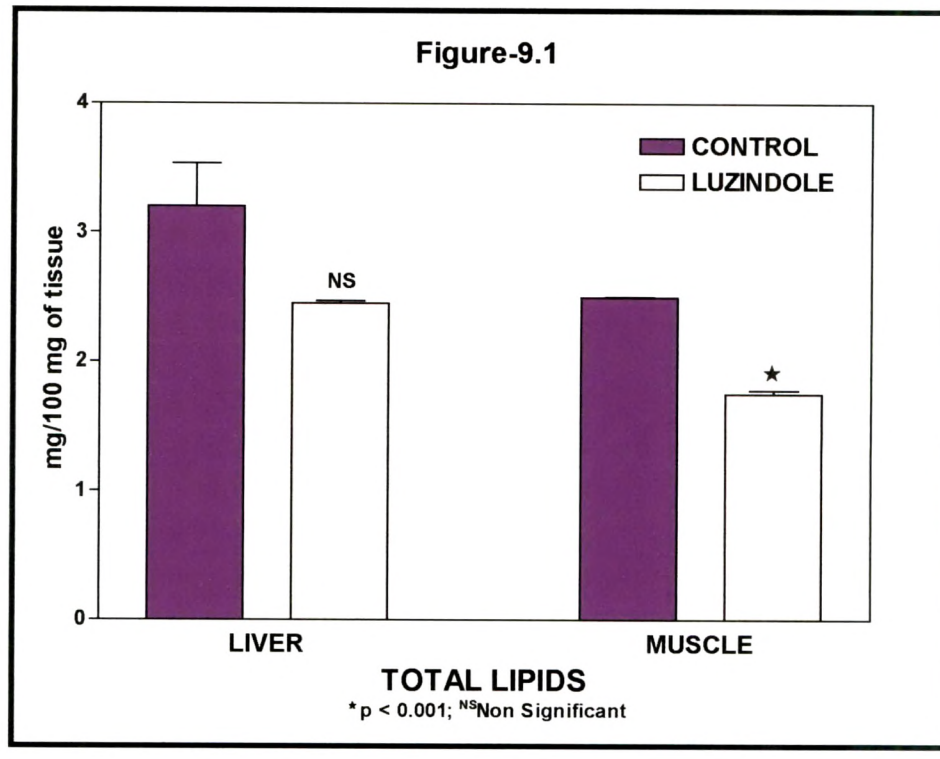

Table 9.1: Hepatic and muscle total lipid content in the adult rats on 60th day subjected to neonatal luzindole treatment:

	CONTROL	LUZINDOLE
LIVER	3.2 ±0.33	2.45[NS] ±0.021
MUSCLE	2.5 ±0.0006	1.75* ±0.031

Values are expressed as mean ± SEM, *p < 0.001; [NS] Non Significant

Figure 9.2: Hepatic and muscle cholesterol content of the adult rats on 60th day subjected to neonatal luzindole treatment:

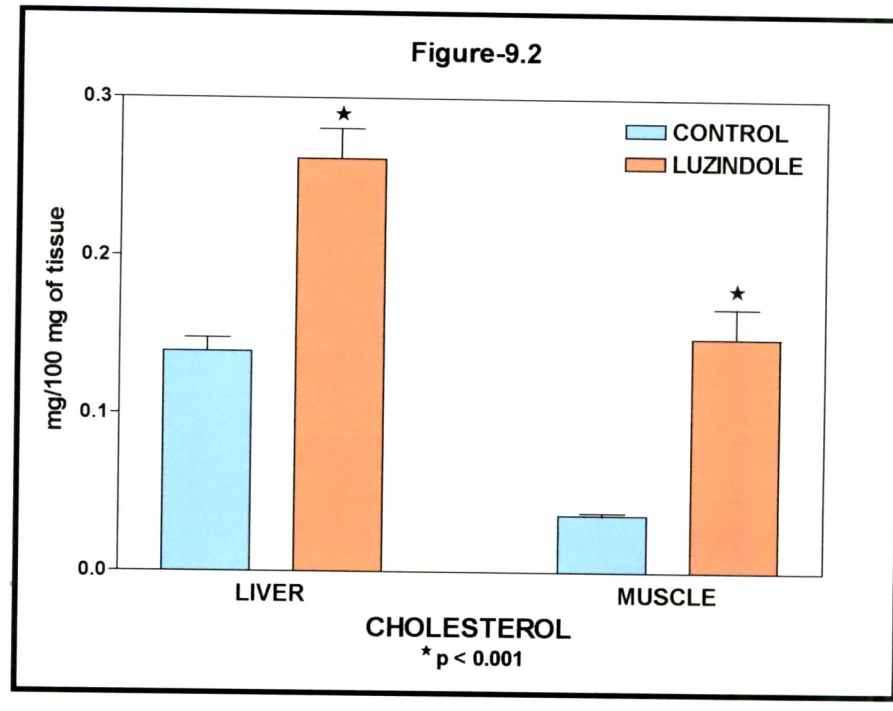

Table 9.2: Hepatic and muscle cholesterol content of the adult rats on 60th day subjected to neonatal luzindole treatment:

	CONTROL	LUZINDOLE
LIVER	0.14 ±0.0084	0.26* ±0.019
MUSCLE	0.036 ±0.0018	0.1486* ±0.021

Values are expressed as mean ± SEM, *p < 0.001

Figure 9.3: Adipose tissue total lipid content in the adult rats on 60th day subjected to neonatal luzindole treatment:

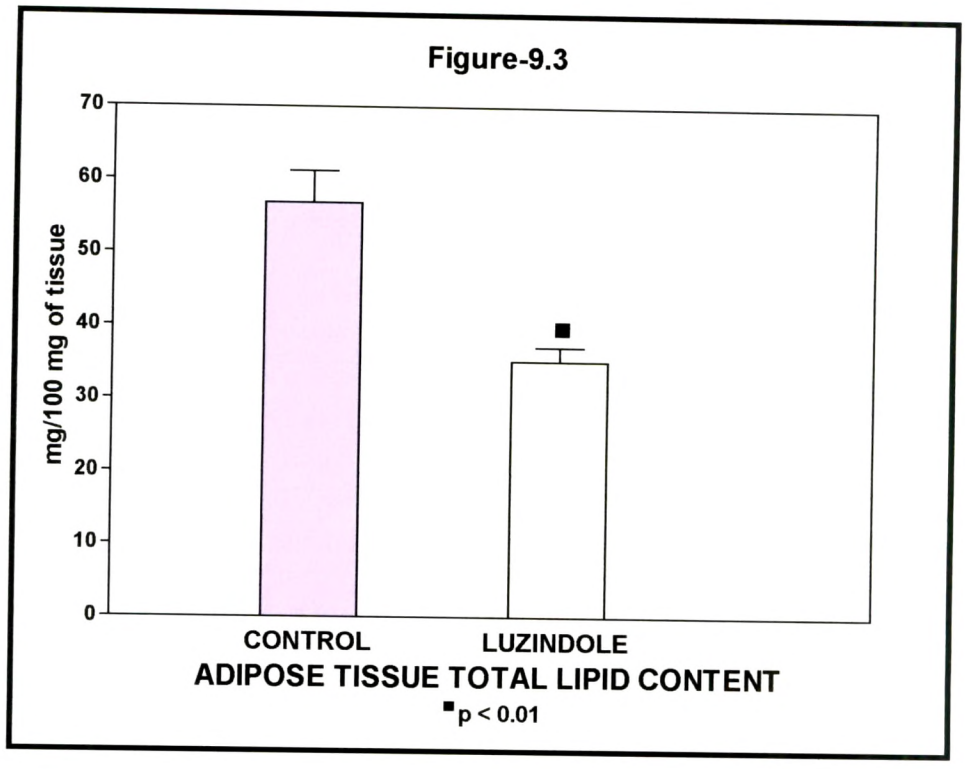

Table 9.3: Adipose tissue total lipid content in the adult rats on 60th day subjected to neonatal luzindole treatment:

	CONTROL	LUZINDOLE
TOTAL LIPID	56.8 ±4.34	35.15■ ±2.02

Values are expressed as mean ± SEM, ■ p < 0.01

Figure 9.4: Cholesterol content in adipose tissue of adult rats on 60th day subjected to neonatal luzindole treatment:

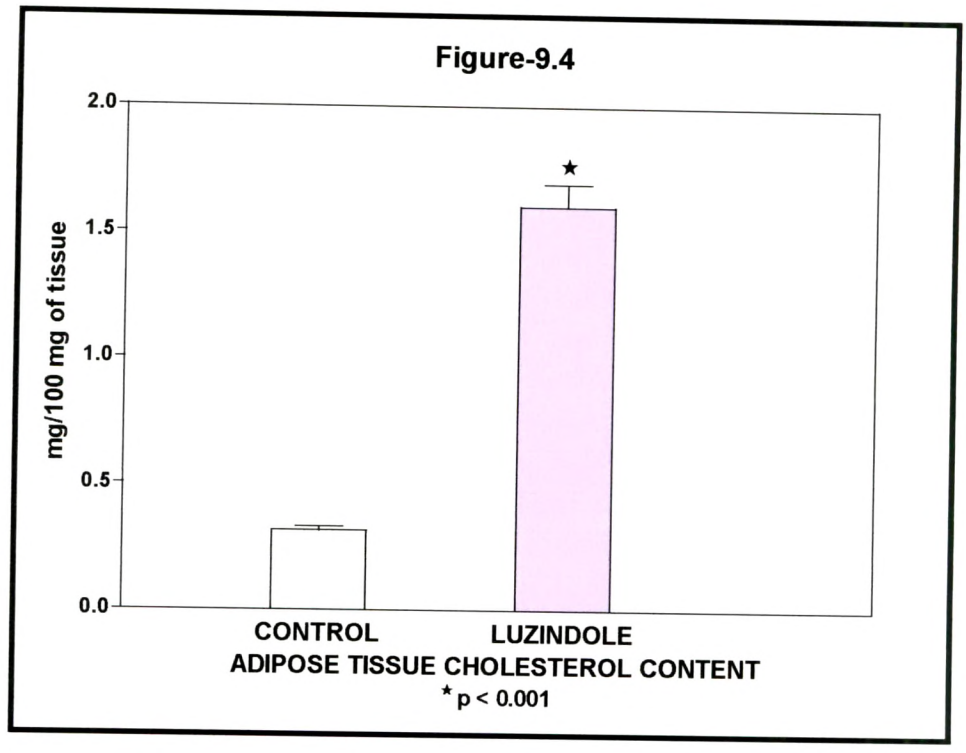

Table 9.4: Cholesterol content in adipose tissue of adult rats on 60th day subjected to neonatal luzindole treatment:

	CONTROL	LUZINDOLE
CHOELSTEROL	0.32 ±0.016	1.60* ±0.090

Values are expressed as mean ± SEM, *p < 0.001

Figure 9.5: Serum lipid fractions of adult rats on 60th day subjected to neonatal luzindole treatment:

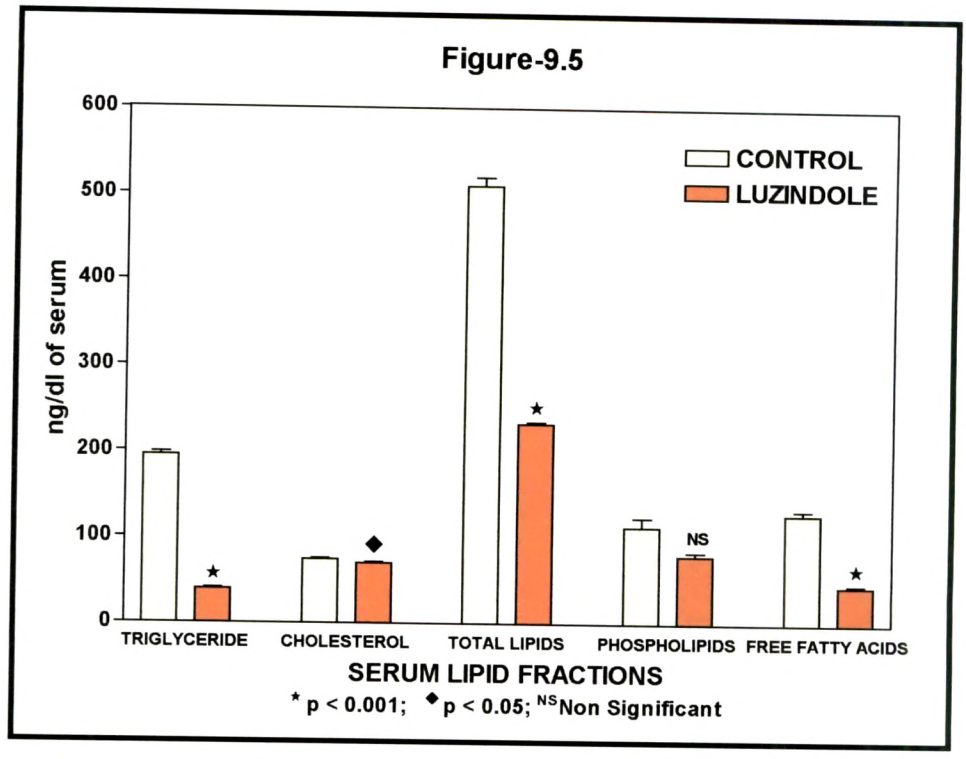

Table 9.5: Serum lipid fractions of adult rats on 60th day subjected to neonatal luzindole treatment:

	CONTROL	LUZINDOLE
TRIGLYCERIDE	194.94 ±3.84	38.88* ±1.65
CHOLESTEROL	74.31 ±1.20	70.13◆ ±1.15
TOTAL LIPIDS	509.75 ±9.78	232.54* ±2.43
PHOSPHOLIPIDS	112.76 ±10.44	79.34NS ±4.11
FREE FATTY ACIDS	127.74 ±4.96	44.19* ±2.13

Values are expressed as mean ± SEM, * p < 0.001; ◆ p < 0.05; NS Non Significant

- **Serum insulin level:** The luzindole treated rats showed a significant decrease in the serum insulin level as compared to control rats (Chapter, 7; Figure and Table; 7.6).

DISCUSSION:

Increase in circulating free fatty acids and fat pad mass have been reported to occur progressively from weaning rats as manifestations of age related increase in peripheral insulin resistance (Pagliassotti *et al.*, 2000). This has been substantiated by the observations of fat accumulation occurring during and after puberty (Banarjee *et al.*, 1997). In the present study involving Charles foster strain of rats, no such increase in insulin resistance or fat load has been seen in control rats from weaning through puberty to young age (see Chapters 3 & 6). Since all the studies have employed Sprague-Dawley strain of rats, the discrepancy in the observations in terms of strain difference in terms of occurrence of insulin resistance and fat accumulation is inferable.

The control rats in the present study has shown a higher adipose tissue, muscle and hepatic lipid contents (120-25/300 days, see Pagliassotti *et al.*, 2000) these changes in tissue lipid contents of control rats are in contrast and can be accounted for the strain difference. These changes in tissue lipid contents of control rats are in contrast to those seen in rats subjected to melatonin antagonism wherein, there was a significant low tissue lipid content in the weaning period, which increased to control levels in the pubertal period (Chapters 3 & 6). This difference was related to an increased lipogenic influence in the pubertal period consequent to an anti-lipogenic phase

in the weaning period due to neonatal blockage of melatonin action (Chapter 6). It was inferred in the above study that an optimal insulin level, as well as, melatonin action are needed to increase lipid contents by induction of lipogenic enzymes. Attainment of increased tissue lipid contents in the pubertal period of experimental rats as against such an attainment in the weaning stage itself in control rats, was suggested to be due to a delayed expression of optimal insulin-melatonin interaction/synergism (Chapter 6). Previously it was seen that, potential for tissue glucose uptake was very high in the weaning, which decreased significantly in the pubertal period and again increased in the young adults as a consequence of neonatal melatonin antagonism (Chapter 2, 5 & 8). Obviously there is an increased potential for tissue glucose uptake from puberty to young adult stage but there is no increase in tissue glycogen contents (Chapters 5 & 7). Since the tissue glycogen contents were significantly lower due to greater glycogenolysis and unchanged higher tissue lipid stores from pubertal to young adult stage of rats subjected to neonatal melatonin antagonism (Chapter 6 and present observations), it is presumable that there is increased glucose/carbohydrate oxidation sparing lipids. A steady state of tissue lipids suggests balanced turn over with no net synthesis. Melatonin is known to decrease fat pads and more specifically visceral fat deposits (Rasmussen *et al.*, 1999; Wolden-Hauson *et al.*, 2000) and as in the present study, decreased tissue lipid contents are recorded in rats subjected to neonatal melatonin antagonism, it is likely that there is potentiated melatonin action in

these animals as long term consequence of neonatal blockade of melatonin action. As against the tissue lipid contents, the tissue cholesterol contents are significantly increased with concomitant decrease in serum cholesterol level suggesting increasing conversion of serum cholesterol into tissue cholesterol esters in the experimental rats. Possibility of decreased cholesterol utilization as a long term impact of neonatal melatonin antagonism is another metabolic feature worth investigating. The previously inferred potentiated melatonin action in the experimental rats as long term effect of neonatal blockade of melatonin action gains validity, from the reported ability of exogenous melatonin to decrease serum cholesterol level and augment tissue cholesterol esterification (Esquifino *et al.*, 1997). Increased tissue lipid contents and serum lipid fractions in the control rats are indicative of age associated increase in lipids/adiposity leading towards the reported insulin resistance in literature. But the significantly decreased tissue lipids and serum lipid fractions in the age matched experimental rats, is suggestive of the long term protective influence of neonatal blockade of melatonin action. Whether this long term consequential effect is due to potentiated melatonin action by increased receptor sensitivity or, due to an increase in the number of melatonin receptors, is a topic of relevance for future investigations.

SUMMARY:

Previous studies on melatonin antagonism using luzindole during the neonatal period showed decreased tissue lipid contents in the weaning

and pubertal periods and increased serum lipid fractions at both the ages. It is in this background that the present study has been undertaken to assess the lipid status of adult animals subjected to neonatal hypomelatonemia. To this end, rat neonates have been treated with Luzindole (An MT_2 receptor blocker) (400 µg/Kg body weight) intra peritoneally from day 1 to day 21 and assessed on the 60^{th} day. The hepatic total lipid content decreased marginally in the luzindole treated rats whereas, the muscle and adipose tissue total lipid content decreased significantly. The hepatic, muscle and adipose tissue cholesterol contents increased significantly in the experimental rats. The serum total lipid, cholesterol, triglyceride, free fatty acid and insulin levels decreased significantly in the luzindole treated rats. The significantly decreased tissue lipids and serum lipid fractions in the age matched experimental rats is suggestive of the long term protective influence of neonatal blockade of melatonin action. Whether this long term consequential effect is due to potentiated melatonin action by increased receptor sensitivity or due to an increase in the number of melatonin receptors is a topic of relevance for future investigations.