CHAPTER – 6

NEONATAL MELATONIN ANTAGONISM IN THE PRE-WEANING PERIOD FAVORS LIPID SYNTHESIS FROM THE WEANING TO PUBERTAL PERIOD.

INTRODUCTION:

Recognition of the fact that different types of melatonin receptors have been localized in the plasma membrane, in the nucleus and even in intracellularly even in neonates, suggests involvement of melatonin in various physiological functions (Dubocovich, 1988; Becker-Andre *et al.*, 1994). The modulatory influence of melatonin on carbohydrate metabolism has been indicated by the many studies involving melatonin administration or pinealectomy (Bailey *et al.*, 1974; Milcu *et al.*, 1978; Diaz and Blazquez, 1986; Iizuka, 1996; Lima *et al.*, 2002; Markova *et al.*, 2003; Jani, 2004; Chapter 1). In contrast, lipid metabolism in relation to melatonin has received only scant attention (Fabis *et al.*, 2002; Mustonen *et al.*, 2002; Markova *et al.*, 2003). An earlier study had reported serum and tissue lipid lowering effect due to administration of pineal extract and the ability of pinealectomy to reverse the same (Esquifino *et al.*, 1997). A definite cholesterol lowering effect has been recognizable as pineal extracts could decrease serum and biliary cholesterol along with serum phospholipids

and, pinealectomy, increased serum cholesterol, total lipids and free fatty acids in the blood (Dhar *et al.*, 1983). Long term administration of melatonin has been shown to decrease plasma cholesterol level as well as prevents fatty liver (Aoyama *et al.*, 1988). Studies from this laboratory on neonatal hypermelatonemia during the entire pre-weaning period have shown decreased tissue lipid and cholesterol contents and increased serum lipid fractions (Jani, 2004). In the above study, these changes in lipid parameters were concurrently paralled by increased glycogenic effect with greater insulin sensitivity suggesting preferential lipid utilization and, conversion of mother's lipid rich milk into carbohydrate reserve: due to neonatal hypermelatonemia. A continuation of the above study, to assess the long term consequence of neonatal hypermelatonemia on pubertal metabolic features had revealed increased tissue lipid contents with increased insulin level compared to the weaning period, indicating a delayed lipogenic influence compared to control animals. Even melatonin antagonism during the neonatal period, decreased tissue lipid contents in the weaning period, suggesting a similar reversed metabolic status coupled to lipid utilization and greater glycogenesis as in hypermelatonemic rats. It is in this background, that the present study has been undertaken to assess the lipid status of pubertal animals subjected to neonatal hypomelatonemia as in these animals increased carbohydrate utilization has been recorded (Chapter 3).

MATERIAL AND METHODS: See page numbers 18-38.

RESULTS:

- **Hepatic lipid and cholesterol contents:** The luzindole treated rats showed a significant increase in the hepatic total lipid content, whereas the hepatic cholesterol content decreased significantly in the luzindole treated rats as compared to the control rats (Figure and Table; 6.1, 6.2).
- **Muscle lipid and cholesterol contents:** The muscle total lipid and cholesterol contents showed no significant alterations as compared to the controls (Figure and Table; 6.1, 6.2).
- **Lipid and cholesterol content in adipose tissue:** The adipose tissue total lipid content decreased significantly in the luzindole treated rats, while the adipose tissue cholesterol content showed no significant alteration as compared to controls (Figure and Table; 6.3, 6.4).
- **Serum lipid fractions:** The luzindole treated rats showed an significant increase in the levels of serum cholesterol and free fatty acids whereas, the serum triglyceride levels decreased significantly in the experimental rats as compared to control rats. The serum total lipid and phospholipid levels remained unaltered in the experimental rats as compared to the control (Figure and Table; 6.5).
- **Serum insulin level:** The serum insulin levels increased significantly in the luzindole treated rats as compared to the control rats (Chapter, 4; Figure and Table; 4.6).

Figure 6.1: Hepatic and muscle total lipid content in the pubertal rats on 45th day subjected to neonatal luzindole treatment:

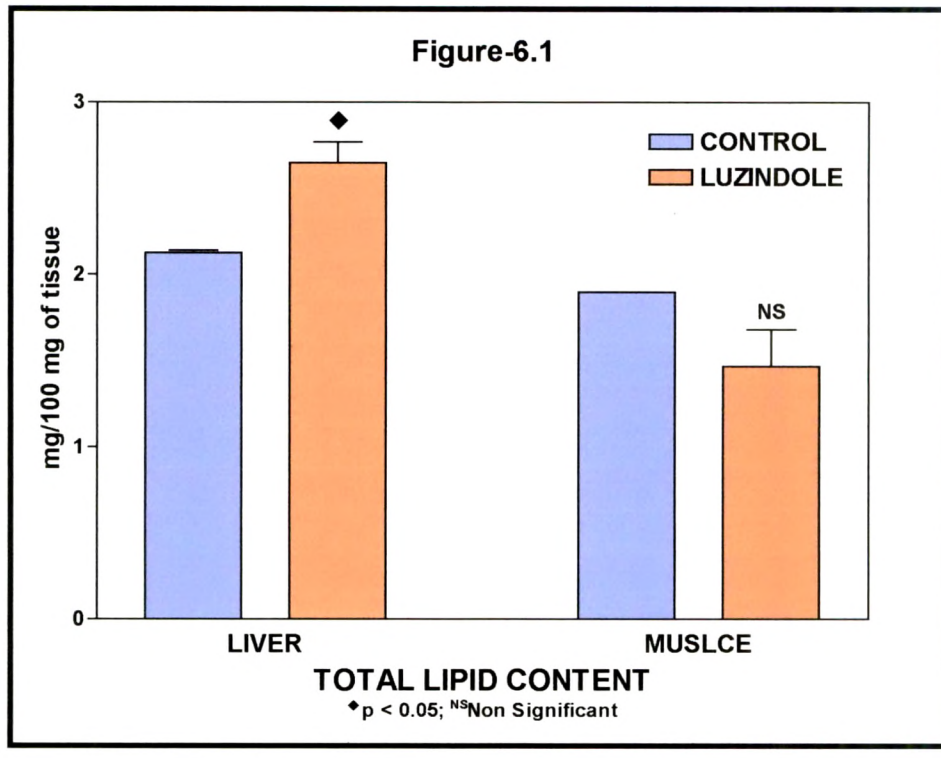

Table 6.1: Hepatic and muscle total lipid content in the pubertal rats on 45th day subjected to neonatal luzindole treatment:

	CONTROL	LUZINDOLE
LIVER	2.125 ±0.017	2.65◆ ±0.1190
MUSCLE	1.9 ±0.00004	1.47NS ±0.2136

Values are expressed as mean ± SEM, ◆p < 0.01; NS Non Significant

Figure 6.2: Hepatic and muscle cholesterol content of the pubertal rats on 45th day subjected to neonatal luzindole treatment:

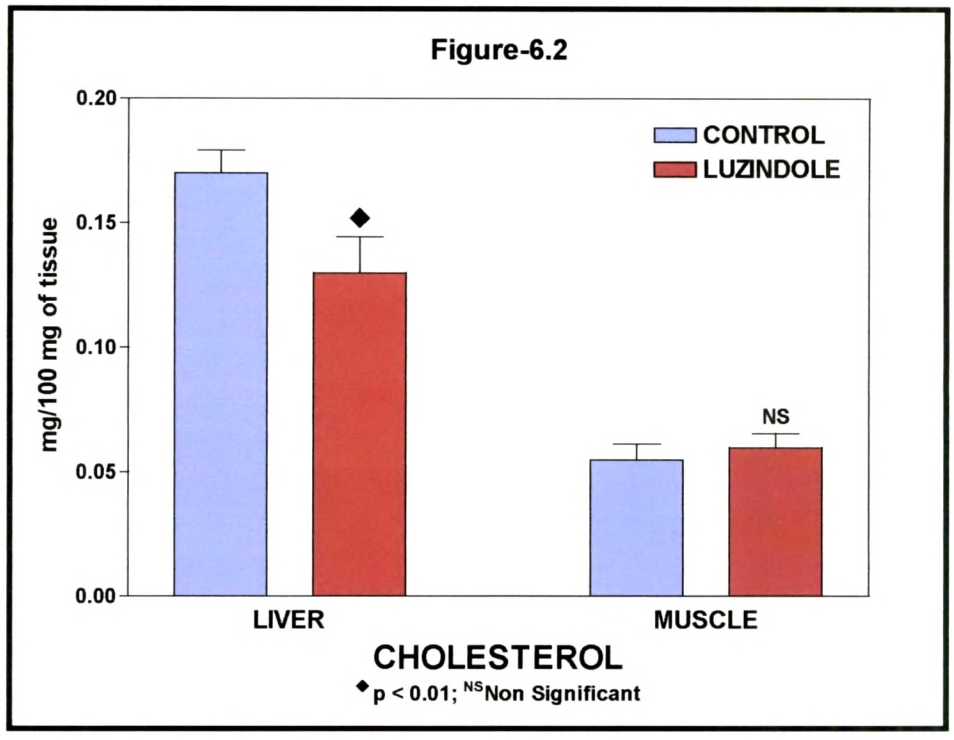

Table 6.2: Hepatic and muscle cholesterol content of the pubertal rats on 45th day subjected to neonatal luzindole treatment:

	CONTROL	LUZINDOLE
LIVER	0.17 ±0.0091	0.13◆ ±0.01435
MUSCLE	0.055 ±0.00645	0.06NS ±0.0057

Values are expressed as mean ± SEM, ◆p < 0.01; NS Non Significant

Figure 6.3: Adipose tissue total lipid content in the pubertal rats on 45th day subjected to neonatal luzindole treatment:

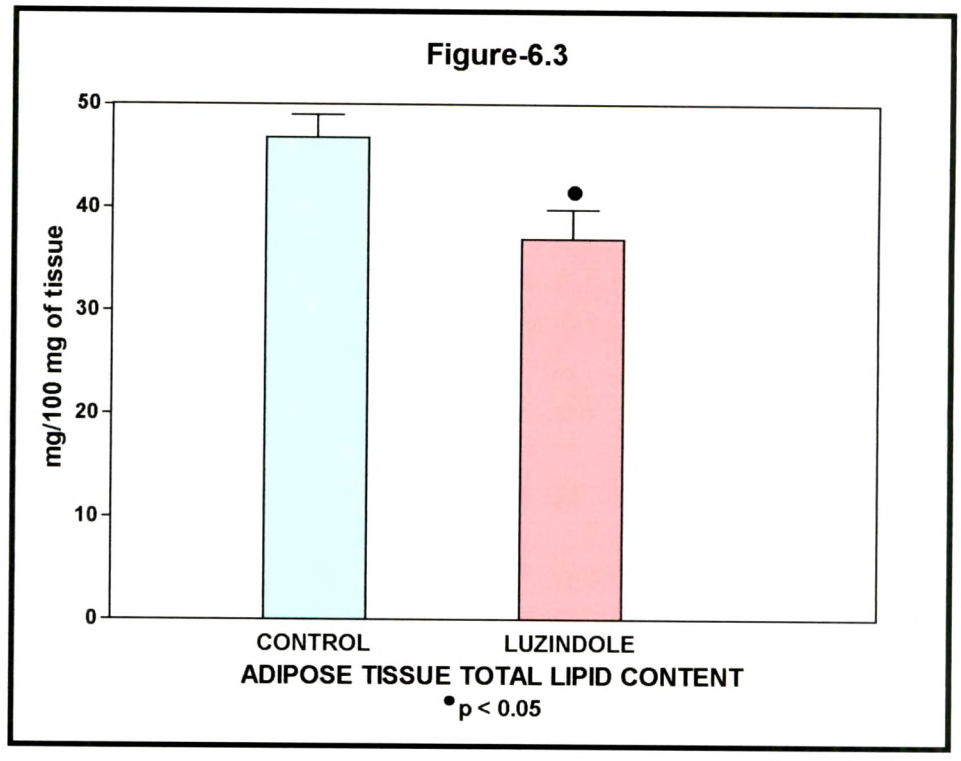

Table 6.3: Adipose tissue total lipid content in the pubertal rats on 45th day subjected to neonatal luzindole treatment:

	CONTROL	LUZINDOLE
TOTAL LIPID	46.8 ±2.218	37.00• ±2.7967

Values are expressed as mean ± SEM, •p < 0.05;

Figure 6.4: Cholesterol content in adipose tissue of pubertal rats on 45th day subjected to neonatal luzindole treatment

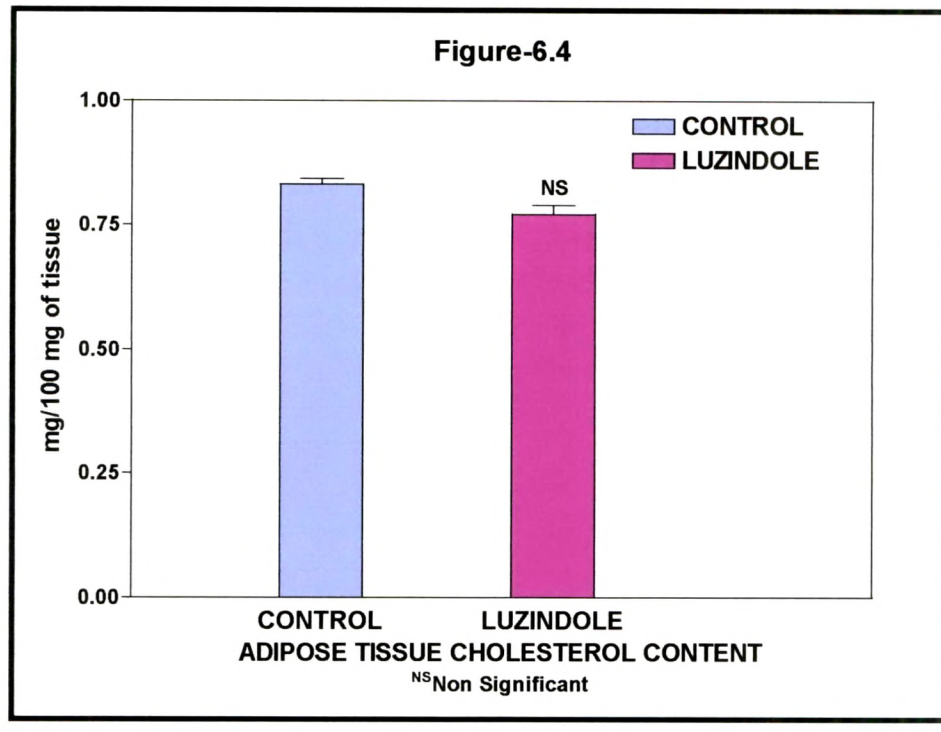

Table 6.4: Cholesterol content in adipose tissue of pubertal rats on 45th day subjected to neonatal luzindole treatment:

	CONTROL	LUZINDOLE
CHOELSTEROL	0.832 ±0.01105	0.772 ±0.0179

Values are expressed as mean ± SEM, NS Non Significant

Figure 6.5: Serum lipid fractions of pubertal rats on 45th day subjected to neonatal luzindole treatment:

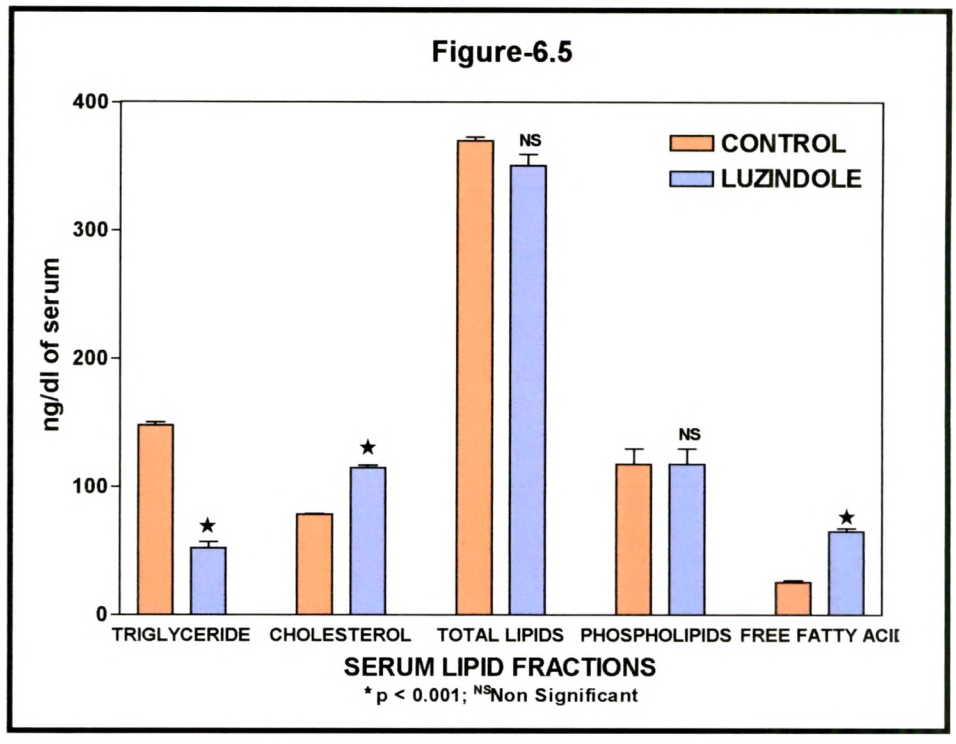

Table 6.5: Serum lipid fractions of pubertal rats on 45th day subjected to neonatal luzindole treatment:

	CONTROL	LUZINDOLE
TRIGLYCERIDE	148.187 ±2.3246	52.31* ±4.622
CHOLESTEROL	78.492 ±0.5883	115.06* ±1.7956
TOTAL LIPIDS	369.98 ±2.6205	351.12NS ±8.3640
PHOSPHOLIPIDS	117.97 ±12.0595	118.08NS ±11.49
FREE FATTY ACIDS	25.34 ±1.16	65.67* ±2.31

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

DISCUSSION:

The present results clearly show that there is a lipogenic influence in the pubertal period consequent to an antilipogenic phase in the weaning period due to neonatal blockage of melatonin action. This is clearly manifested by the significantly increased tissue lipid contents compared to the weaning period and more comparable with the tissue lipid contents of control animals. The significant decrease in serum triglyceride content supports the lipogenic status. Since the lipogenic influence is delayed in hypomelatonemic rats compared to control rats, it is presumable that induction of lipogenic enzymes requires an optimal melatonin action. A similar delay observed even in hypermelatonemic rats (Jani, 2004) was inferred to be due to melatonin receptor down regulation as well as the hypoinsulinemic status (Chapter 3). The significantly higher insulin level in the control weanings compared to hypermelatonemic weanings was also taken to suggest the requirement for an optimal insulin level for induction of lipogenic enzymes (Jani, 2004). However, luzindole treated rats had significantly higher insulin level in the weaning period but there was no lipogenic effect. Apparently, induction of lipogenic enzymes during postnatal development requires not only an optimal insulin level but also an optimal background of melatonin action. Obviously, a synergistic influence of melatonin and insulin can be presumed to be of significance in the induction of lipogenic enzymes and tissue lipid deposition in the postnatal period. Most of the recorded effects of pineal and melatonin on lipid metabolism involve different

treatment/experimental regimes and that too on pubertal or adult animals and hence, are not comparable with the present studies (Fabis *et al.*, 2002; Mustonen *et al.*, 2002; Markova *et al.*, 2003). The present study together with our previous studies (Chapter, 3) are the only studies conducted in the neonatal period with the objective of understanding the impact of altered melatonin status in the critical pre-weaning period as an immediate as well as a long term basis. A novel inference that is emerging from these studies is the need for an optimal melatonin-insulin interaction/synergism in induction of lipogenesis and build up of tissue lipid stores.

The increased serum cholesterol level recorded herein is a clear persisting manifestation of the hypercholesterolemic effect of absence of melatonin, in the wake of the known hypocholesterolemic effect of melatonin (Aoyama *et al.*, 1988; Mori *et al.*, 1989). The higher serum free fatty acids seen in the experimental animals could not only serve to meet the energy needs but also serve as precursors for hepatic and muscle lipid synthesis. Overall, the present study clearly shows that, neonatal blockage of melatonin action could delay the process of lipogenesis due to the absence of an optimal melatonin-insulin interaction and that, this interaction is significant in the postnatal periods for effective development of metabolic homeostasis.

SUMMARY:

Melatonin administration during the pre-weaning period decreased tissue lipid contents in the weaning period. It is in this background that the present study has been undertaken to assess the lipid status of

pubertal rats subjected to neonatal melatonin blockage. 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 45^{th} day. The hepatic lipid content in the luzindole treated rats increased significantly while, the hepatic cholesterol content decreased significantly. The muscle lipid and cholesterol contents of the experimental rats showed no significant alteration as compared to controls. The adipose tissue total lipid content decreased significantly whereas, the adipose tissue cholesterol content in the experimental rats showed no significant alteration as compared to controls. The serum cholesterol and free fatty acid levels increased significantly in the experimental rats. The serum total lipid and phospholipid level showed no significant alteration in the luzindole treated rats as compared to control rats. While the serum triglyceride level decreased significantly in the luzindole treated rats, the serum insulin levels increased significantly. The present study clearly shows that, neonatal blockage of melatonin action could delay the process of lipogenesis due to the absence of an optimal melatonin-insulin interaction and that, this interaction is significant in the postnatal periods for effective development of metabolic homeostasis.