CHAPTER – 3

DIFFERENTIAL EFFECTS OF LUZINDOLE (MT2 RECEPTOR BLOCKER) ON TISSUE LIPID AND CHOLESTEROL CONTENTS AND SERUM LIPID PARAMETERS IN RAT NEONATES.

INTRODUCTION:

The involvement of the pineal hormone melatonin in the control of circadian and other physiological functions in adult mammals is known to be modulated by the circadian rhythmicity of the melatonin secretion. The development of circadian rhythm and the entrainment of these rhythms with the ambient photoperiod are of significance. In this respect the entrainment of neonatal and fetal rhythms could be of great relevance in the regulation of fetal and neonatal physiology. The suprachiasmatic nucleus is known to be intrinsically rhythmic in the fetal rat (Reppert & Weaver, 1991) but direct entrainment of the SCN by light does not develop until the sixth post natal day (Eart *et al.*, 1985). Apparently, the maternal control in establishing appropriate phase relationship between the fetal suprachiasmatic nucleus and the environment and, reinforcing the same in the neonate until photoperiodic information can directly entrain the circadian rhythms, would be more meaningful (Moore, 1991). Many developmental studies

have shown that synthesis and secretion of melatonin by the pineal gland of rat pups does not appear until the second postnatal week (Tang and Pang,1988; Blazquez *et al.*, 1989; Stehle *et al.*, 1995; Laakso *et al.*,1996) Robust rhythm of melatonin in the maternal circulation is sustained throughout pregnancy and, placental transfer of melatonin to the fetus has been demonstrated in mammals (Klein, 1972; Reppert *et al.*, 1978; Kennaway *et al.*,1981; Weaver *et al.*, 1988; Zemdegs *et al.*, 1988; Velazquez *et al.*,1992). Other than maternal melatonin even other hormonal or behavioral signals are considered possible in establishing maternal-fetal circadian rhythm by synchronization (Reppert and Schwartz, 1986; Viswanathan *et al.*, 1994; Weaver and Reppert, 1989). Postnatal transfer of melatonin via milk to the neonates has been proposed as a maternal entraining signal (Ilverova *et al* 1993), but a recent study has shown the transfer of melatonin via milk may be unlikely to provide an entraining signal for rat pups (Rowe & Kennaway, 2002). Effects of melatonin on various physiological functions in neonates can be considered feasible as various types of melatonin receptor have been localized in the plasma membrane and nucleus and even intracellularly (Dubocovich, 1988; Becker-Andre' *et al.*, 1994). The role of melatonin in glucose and carbohydrate metabolism has been studied by a number of workers by either melatonin administration or by pinealectomy (Bailey *et al.*, 1974; Milcu *et al.*, 1958, Diaz and Blazquez, 1986; Iizuka, 1996; Lima *et al.*, 1998; Van-Cauter, 1998; Fleur *et al.*, 2001; La –Fleur *et al.*, 2001; Fabis *et al.*, 2002; Markova *et al.*, 2003; Jani, 2004; Chapter 1).

Relatively fewer studies have been carried out in relation to melatonin and lipid metabolism (Fabis *et al.*, 2002; Mustonen *et al.*, 2002; Markova *et al.*, 2003). Administration of pineal extracts was shown to lower serum, hepatic, adrenal and testicular cholesterol levels, whereas pinealectomy could reverse these effects (Esquifino *et al.*, 1997). Pineal extracts have also been shown to decrease serum and biliary cholesterol and, serum phospholipids while, the absence of pineal gland has been shown to increase cholesterolemia as well as concentration of 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 and prevent fatty liver (Aoyama *et al.*, 1988). A parallel study on neonatal hypermelatonemia has been shown to decrease tissue lipid and cholesterol contents, and increase serum lipid and cholesterol contents and increase serum lipid functions (Jani, 2004). In the present study, the effect of blockage of melatonin action by a receptor antagonist (luzindole) has been evaluated in terms of tissue and serum lipid parameters.

MATERIAL AND METHODS: See page numbers 18-38.

RESULTS:

- **Hepatic lipid and cholesterol contents:** The lipid content in the liver of luzindole treated weanings showed a significant decrease as compared to control weanings. The liver cholesterol content of the experimental animals showed significantl decrease (Figure and Table; 3.1, 3.2).

- **Muscle lipid and cholesterol contents:** The muscle of experimental animals showed a significant decrease in lipid content as compared to that of the control animals. There is no alteration in the cholesterol content in the muscle of experimental neonates as compared to control neonates (Figure and Table; 3.1, 3.2).
- **Adipose tissue content of lipid and cholesterol:** The total lipid content of the adipose tissue showed a significant decrease in the luzindole treated rat neonates as compared to control neonates. The cholesterol content in the adipose tissue of both control and experimental animals did not show any variation (Figure and Table; 3.3, 3.4).
- **Serum lipid fractions:** The level of phospholipids and free fatty acids in the serum of luzindole treated rat neonates decreased significantly as compared to control neonates. Serum total lipids and cholesterol levels showed no significant alteration while the, serum triglyceride levels increased significantly in the experimental animals as compared to controls (Figure and Table; 3.5).
- **Serum insulin levels:** Luzindole treated rat neonates had significantly increased insulin levels as compared to controls (Chapter 1, Figure and Table; 1.5).

Figure 3.1: Hepatic and muscle total lipid content in the weaning rats on 22nd day subjected to neonatal luzindole treatment:

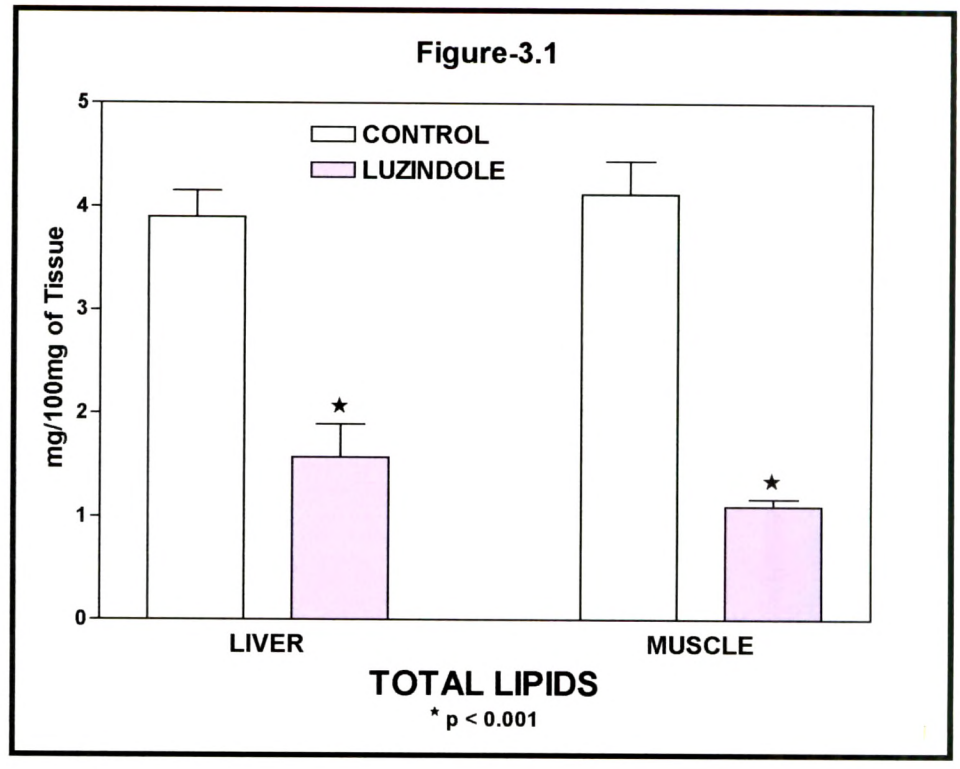

Table 3.1: Hepatic and muscle total lipid content in the weaning rats on 22nd day subjected to neonatal luzindole treatment:

	CONTROL	LUZINDOLE
LIVER	3.9 ±0.25	1.575* ±0.32
MUSCLE	4.12 ±0.33	1.1* ±0.07

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

Figure 3.2: Hepatic and muscle cholesterol content of the weaning rats on 22nd day subjected to neonatal luzindole treatment:

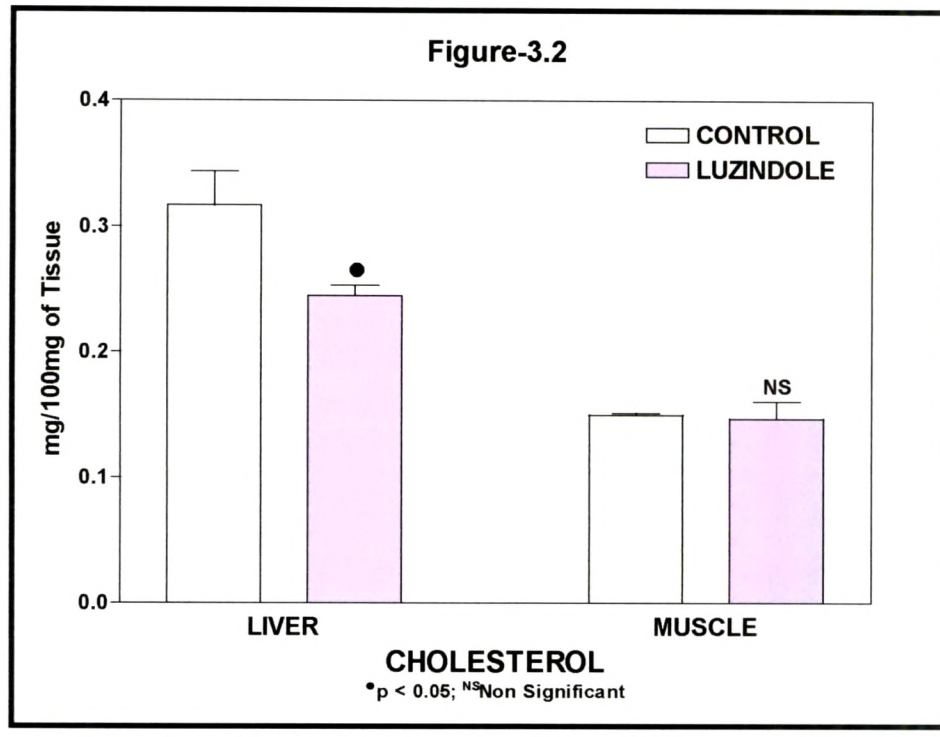

Figure 3.2: Hepatic and muscle cholesterol content of the weaning rats on 22nd day subjected to neonatal luzindole treatment:

	CONTROL	LUZINDOLE
LIVER	0.3168 ±0.027	0.2449• ±0.0081
MUSCLE	0.15 ±0.0015	0.147NS ±0.014

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

Table 3.3: Adipose tissue total lipid content in the weaning rats on 22nd day subjected to neonatal luzindole treatment:

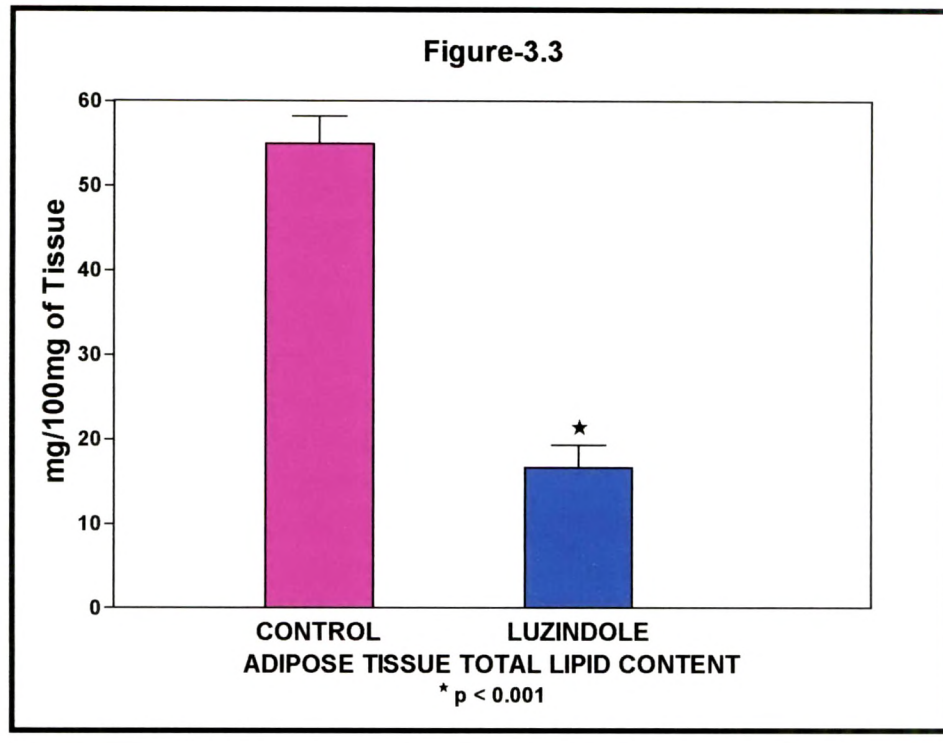

Table 3.3: Adipose tissue total lipid content in the weaning rats on 22nd day subjected to neonatal luzindole treatment:

	CONTROL	LUZINDOLE
TOTAL LIPID	54.95 ±3.2213	16.65* ±2.6503

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

Figure 3.4: Cholesterol content in adipose tissue of weaning rats on 22nd day subjected to neonatal luzindole treatment:

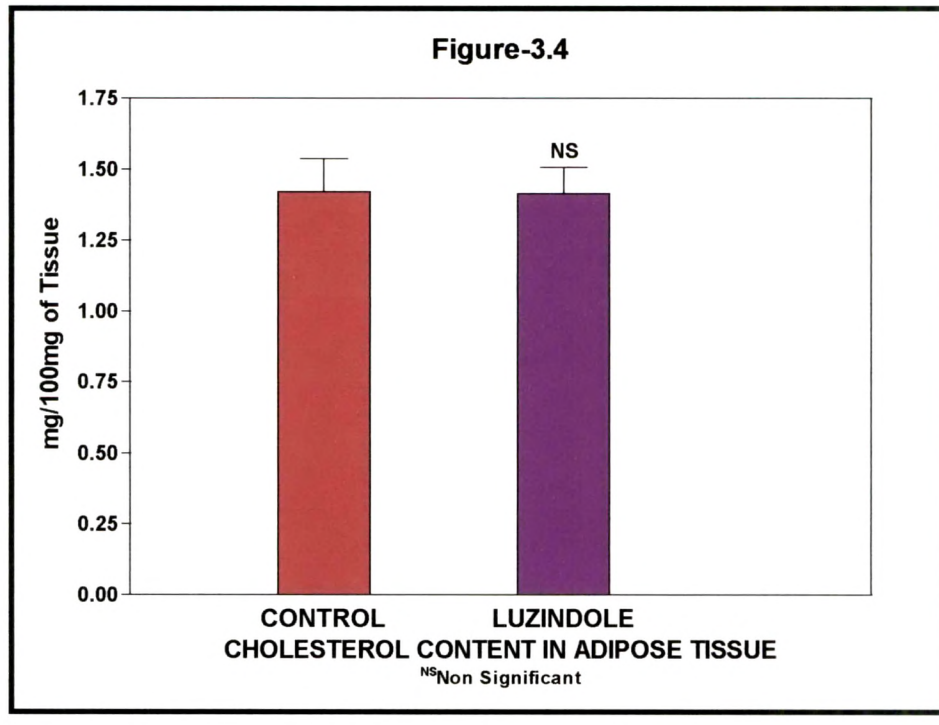

Table 3.4: Cholesterol content in adipose tissue of weaning rats on 22nd day subjected to neonatal luzindole treatment:

	CONTROL	LUZINDOLE
CHOELSTEROL	1.4194 ±0.1176	1.4129[NS] ±0.0919

Values are expressed as mean ± SEM, [NS]Non Significant

Figure 3.5: Serum lipid fractions of weaning rats on 22nd day subjected to neonatal luzindole treatment:

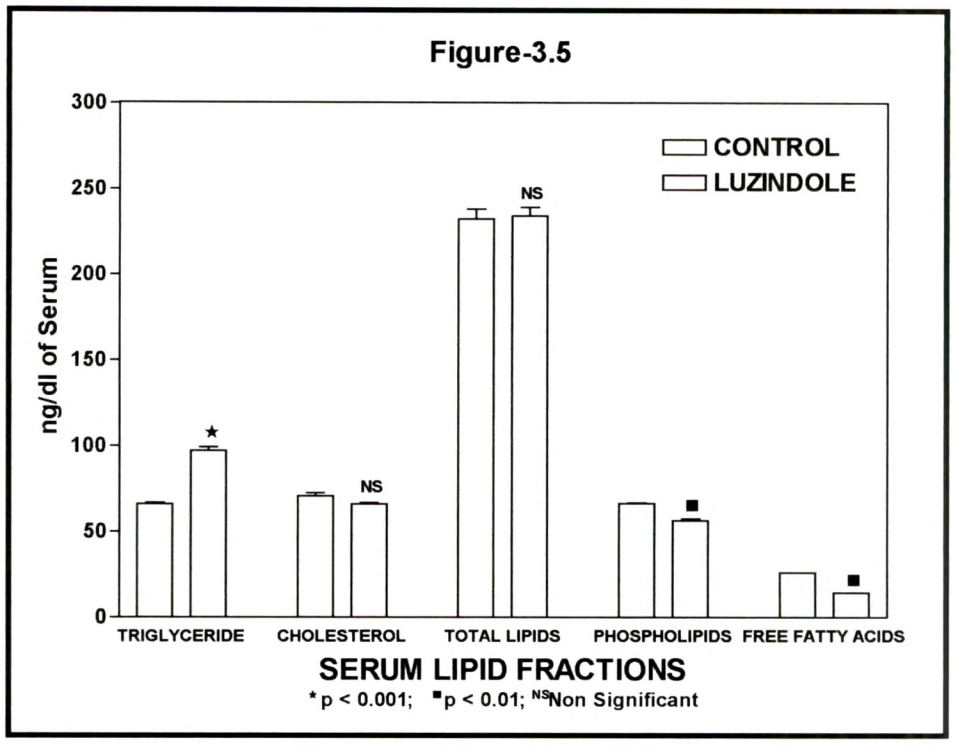

Table 3.5: Serum lipid fractions of weaning rats on 22nd day subjected to neonatal luzindole treatment:

	CONTROL	LUZINDOLE
TRIGLYCERIDE	69.58 ±0.678	97.052* ±2.2545
CHOLESTEROL	70.455 ±1.8719	66.045NS ±0.7434
TOTAL LIPIDS	232.24 ±5.647	234.01NS ±5.0645
PHOSPHOLIPIDS	66.315 ±0.545	56.52■ ±0.7743
FREE FATTY ACIDS	25.86 ±1.13	14.393■ ±0.98

Values are expressed as mean ± SEM, *p < 0.001; ■ p < 0.01; NS Non Significant

DISCUSSION:

The present study on blockage of melatonin action by luzindole has revealed differential effects with tissue total lipid contents being significantly decreased without significantly altering the cholesterol content (Figure and Table; 3.1, 3.2). Though there was no significant difference in the serum total lipid and cholesterol contents between the control and luzindole treated weanings, the triglyceride content was significantly increased and phospholipid, cholesterol and free fatty acid levels decreased in luzindole treated weanings (Figure and Table; 3.5). The unchanged tissue cholesterol contents are very much in keeping with the cholesterol lowering effect of melatonin (Esquifino *et al.*, 1997). The lower serum cholesterol level seen in the present study as against unchanged tissue cholesterol contents may suggest a differential effect of melatonin on serum and tissue cholesterol contents in neonates as a previous study on melatonin administration had tended to increase serum cholesterol level (Jani, 2004). As the neonates are dependent on maternal milk, the prevailing serum lipid fractions in the control pups would be a reflection of the maternal milk derivation. The increased tissue glycogen and lipid contents at the time of weaning are a reflection of the gradual build up of the energy reserves towards adult metabolic homeostasis from the lipid rich milk, besides meeting the routine energy requirement of the pups. Since the tissue lipid contents of luzindole treated weanings are significantly lower than the control weanings, and at the same time the tissue glycogen contents are significantly higher (Chapter-I), melatonin antagonism seems to

imbalance the metabolic machinery of neonates by excessive glycogenesis at the cost of lipogenesis. Apparently, reduced melatonin action in the neonatal period may up regulate glycogenic machinery and down regulate lipogenic machinery. Interestingly, even neonatal hypermelatonemia was seen to induce a similar set of changes of increased tissue glycogen contents and reduced tissue lipid contents (Jani, 2004). Obviously, altered melatonin status, either increased or decreased melatonin levels in the neonates can disturb the normal ontogenic metabolic adaptations in one direction at the expense of the other. However, the degree of this imbalance seems to be more pronounced in hypomelatonemic status than in hypermelatonemic. One possible explanation for the similar set of changes could be the higher level of melatonin induced down regulation of melatonin receptors in the hypermelatonemic neonates as down regulation of melatonin receptors is known to occur under high melatonin concentration (Rowe and Kennaway, 2002). This could easily explain the reduced lowering effect of tissue lipid contents in hypermelatonemic neonates and greater degree of lowering effect of tissue lipid contents in luzindole treated neonates. The same explanation could also be valid for the observed differential degrees of glycogenic effect. Hyperinsulinemia seen in the present study contrasted with hypoinsulinemia in hypermelatonemic neonates (Jani, 2004), indicates the ability of chronic melatonin treatment to suppress insulin secretion (Peschke *et al.*, 1997) and conversely, absence of melatonin action leads to increased insulin release. Similarly,

hypermelatonemia potentiated tissue insulin sensitivity and melatonin receptor antagonism resulted in decreased insulin sensitivity (Chapter-2). Both these features of melatonin related to, insulin release and tissue insulin sensitivity, have shown diametrically opposite patterns due to hypermelatonemia and melatonin receptor blockage, unlike the similarity in terms of proglycogenic and antilipogenic effects. The latter similarity was explainable in terms of receptor down regulation under hypermelatonemia producing results similar to receptor blockage. However in the former case, of diametrically opposite responses, this explanation seems invalid and may have to be seen as an expression of higher receptor concentration in pancreas, liver and muscle thereby offsetting the receptor down regulation effect of melatonin at the dose used in the present study. Though there are no other studies of this type to cite and make meaningful discussion, there are however certain studies on lipid metabolism involving a single dose of melatonin administration or even implantation and long term discontinuous melatonin intake, or even, pinealectomy (Fabis *et al.*, 2002; Mustonen *et al.*, 2002; Markova *et al.*, 2003; Zanquetta *et al.*, 2003). Finally it can be inferred from the present study that neonatal disruption of melatonin action can result in hyperinsulinemia and decreased tissue lipogenic response, probably by some direct surface receptor independent actions of melatonin on lipogenic enzymes.

SUMMARY:

A parallel study on neonatal hypermelatonemia has been shown to decrease tissue lipid and cholesterol contents and increase serum lipid

fractions. The present study is designed to fathom the effect of blockage of melatonin action by a receptor antagonist (luzindole) in terms of tissue and serum lipid parameters. 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 22^{nd} day. The hepatic and muscle total lipid contents decreased significantly in the luzindole treated rats while the, cholesterol content decreased in the liver and the, muscle cholesterol content remained unaltered in the experimental rats. The total lipid content in the adipose tissue of luzindole treated rats decreased significantly while, the cholesterol content in the adipose tissue of the luzindole treated rats showed no significant alteration as compared to the controls. The serum phospholipid and free fatty acid levels decreased significantly while the, serum triglyceride level increased in the experimental rats. The luzindole treated rats showed a significant increase in the serum insulin level as compared to controls. It is inferred from the present study that neonatal disruption of melatonin action can result in hyperinsulinemia and decreased tissue lipogenic response probably by some distinct surface receptor independent actions of melatonin on lipogenic enzymes.