CHAPTER – 3

NEONATAL HYPERMELATONEMIA IN THE PREWEANING PERIOD DECREASES TISSUES LIPID AND CHOLESTERAL CONTENTS AND INCREASES SERUM LIPID FRACTIONS.

INTRODUCTION:

Melatonin, the pineal hormone is light dependent and its secretion pattern is clearly circadian. Accordingly, its serum level is low during the day and high during the night This circadian pattern of melatonin synthesis and secretion is under the control of hypothalamic suprachiasmatic nucleus and is synchronized by circadian light-dark circle. The length and amplitude of the nocturnal melatonin pulse convey the photoperiodic information to the organism and so it functions as a daily (for all species) or annual (for seasonal breeders) calendar (Yu and Reiter, 1993; Vijayalakshmi *et al.*, 2002). Apart from circadian and circannual functions, melatonin is also known to modulate various physiological functions in animals and man (Hill and Blask, 1988; Tan *et al.*, 1993a,b; Acuna-Castroviejo *et al.*, 1994; Maestroni, 1993; Song *et al.*, 1995, Williams *et al.*, 1997; Ebadi *et al.*, 1998; Martin *et al.*, 1998). The modulatory influence of melatonin on intermediary metabolism is suspected from relevant observations

(Mustonen *et al.*, 2002) In strongly social animals, the annual changes in the body weight, adipocity and food intake are known to be influenced by the pattern of melatonin secretion (Wade and Bartness, 1984). Though the mechanism of action of melatonin on energy and intermediary metabolism is not clear, the distribution of melatonin receptors in the brain and many systemic organs suggests possible central as well as peripheral mode of action (Shima *et al.*, 1997; Van Cauter, E., 1998; Acuna-Castroviejo *et al*, 1994; Song *et al.*, 1995; Williams *et al*, 1997; Martin *et al.*, 1998). Because of the presence of receptors in the liver, muscle and adipose tissue, it is presumable that melatonin can influence metabolism through its effect on these metabolic organs. Based on the hormone's influence on blood glucose and tissue glycogen contents in various species, a definite role for it on glycemic status and carbohydrate homeostasis in vertebrate animals is clearly established (Ramachandran,2002). Previous studies on neonatal hypermelatonemic status has shown significant hypoinsulinemia and hypoglycemia together with tissue glycogenic effect and reduced tissue sensitivity to insulin and other agonists for glucose uptake (Chapters 1,2).

Compared to its effect on carbohydrate metabolism, effects on lipid metabolism have been less studied (de Vlaming *et al.*, 1974). Some studies have suggested an action of pineal gland on lipid metabolism and, administration of pineal extracts has been shown to lower the serum, hepatic, adrenal and testicular cholesterol level. In rabbits, pineal extracts could decrease cholesterolemia, biliary

cholesterol and serum phospholipids (Esquifino *et al*, 1997). Cholesterol lowering effect of melatonin has been considered a potent effect as long term melatonin administration could significantly decrease the plasma cholesterol level and prevent fatty liver in genetic hypercholesterolemic rats (Ayoama *et al*, 1988). The role of melatonin on lipid metabolism is also suggested by the observation of delayed post prandial clearance of triacylglycerol indicating possible lipid intolerance in human subjects under stimulated nine hour phase-shifts (Hampton *et al.*, 1996). Melatonin could also prevent hyperlipidemia caused by glucocorticoid administration in rats (Ayoama *et al* 1988) or by cholesterol rich feed (Mori *et al.*, 1989). It is also recorded that melatonin cannot prevent hypercholesterolemia in old rats (Vaughan *et al.*, 1982). The aim of the present study is to evaluate the effect of neonatal hypermelatonemia on tissue and blood lipid parameters so as to contribute to the knowledge on neonatal metabolic features and also to relate with the previously observed effects on carbohydrate metabolism.

MATERIAL AND METHODS: See page Nos. 16 to 37.

RESULTS:

- **Hepatic lipid and cholesterol contents:** The hepatic lipid and cholesterol contents decreased significantly in the melatonin treated animals as compared to controls. This decrease was more pronounced in the cholesterol content as compared to lipid content (Figure and Table; 3 1, 3.2).

- **Muscle lipid and cholesterol contents:** In the melatonin treated animals there is a significant decrease in muscle cholesterol contents as compared to the control animals. The decrease in total lipid content did not show any significant alteration as compared to controls (Figure and Table; 3.1, 3.2).
- **Lipid and cholesterol contents in the adipose tissue:** The adipose tissue of melatonin treated neonates showed a significant decrease in both lipid and cholesterol contents, the latter showing a much more hypocholesterolemic effect as compared to controls (Figure and Table; 3 3, 3.4).
- **Serum lipid fractions:** Serum total lipids, phospholipids and free fatty acid levels increased significantly in the hypermelatonemic rat neonates as compared to controls. Whereas the serum cholesterol and triglyceride levels did not show any significant alteration as compared to controls (Figure and Table; 3 5)
- **Serum Insulin levels:** The serum insulin levels decreased significantly in the melatonin treated rat neonates as compared to control animals (Chapter 1; Figure and Table 1.5).

DISCUSSION:

The results of the present study clearly show a lowering of tissue load of lipid and cholesterol and an increase in serum lipid fractions

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

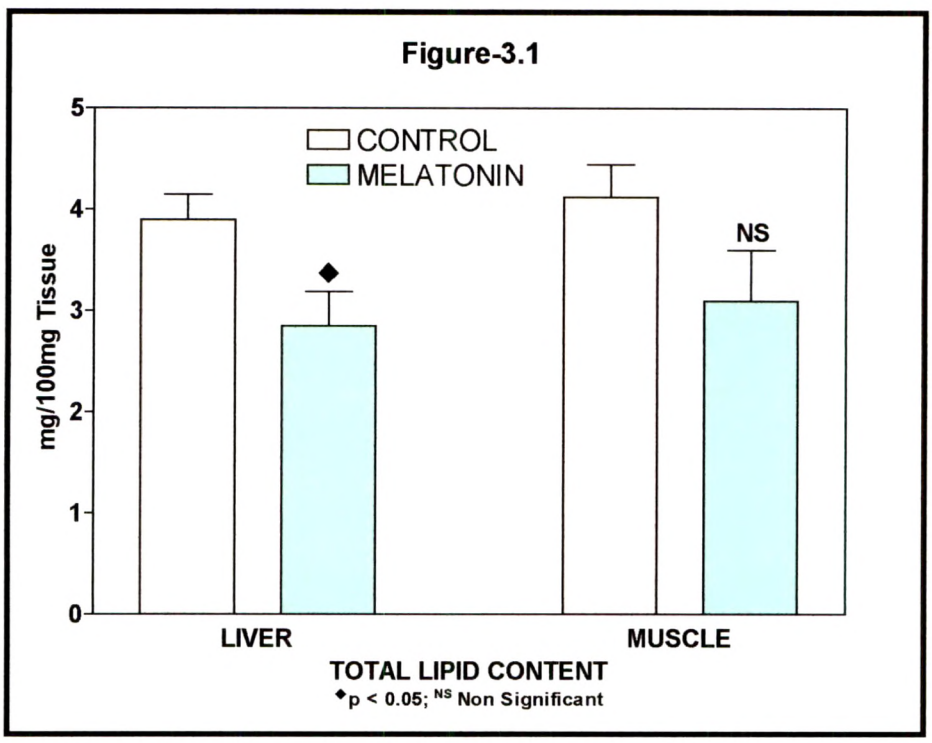

Table 3.1: Hepatic and muscle total lipid content in the weaning rats on 22nd day subjected to neonatal melatonin treatment:

	CONTROL	MELATONIN
LIVER	3.9 ±0.25	2.85◆ ±0.34
MUSCLE	4.12 ±0.33	3.097NS ±0.51

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

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

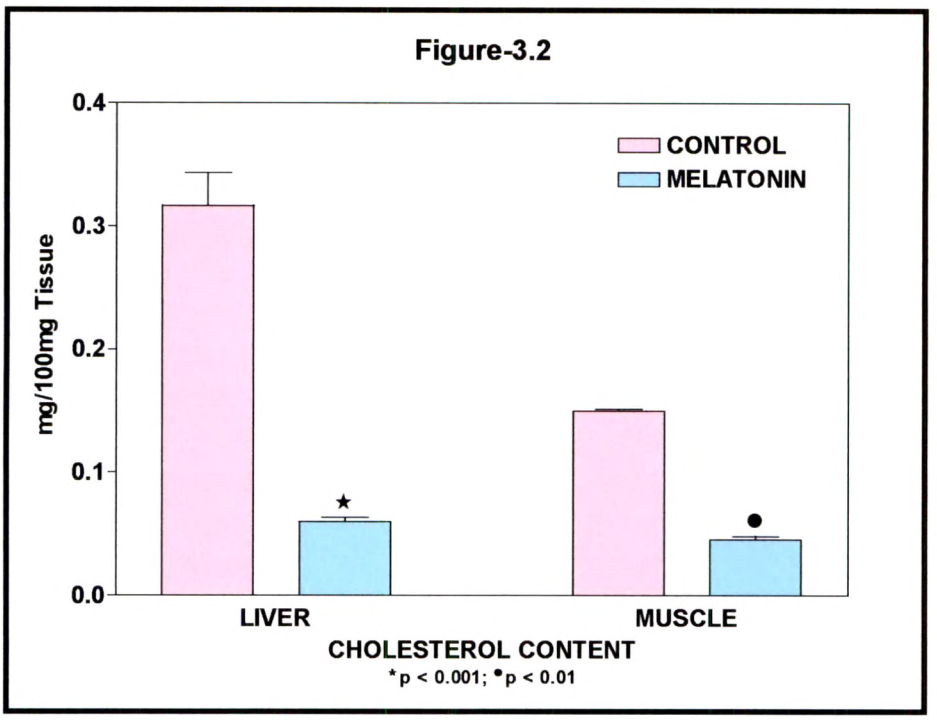

Figure 3.2: Hepatic and muscle cholesterol content of the weaning rats on 22nd day subjected to neonatal melatonin treatment:

	CONTROL	MELATONIN
LIVER	0.3168 ±0.027	0.0599* ±0.0034
MUSCLE	0.15 ±0.0015	0.0454• ±0.0027

Values are expressed as mean ± SEM, *p < 0.001; • p < 0.01

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

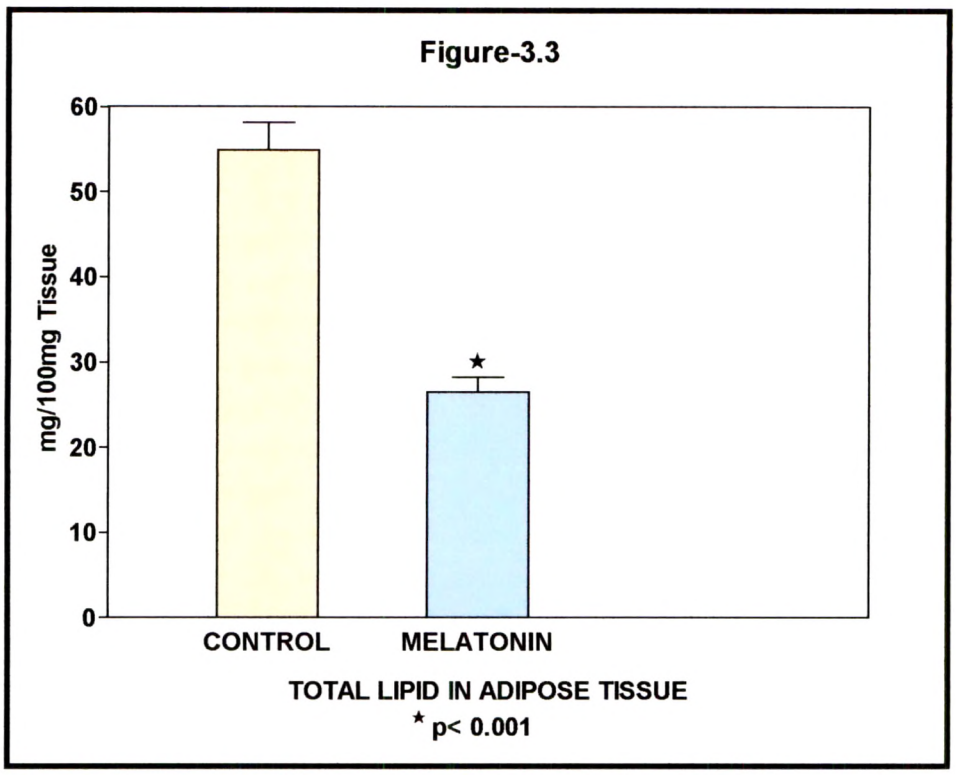

Table 3.3: Adipose tissue total lipid content in the weaning rats on 22nd day subjected to neonatal melatonin treatment:

	CONTROL	MELATONIN
TOTAL LIPID	54.95 ±3.22	26.5* ±1.75

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 melatonin treatment:

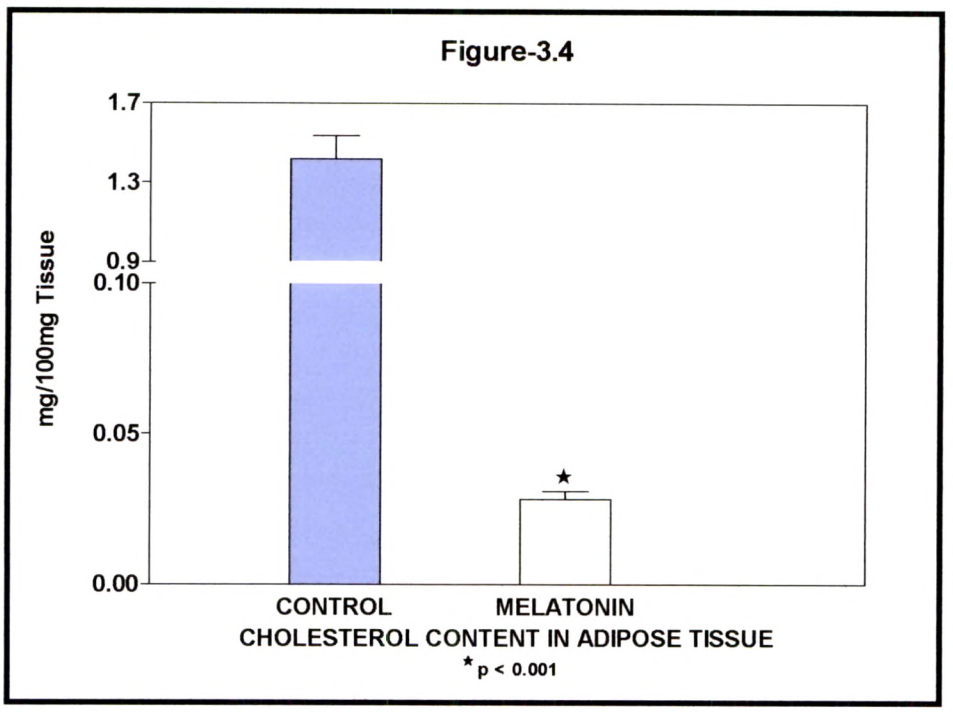

Table 3.4: Cholesterol content in adipose tissue of weaning rats on 22nd day subjected to neonatal melatonin treatment:

	CONTROL	MELATONIN
CHOELSTEROL	1.42 ±0.12	0.027* 0.0027

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

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

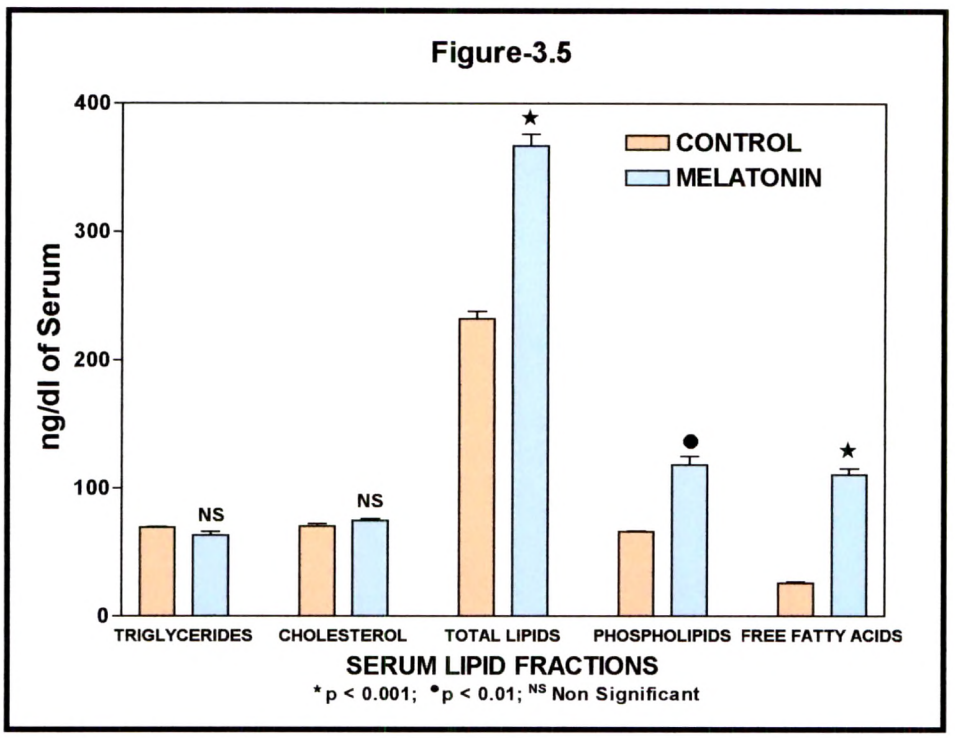

Table 3.5: Serum lipid fractions of weaning rats on 22nd day subjected to neonatal melatonin treatment:

	CONTROL	MELATONIN
TRIGLYCERIDE	69.58 ±0.68	63.19^{NS} ±2.71
CHOLESTEROL	70.45 ±1.8719	74.99^{NS} ±1.292
TOTAL LIPIDS	232.24 ±5.65	366.98* ±9.39
PHOSPHOLIPIDS	66.315 ±0.55	118.48$^{•}$ ±6.46
FREE FATTY ACIDS	25.86 ±0.85	110.32* ±5.31

Values are expressed as mean ± SEM, $^{*}p < 0.001$; $^{•}p < 0.01$; ^{NS}Non Significant

in hypermelatonemic rats. Neonatal pre-weaning period is a period during which the young ones make necessary arrangements to shift from a lipid rich milk diet to a carbohydrate diet. It is also the period during which the neonate builds up its carbohydrate and lipid reserves. The levels of tissue glycogen and lipid at the time of weaning would in this respect represent the gradually built up reserves. The levels of serum glucose and lipid fractions at the weaning period could also represent the transition phase in the metabolic homeostasis, between dependence on mother's milk and, self dependence. Looked in this perspective, the decreased hepatic, muscle and adipose tissue lipid and cholesterol contents in the hypermelatonemic neonates (Fig. and Tab.; 3.1, 3.2, 3.3, 3.4), could indicate either a retarded build up of lipid reserves or, an increased utilization. Since there is no change in serum triglyceride and cholesterol contents in control and experimental neonates, the observed significant increment in the serum total lipids is essentially due to an increased titre of phospholipids and free fatty acids (Fig. and Tab.; 3.5). Since in the previous studies a glycogenic effect with hypoglycemia and increased tissue sensitivity for glucose uptake were recorded (Chapters 1,2), the presently observed increased serum free fatty acids and decreased tissue lipid content could suggest an adaptive increased lipid utilization to counteract the carbohydrate sparing effect of neonatal hypermelatonemia. The low tissue lipid stores could be easily correlated with the prevailing hypoinsulinemia. In a study on the effect of single injection of melatonin in young rats Fabis *et al.* (2002) observed a decrease in

serum free fatty acid levels and an increase in total, free, esterified and HDL cholesterol though, serum triglycerides and liver triglycerides and phospholipid contents were unchanged. Obviously, effects of single injections are not comparable with treatment for longer duration. The present observations on serum cholesterol and lipid levels are consistent with the observation of Mori *et al*, (1989) on anti-hypercholesterolemic effect of melatonin and of Hoyos *et al.* (2000) on decreased serum cholesterol and lipid peroxidations by melatonin, in diet induced hypercholesterolemic rats. In another study involving melatonin implantation for 28 days in adult rats, it has been shown that there is a decreased hepatic lipase and esterase activities (Mustonen *et al.*, 2002), which was explained as reduced utilization of triacylglycerols and the deposition as body energy reserve. Exogenous melatonin has been shown to stimulate accumulation of fat in several mammalian species such as the Syrian hamster (Wade and Bartness, 1984) and the garden dormouse (Le Gouic *et al.*, 1996). Decreased rate of liver lipogenesis due to melatonin treatment in chickens has also been reported (Osei *et al.*, 1989). The report of increased serum phospholipid levels by melatonin treatment in rats (Esquifino *et al.*, 1997) tallies with the present observations. In another study involving long term discontinuous melatonin treatment through drinking water, reduced serum triglyceride and reduced serum and liver cholesterol levels were reported (Markova *et al.*, 2003). Increased hepatic phospholipid and diacylglycerol concentrations due to continuous melatonin administration have also been reported (Mustonen *et al.*,

2002). The differences in the observations of various workers once again emphasize the importance of age, sex, time, dose and duration of melatonin treatment employed, and the need to be cautious in contradicting and interpreting the data.

The significantly low content of lipid and cholesterol in tissues noted in the present study, suggests reduced tissue adiposity by way of reduced tissue lipogenesis The concurrently higher serum free fatty acid could be considered as an energy source rather than as a precursor for tissue lipid synthesis. Since the control weanings show a much higher tissue lipid and cholesterol stores and significantly low serum free fatty acids, low lipid oxidation and increased lipid deposition are the features under normal melatonin status. The lower tissue glycogen contents and high blood glucose level recorded previously (Chapter-I), confirms dominant carbohydrate utilization in control weanings.

It can be concluded from the present observations that neonatal hypermelatonemia decreases lipid synthesis and increases lipid utilization as against increased lipid synthesis and decreased utilization in control weanings.

SUMMARY:

Previous studies on neonatal hypermelatonemic status have shown significant hypoinsulinemia and hypoglycemia together with tissue glycogenic effect and reduced tissue sensitivity to insulin and other agonists for glucose uptake Chapters 1, 2). The aim of the present study is to evaluate the effect of neonatal hypermelatonemia on tissue

and blood lipid parameters. To this end, rat neonates have been treated with melatonin in graded doses of 200 µg/animal from day 1 to day 7; 400 µg/animal from day 8 to day 14 and 600 µg/animal from day 15 to day 21 and assessed on the 22nd day. The melatonin treated animals showed significantly decreased hepatic and adipose tissue lipid and cholesterol contents. Whereas serum total lipids, phospholipids and free fatty acids level increased significantly, the serum triglyceride and cholesterol levels did not show any significant alteration. The serum insulin level decreased significantly in the experimental rats. It can be concluded from the present observations that neonatal hypermelatonemia decreases lipid synthesis and increases lipid utilization as against lipid synthesis and decreased utilization in control weanlings.