# CHAPTER – 6

## NEONATAL HYPERMELATONEMIA INCREASES INSULIN SENSITIVITY AND POTENTIATES LIPOGENESIS FROM WEANING TO PUBERTAL PERIOD.

### INTRODUCTION:

The modulatory influence of melatonin on intermediary metabolism is getting clearly established (Mustonen *et al.*, 2002; Ramachandran, 2002; Markova *et al.*, 2003). The localization of melatonin receptors in liver, muscle and adipose tissue strengthens the reported influence of melatonin in recent times on intermediary metabolism Relatively more data is generated on its effect on carbohydrate metabolism but less well studied with reference to lipid metabolism. Studies with administration of pineal extracts have shown a lowering effect on serum, hepatic, adrenal and testicular cholesterol levels. Esquifino *et al.* (1997) have demonstrated decreased cholesterolemia, biliary cholesterol and serum phospholipids in rabbits treated with pineal extracts. A potent effect of long term melatonin in lowering plasma cholesterol level and prevent fatty liver development in genetic hypercholesterolemic rats has also been reported (Aayoma *et al.*, 1988). Suggestion of the influence of melatonin on lipid metabolism has also come from the observations of delayed post prandial

clearance of triacylglycerol indicating possible lipid intolerance in human subjects under simulated photoperiodic phase shifts (Hampton *et al.*, 1996). Its influence on lipid metabolism is further indicated by the ability of melatonin to resist glucocorticoid induced hyperlipidemia in rats (Aayoma *et al.*, 1988) or even by a cholesterol rich food (Mori *et al.*, 1989). Age dependent resistance is also suggested by its inability to prevent hypercholesterolemia in old rats (Vaughan *et al.*, 1982).

Previously it was shown that neonatal hypermelatonemic in the pre-weaning period could decrease tissue lipid and cholesterol content and increase serum lipid fractions in the weaning period (Chapter 3). The above observations were taken to suggest that neonatal melatonin excess can lead to decreased lipid synthesis and increased lipid utilization as against increased lipid synthesis and decreased lipid utilization in control weanings (Chapter 3). It was pertinent in this content to evaluate the effect of neonatal melatonin excess on pubertal lipid metabolism essentially to test the prolonged effect of the hormone if any.

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

## RESULTS:

- **Hepatic lipid and cholesterol contents:** The hepatic lipid content increased significantly while, the hepatic cholesterol content decreased significantly in the hypermelatonemic rats as compared to controls (Figure and Table; 6.1, 6.2).

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

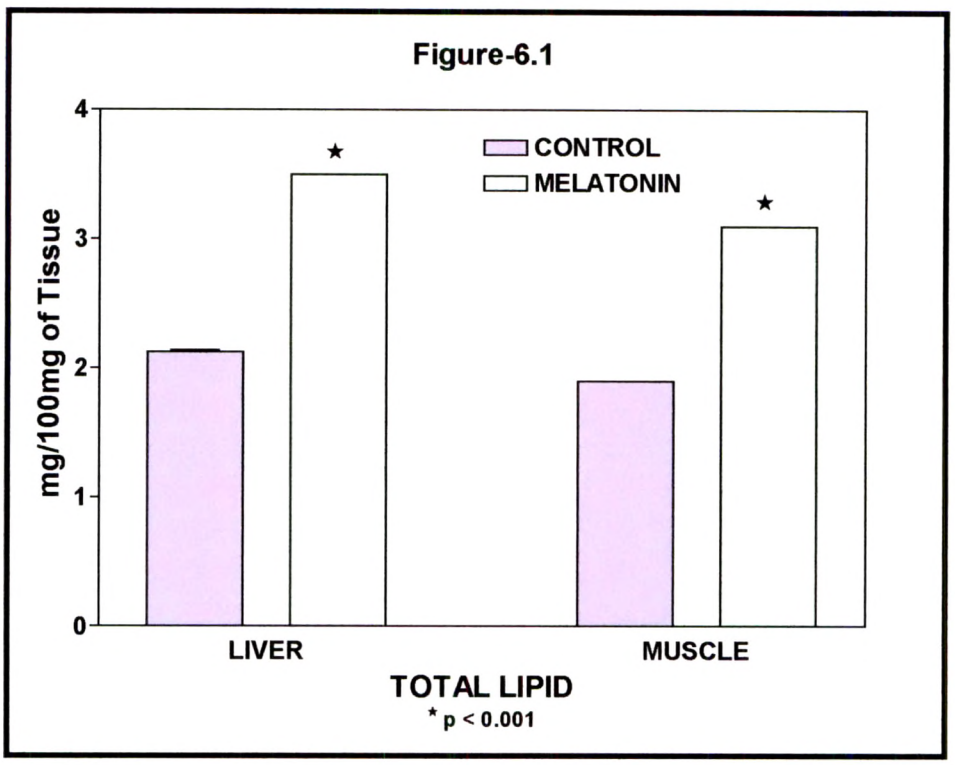


**Table 6.1: Hepatic and muscle total lipid content in the pubertal rats on 45th day subjected to neonatal melatonin treatment:**

| | CONTROL | MELATONIN |
|---|---|---|
| LIVER | 2.125 ±0.017 | 3.5* ±0.00085 |
| MUSCLE | 1.9 ±0.00004 | 3.1* ±0.000465 |

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

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

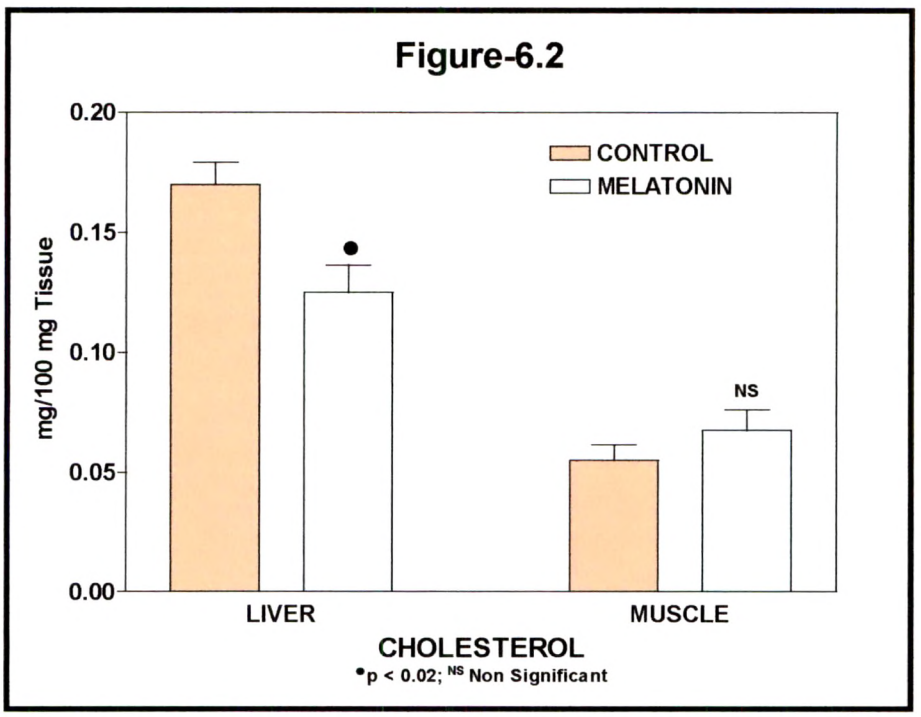


**Table 6.2: Hepatic and muscle cholesterol content of the pubertal rats on 45th day subjected to neonatal melatonin treatment:**

| | CONTROL | MELATONIN |
|---|---|---|
| LIVER | 0.17 ±0.0091 | 0.125• ±0.0115 |
| MUSCLE | 0.055 ±0.00645 | 0.0675NS ±0.0085 |

**Values are expressed as mean ± SEM, *p < 0.02; NS Non Significant**

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

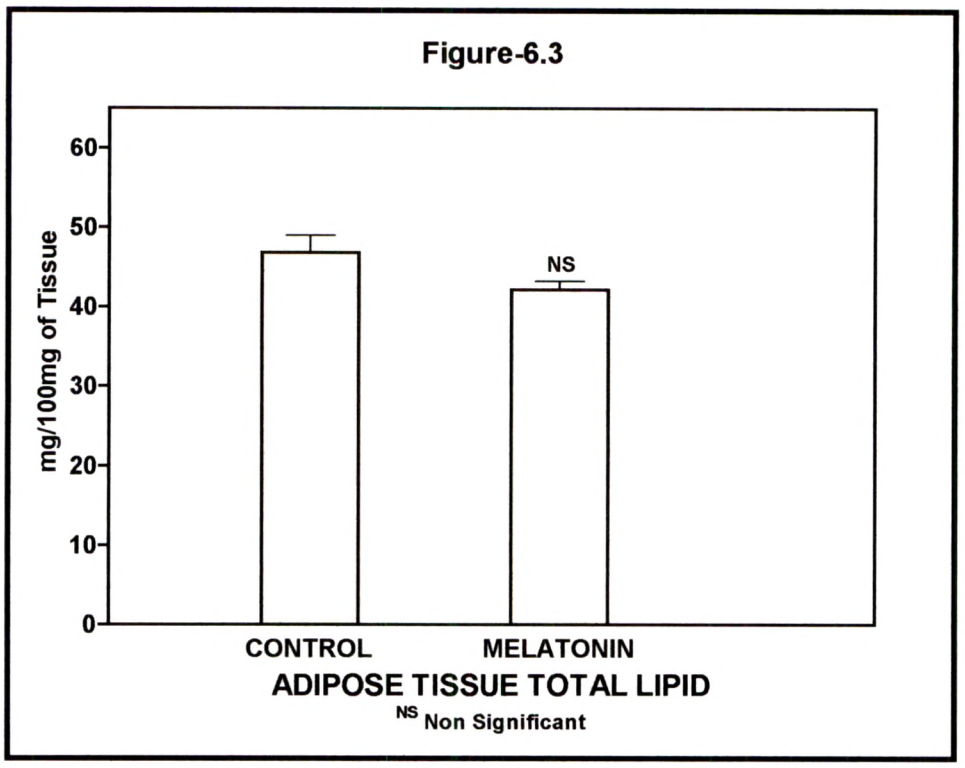


**Table 6.3: Adipose tissue total lipid content in the pubertal rats on 45th day subjected to neonatal melatonin treatment:**

| | CONTROL | MELATONIN |
|---|---|---|
| TOTAL LIPID | 46.8 ±2.218 | 42.15[NS] ±1.085 |

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

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

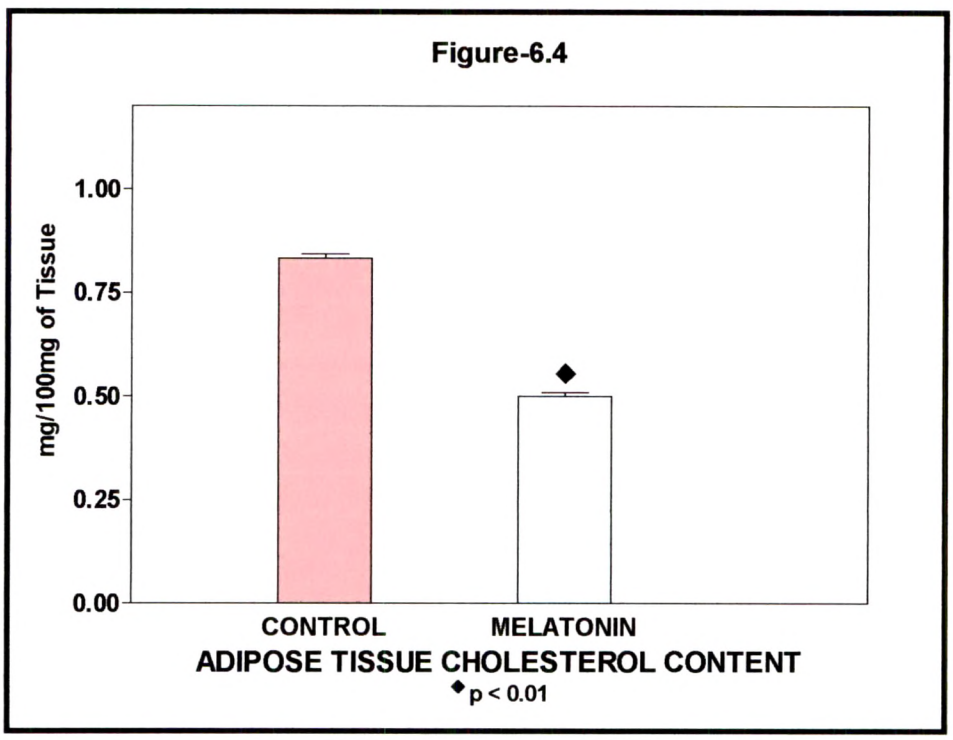


**Table 6.4: Cholesterol content in adipose tissue of pubertal rats on 45th day subjected to neonatal melatonin treatment:**

| | CONTROL | MELATONIN |
|---|---|---|
| CHOELSTEROL | 0.832 ±0.01105 | 0.5◆ 0.0.0091 |

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

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

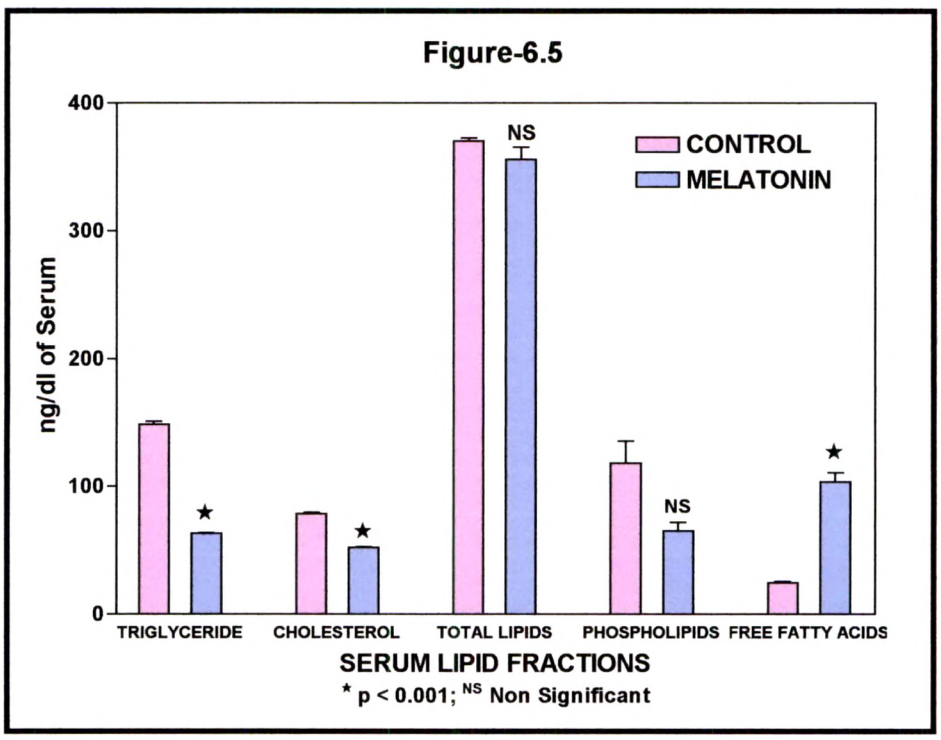


**Table 6.5: Serum lipid fractions of pubertal rats on 45th day subjected to neonatal melatonin treatment:**

| | CONTROL | MELATONIN |
|---|---|---|
| TRIGLYCERIDE | 148.187 ±2.32 | 63.19* ±2.71 |
| CHOLESTEROL | 78.492 ±0.59 | 74.99* ±1.29 |
| TOTAL LIPIDS | 369.98 ±2.6205 | 366.98NS ±9.39 |
| PHOSPHOLIPIDS | 117.97 ±12.05 | 118.48NS ±6.46 |
| FREE FATTY ACIDS | 25.34 ±0.98 | 110.32* ±7.16 |

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

- **Muscle lipid and cholesterol contents:** The muscle of hypermelatonemic rats showed a significant increase in the lipid content but, only a marginal increase though insignificant, in the cholesterol content as compared to control rats (Figure and Table; 6.1, 6.2).
- **Lipid and cholesterol content in the adipose tissue:** There is a decrease in the lipid and cholesterol content of the adipose tissue in the hypermelatonemic rats as compared to control rats (Figure and Table, 6.3, 6.4).
- **Serum lipid fractions:** The serum triglyceride and cholesterol levels decreased significantly in the melatonin treated rats. Whereas, the total lipid and phospholipid levels decreased marginally, the serum free fatty acid level increased significantly in the hypermelatonemic rats as compared to controls (Figure and Table; 6.5).
- **Serum insulin level:** The serum insulin level decreased significantly in the melatonin treated rats as compared to the control rats (Chapter 4; Figure and Table; 4.6).

**DISCUSSION:**

The present results reveal that tissue lipid contents are significantly increased in hypermelatonemic rats while those of control rats have shown significant reductions (Fig. and Tab.; 6.1, 6.2, 6.3, 6.4). Clearly, the control rats had increased their lipid contents by the time of weaning itself when they were feeding on a lipid rich mother's milk. In contrast the hypermelatonemic rats converted the lipid content of milk

into tissue lipids very poorly probably channelising most of the lipid nutrients into tissue glycogen and also using partly for their energy need (Chapter 1). This was well reflected in the serum free fatty acid levels which was significantly higher compared to controls (Fig. and Tab.; 6 5). Simultaneously in the weaning age the serum glucose level was also very high suggesting a possible gluconeogenic status. The significant reduction in tissue lipid contents coupled with increased serum lipid fractions but low fatty acid level in control rats suggest utilization of lipids as a source of energy proceeding from the weaning to pubertal age. The hypermelatonemic rats in contrast seems to have delayed their lipogenic activity to the weaning to pubertal age probably converting the carbohydrate rich diet into more of lipids. Apparently the hypermelatonemic rats in the weaning to pubertal age channelise quite a bit of carbohydrate matter into lipogenic pathways and the rest into catabolic pathways to meet the energy needs. The high serum glucose as well as fatty acid levels tends to indicate balanced optimized utilization of both for energy requirements. However, the hypermelatonemic animals seem to be geared to a highly anabolic state between weaning and puberty as tissue glycogen, lipid and protein reserves have all shown significant elevation. This apparent anabolic *milieu* could be related with the increased serum insulin level to the tune of 176% as against a 72% increment in controls compared to the weaning period. The significantly increased insulin levels as well as the potentiated insulin sensitivity that the hypermelatonemic animals manifest (Chapters 2 & 5) are responsible for the overall anabolic state.

It is presumable that the poor lipogenesis seen in the hypermelatonemic weaning rats is essentially due to the inability of the animals to induce higher levels of lipogenic enzymes due to the very low level of insulin. A need for an optimal minimal level of insulin for induction of lipogenic enzymes could be considered relevant as with the increase in serum insulin level from weaning to puberty; these rats show the expected level of tissue lipid deposition. A survey of literature on melatonin and lipid metabolism reveals that differential effects are reported. The varying and at times contradictory results are essentially due to the mere modulatory role of melatonin in a given set of conditions. Apparently as melatonin does not have a defined committed role in intermediary metabolism, its modulatory influence varies with change in conditions like, habit and habitat of the animals, seasonal or non-seasonal nature, prevailing environmental conditions like photoperiod, temperature, humidity, etc and time of the day, duration and dosage of melatonin used and, also the age of the animals. Single injection of melatonin in young rats has reported decreased serum free fatty acid levels and increased total, free and esterified and HDL cholesterol without any change in serum and tissue triglyceride and phospholipid levels (Fabis *et al.*, 2002). Long term treatment with melatonin has been shown to decrease serum cholesterol and lipid levels in rats (Mori *et al.*, 1989; Hoyos *et al.*, 2000). In another study involving implantation of melatonin for 28 days, increased lipid reserves with reduced lipase and esterase activities have been reported (Mustonen *et al.*, 2002). In seasonal

breeders, exogenous melatonin has been shown to stimulate accumulation of fat (Wade and Bartness, 1984; Le Gouic *et al.*, 1996). In chickens decreased liver lipogenesis due to melatonin treatment has been recorded (Osei *et al.*, 1989). Increased serum phospholipid levels in rats treated with melatonin have also been reported (Esquifino *et al.*, 1997). Long term discontinuous melatonin treatment through drinking water reportedly reduced serum triglyceride and liver cholesterol levels (Markova *et al.*, 2003). Continuous administration of melatonin also increased hepatic phospholipid and diacylglycerol concentrations (Mustonen *et al.*, 2002),

The present study is not comparable with any of the recorded studies in literature as there is no report on the effect of continuous neonatal hypermelatonemia for the entire pre-weaning period on, pubertal status of lipid metabolism. This is more of a consequential study on later effects, of melatonin given in a sensitive period as the neonatal period, and evaluated much after cessation of melatonin administration. Clearly, neonatal melatonin administration in the neonatal period and has definite long term modulatory influence on intermediary metabolism by altering the overall hormonal *milieu* and tissue sensitivity and even probably by influencing activity of various enzymes. The reduction in serum levels of various lipid fractions other than free fatty acids suggests a hypolipidemic effect of chronic melatonin treatment. Cholesterol lowering effect of melatonin seems to be a characteristic feature as at both weaning (Chapter 3) and in the pubertal stage (Present study) the cholesterol content of the body is

significantly less in neonatal hypermelatonemic rats. It is apparent from the present results that neonatal hypermelatonemia has the ability to alter the normal metabolic homeostasis to a new level with effects on all aspects of metabolism impinging as carbohydrates, lipids and proteins. The favorable or unfavorable consequences of these alterations to the animal/individual as a whole on a long term basis need to be evaluated.

## SUMMARY:

Previously it was shown that neonatal hypermelatonemia in the pre-weaning period could decrease tissue lipid and cholesterol contents and increase serum lipid fractions in the weaning period (Chapter 3). It was pertinent in this context, to evaluate the effects of neonatal melatonin excess on pubertal lipid metabolism essentially to test the prolonged effect of the hormone if any. 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 45$^{th}$ day. The hepatic and muscle lipid contents increased significantly in the experimental rats however, the hepatic and muscle cholesterol contents showed a marginal decrease. While the cholesterol content in the adipose tissue of experimental rats decreased significantly, the lipid content remained more or less unaltered. Whereas the serum free fatty acid, triglyceride and cholesterol levels decreased significantly, the total lipid and phospholipid levels remained unaltered in the experimental rats. It is

apparent from the present results that neonatal hypermelatonemia has the ability to alter the normal metabolic homeostasis to a new level with effects on all aspects of metabolism. The favorable or unfavorable consequences of these alterations to the animal/individual as a whole on a long term basis needs to be evaluated.