CHAPTER – 9

NEONATAL HYPERMELATONEMIA ALTERS LIPID STATUS OF YOUNG ADULT RATS: LONG TERM EFFECTS OF MELATONIN.

INTRODUCTION:

Melatonin is a neurohormone synthesized and secreted at night mainly by the pineal gland in vertebrates (Arendt, 1995; Reiter, 1991). It affects various physiological functions such as seasonal reproduction, thermoregulation and energy metabolism in mammals and particularly in seasonal mammalian species. In the latter, melatonin is known to affect body mass, adiposity and both energy intake and expenditure (Himms-Hagen, 1984; Wade and Bartness, 1984; McElroy and Wade, 1986, Bartness, 1995). These effects may vary according to the species Thus opposite results are observed in Siberian and Syrian hamsters in which melatonin decreases or increases body fat mass respectively (Wade and Bartness, 1984; McElroy and Wade, 1986; Bartness and Wade, 1985; Bartness, 1995). Furthermore, a melatonin agonist or antagonist stimulates or lowers seasonal obesity in the garden dormouse (Le Gouic *et al.*, 1996). Also a circadian rhythm of low density lipoprotein (LDL) receptor activity has been demonstrated

which is influenced by cortisol, but not mediated by it (Balasubramaniam *et al.*, 1994). Melatonin itself has been shown to inhibit LDL receptor activity and cholesterol synthesis in human mononuclear leucocytes (Muller-Wieland *et al.*, 1994). Chapman. (1997) indicated that melatonin also influences lipoprotein lipase (LPL) activity, a key regulatory enzyme in circulating triacylglycerol and adipose tissue. A recent study involving long term discontinuous melatonin treatment through drinking water, reduced serum triglyceride and, serum and liver cholesterol levels (Markova *et al.*, 2003). Also increased hepatic phospholipid and diacylglycerol concentrations due to melatonin administration have been reported (Mustonen *et al.*, 2002). 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 inhibiting 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 mediated mechanism (Sauer *et al.*, 2001). The differences in observations of various workers once again emphasize the importance of age, sex, time, dose and duration of melatonin treatment employed.

Previous studies have demonstrated that neonatal hypermelatonemia decreases lipid synthesis and increases lipid utilization in the weaning period (Chapter 3). Similar results were obtained in pubertal period (Chapter 6). The aim of the present study is to evaluate the long term

effect of neonatal hypermelatonemia on tissue and blood lipid parameters if any.

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

RESULTS:

- **Hepatic lipid and cholesterol content:** The melatonin treated animals showed significantly decreased hepatic lipid and cholesterol contents as compared to the control animals (Figure and Table; 7.1 and 7 2).
- **Muscle lipid and cholesterol content:** The muscle lipid content decreased significantly while, the muscle cholesterol contents increased significantly in the hypermelatonemic rats as compared to control rats (Figure and Table; 7.1 & 7.2).
- **Lipid and cholesterol content in the adipose tissue:** Whereas the lipid content in the adipose tissue decreased significantly, the cholesterol content increased significantly in the hypermelatonemic rats as compared to the control rats (Figure and Table; 7.3 and 7.4).
- **Serum lipid fractions:** There was a significant decrease in serum triglyceride, cholesterol, phospholipids and free fatty acid levels in the melatonin treated rats as compared to control rats (Figure and Table; 7.5).
- **Serum insulin level:** The serum insulin level increased significantly in the melatonin treated rats as compared to the control rats (Chapter 7; Figure and Table; 7.6).

DISCUSSION:

An age associated peripheral insulin resistance with concomitant increase in fat pad mass and circulating free fatty acid levels has been reported to occur in weaning to young, young to mature and mature to old rats (Pagliassotti *et al.*, 2000). This observation on Sprague-Dawley rats has been confirmed by the reported fat accumulation occurring during and after puberty (Banarjee *et al.*, 1997). A strain difference in terms of occurrence of insulin resistance and fat accumulation is inferable as no significant insulin resistance or increase in fat load could be recorded from weaning through puberty to young age (Chapter 3 & 6), in the Charles foster strain of rat. The control Charles foster rats showed a higher adipose tissue, muscle and hepatic lipid content in the weaning age itself (Chapter 3). There was a significant decrement from the weaning to pubertal age, which was related with the increased energy provision in association with pubertal changes sparing carbohydrate reserves (Chapter 3 & 6) In the current study involving young Charles foster rats, the tissue lipid contents have increased significantly from the pubertal stage (Fig. and Tab.; 9.1, 9.3), though still not more than those recorded in the weaning age. Apparently these animals are more resistant towards developing insulin resistance and increasing their body lipid loads/adipocity and these age related changes are more likely to be manifested only during the transition from young to mature (60-120 days) and mature to old ages (120-250/300 days; see Pagliassotti *et al.*, 2000).

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

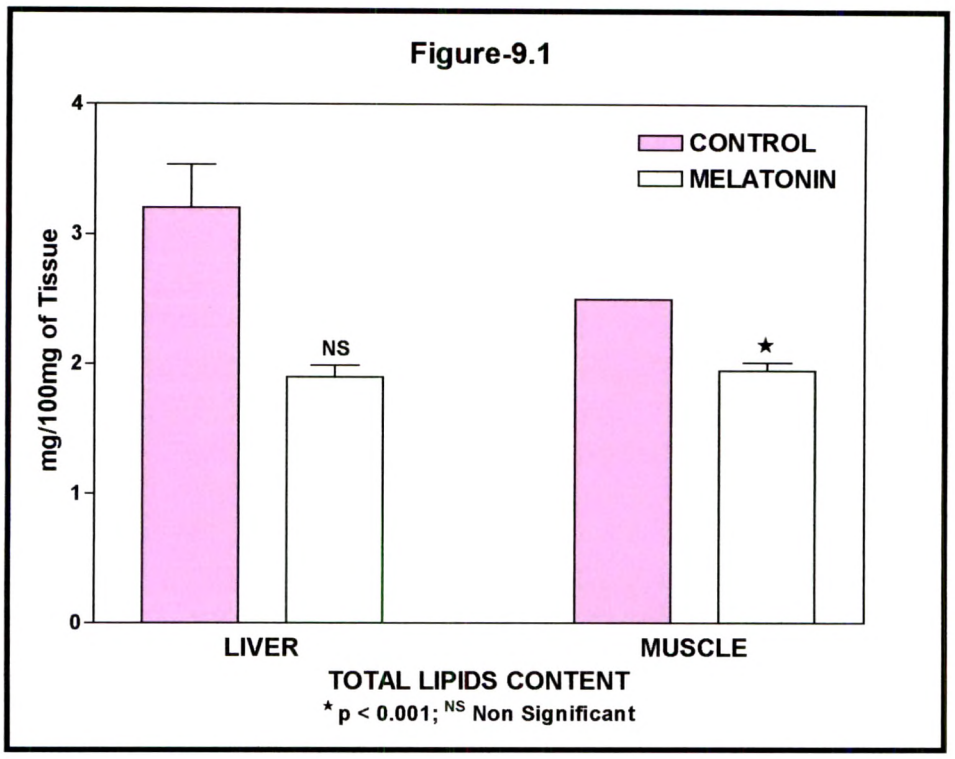

Table 9.1: Hepatic and muscle total lipid content in the adult rats on 60th day subjected to neonatal melatonin treatment:

	CONTROL	MELATONIN
LIVER	3.2 ±0.33	1.9[NS] ±0.91
MUSCLE	2.5 ±0.0006	1.95* ±0.064

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 melatonin treatment:

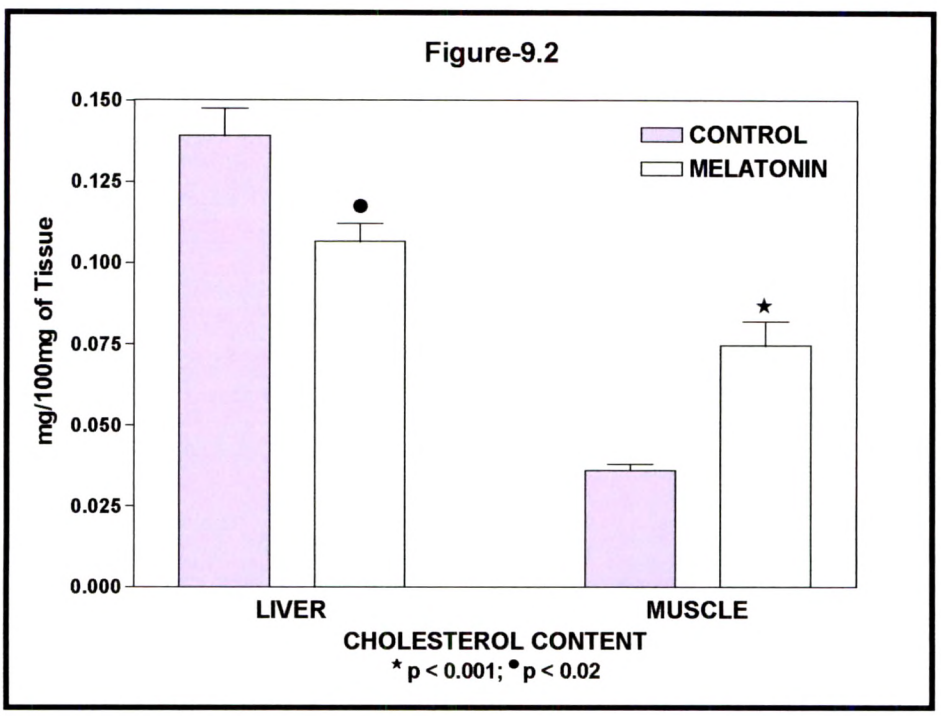

Table 9.2: Hepatic and muscle cholesterol content of the adult rats on 60th day subjected to neonatal melatonin treatment:

	CONTROL	MELATONIN
LIVER	0.14 ±0.0084	0.11• ±0.0056
MUSCLE	0.036 ±0.0018	0.074* ±0.0075

Values are expressed as mean ± SEM, *p < 0.001; •p < 0.02

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

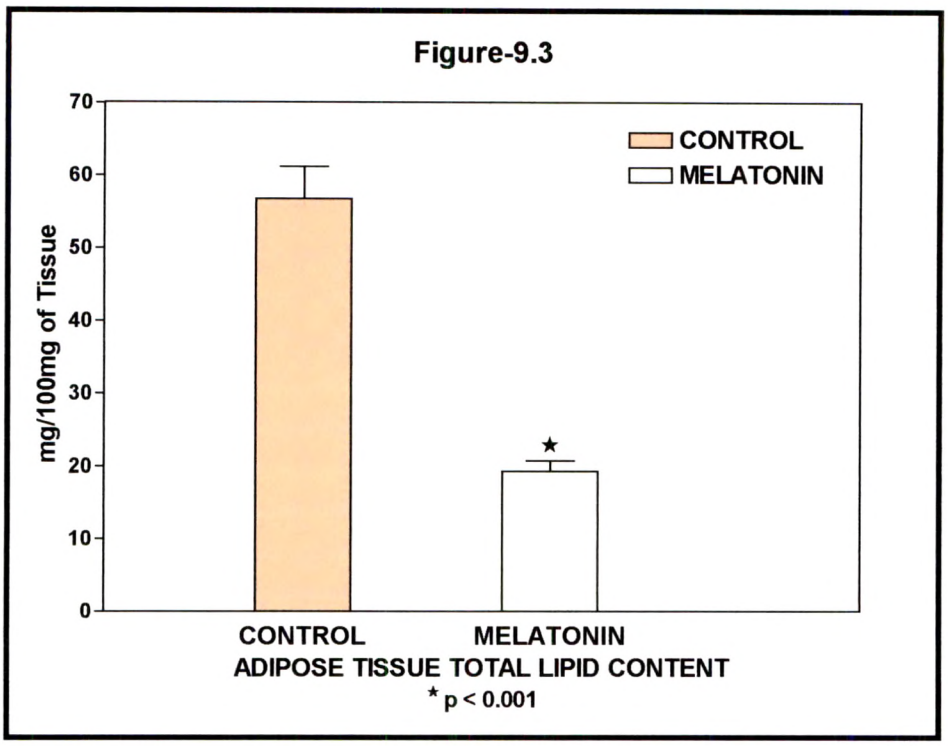

Table 9.3: Adipose tissue total lipid content in the adult rats on 60th day subjected to neonatal melatonin treatment:

	CONTROL	MELATONIN
TOTAL LIPID	56.8 ±4.34	19.27* ±1.48

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

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

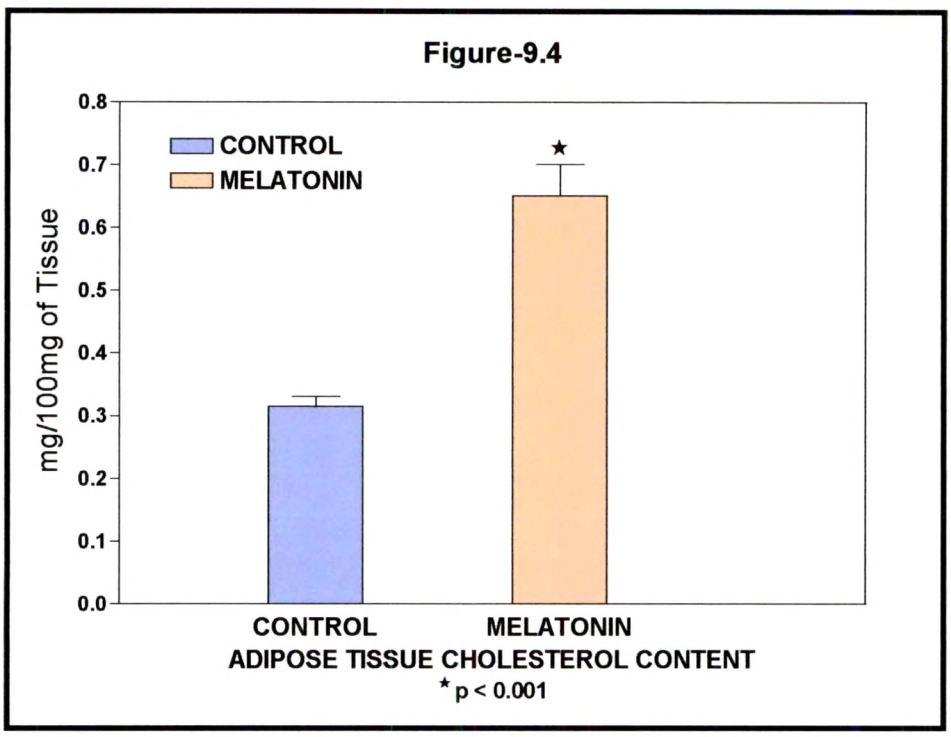

Table 9.4: Cholesterol content in adipose tissue of adult rats on 60th day subjected to neonatal melatonin treatment:

	CONTROL	MELATONIN
CHOELSTEROL	0.31 ±0.016	0.65* ±0.05

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

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

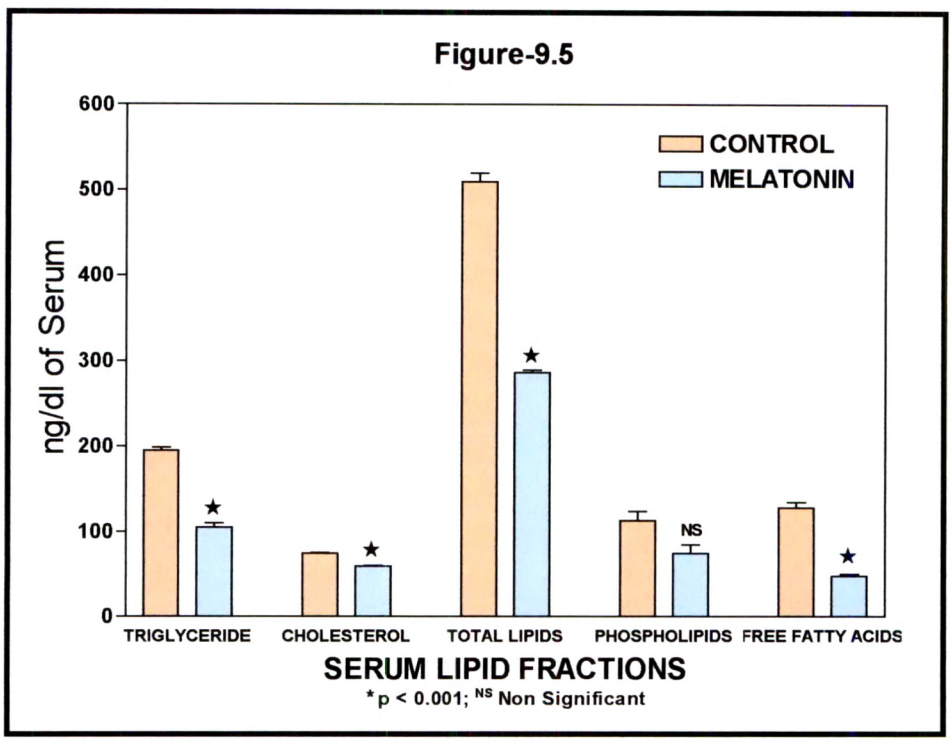

Table 9.5: Serum lipid fractions of adult rats on 60th day subjected to neonatal melatonin treatment:

	CONTROL	MELATONIN
TRIGLYCERIDE	194.94 ±3.8433	104.83* ±4.963
CHOLESTEROL	74.31 ±1.2021	59.25* ±1.0557
TOTAL LIPIDS	509.75 ±9.78	286.05* ±3.348
PHOSPHOLIPIDS	112.76 ±10.4413	74.47NS ±10.44
FREE FATTY ACIDS	127.74 ±6.24	47.49* ±2.82

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

The above changes in tissue lipid contents of control rats are in contrast to those seen in neonatal hypermelatonemic rats and wherein, there was a significantly low tissue lipid contents in the weaning period and, which increased in the pubertal period (Chapters 3 & 6), and again decreased significantly low levels at the young age (present chapter). Inferably, neonatal exposure to higher melatonin levels brings about long lasting alterations in lipid metabolism/homeostasis resulting in significant decrement in tissue lipid load. In keeping with the known fact that high lipid levels are characterized by insulin resistance, the presently observed low lipid content in hypermelatonemic rats should suggest increased insulin sensitivity. Though there was no apparent difference in hepatic or muscle sensitivity to insulin induced glucose uptake at 10 minutes, the significantly increased glucose uptake manifested at 90 minutes by tissues of control rats was not shown by hypermelatonemic rat tissues (Chapter 8). Since uptake promoted by acetylcholine or melatonin also did not record the increase at 90 minutes, rather than insulin resistance, an overall reluctance for tissue uptake of glucose by all agonists as an adaptive metabolic peculiarly induced by neonatal hypermelatonemia should be considered the most feasible explanation. The concomitant decrease in hepatic glycogen as well as tissue lipid contents seen in 60 day old hypermelatonemic rats could suggest negative carbohydrate and lipid balance due to increased energy expenditure. However, the significance/exigency of this increased energy expenditure defies any valid explanation. This is in contrast to

the control rats where there was an increase in lipid stores with only depletion of hepatic glycogen store (Chapter 7) suggesting a glycogenolytic and lipogenic *milieu* at this stage. The depletion in adipose, hepatic and muscle lipid contents seen in hypermelatonemic rats may suggest increased activity of lipolytic enzymes. It is pertinent to note that Mustonen. *et al*, (2002) have reported increased lipase and esterase activity in the liver of melatonin treated rats. Moreover, a decrease in brown adipose tissue has been correlated with a direct or indirect activation of sympathetic tone (Prunet-Marcassus *et al.*, 2001; Bartness *et al.*, 2002). Since in the previous study on *in vitro* glucose uptake, acetylcholine was shown to promote significant glucose uptake by control tissues which was attenuated in the hypermelatonemic rats (Chapter 8), suggesting decreased parasympathetic tone, it is reasonable to speculate a possible hyperactivity of sympathetic system and hence worthwhile to investigate. The decreased tissue lipid contents in hypermelatonemic rats is also supported by the recorded ability of melatonin to decrease fat pads and more specifically visceral fat deposits (Rasmussen *et al.*, 1999; Wolden-Hanson *et al.*, 2000).

As against the changes in tissue lipid content of control and hypermelatonemic rats, are the changes in tissue cholesterol contents which show a decrease in the former and an increase in the latter animals (Fig. and Tab.; 9.2, 9.4). These changes in tissue cholesterol contents are accompanied by complementary high serum level in the control and low serum level in hypermelatonemic rats (Fig. and Tab.; 9.5). This would suggest loss of tissue cholesterol stores leading to

hypermelatonemia in control rats and a reverse condition of storage of cholesterol in tissues resulting in hypocholesterolemia. The role of melatonin in the regulation of cholesterol metabolism is suggested by the observations of exogenous melatonin decreasing circulating cholesterol levels and augmented tissue cholesterol esterification (Esquifino *et al.*, 1997). A generalized long duration protective action of neonatal hypermelatonemia on adiposity is clearly suggested by the herein observed significant decrement in all serum lipid fractions (triglycerides, cholesterol, phospholipid, free fatty acids and total lipids). The increased tissue lipids and serum lipid fractions in the control rats are indicative of age associated increasing lipid level/adiposity leading towards the reported insulin resistance in literature. How neonatal melatonin excess can be translated into protective mechanisms of delayed expression of age associated lipid load and insulin resistance should be a future line of investigation of great relevance.

SUMMARY:

Previous studies have demonstrated that neonatal hypermelatonemia decreases lipid synthesis and increases lipid utilization in the weaning period (Chapter 3) Similar results were obtained in pubertal period (Chapter 6). The aim of the present study is to evaluate the long term effect of neonatal hypermelatonemia on tissue and blood lipid parameters it 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 60th day. The melatonin treated animals

showed significantly decreased hepatic and adipose tissue lipid contents. Whereas, the muscle lipid and cholesterol contents increased significantly, the hepatic cholesterol contents decreased significantly in the experimental rats. The adipose tissue cholesterol content increased significantly in the hypermelatonemic rats. There is a significant decrease in serum triglyceride, cholesterol, total lipid, phospholipid and free fatty acid levels in the melatonin treated rats The serum insulin level increased significantly in the experimental rats. A generalized long duration protective action of neonatal hypermelatonemia on adiposity is clearly suggested by the herein observed significant decrement in all serum lipid fractions. How neonatal melatonin excess can be translated into protective mechanisms of delayed expression of age associated lipid load and insulin resistance should be a future line of investigation of great relevance.