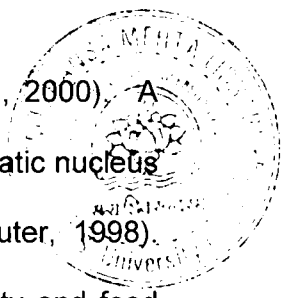
CHAPTER – 7

INCREASED TISSUE GLYCOGEN AND PROTEIN CONTENTS AND SERUM INSULIN LEVEL IN POST PUBERTAL RATS AS LONG TERM EFFECTS OF NEONATAL HYPERMELATONEMIA.

INTRODUCTION:

With regard to carbohydrate metabolism, both hyper and hypoglycemic effects of melatonin have been reported in a variety of animals (Ramachandran, 2002). Melatonin administration has been reported to increase (Delahaunty *et al.*, 1978, Dhar *et al.*, 1983; Mahata *et al.*, 1988; Zemen *et al.*, 1993) or decrease (Mahata *et al.*, 1988) or have no effects on blood glucose level (John *et al.*, 1990; Ramachandran, 2002). The mechanism by which melatonin modulates glycemic status is not clear. It is suggested that the hormone may act by interactions with other metabolic hormones like insulin, glucagon, growth hormone, corticosterone or catecholamines (Ramachandran, 2002). Melatonin has been shown to influence the plasma insulin level (Diaz and Blazquez, 1986), insulin secretion (Bailey *et al.*, 1974; Peschke *et al.*, 1997) and even possibly insulin action (Frankel and Trandberg, 1991). It is also known to modulate the liver insulin and glucagon receptor concentrations (Rodriguez *et al.*, 1989) and increase the



catecholamine content (Mahata *et al.*, 1988; Maitra *et al.*, 2000). A central site of action in the brain, possibly the supra chiasmatic nucleus has also been considered (Shima *et al.*, 1997; Van Cauter, 1998). Though melatonin is known to affect body weight, adiposity and food intake in seasonal animals (Wade and Bartness, 1984; Valtonen, M. *et al.*, 1995; Le Gouic S. *et al.*, 1996), the mechanism of melatonin action on energy metabolism in mammals is not well known. As a focal centre of intermediary metabolism, it is possible that melatonin might interfere with lipid metabolism. Melatonin is assumed to act directly on hepatocytes and pancreatic B cells (Acuna-Castroviejo *et al.*, 1994; Peschke *et al.*, 2000) or even indirectly through the supra-chiasmatic nucleus (Fleur *et al.*, 2001). The effects of melatonin administration to laboratory animals are at best controversial with differing effects on insulin secretion and glucose metabolism (Bailey *et al.*, 1974; Frankel and Strandberg, 1991; Iizuka, 1996; Bizot-Espiard *et al.*, 1998). The apparently contradictory results of melatonin action on carbohydrate metabolism are likely to be due to the various treatment schedules, doses, time and duration of administration as well as the animal species used and the maintenance conditions. Most of the studies pertain to adult or pubertal animals and there is no systematic study on short term or long term consequences of neonatal hypermelatonemia on glycemic status and carbohydrate metabolism. Earlier studies have shown significant higher insulin sensitivity with hyperinsulinemia and stimulated tissue glycogenesis in the weaning period and persistent higher glycogenic effect even in the pubertal period due to transient

pre-weaning hypermelatonemia (Chapter 1 & 4). In the present investigation, long term consequences of neonatal hypermelatonemia have been assessed in the mature stage (60 days) in terms of tissue glycogen and protein load, glycemic and insulinemic status and activities of enzymes related to carbohydrate metabolism.

MATERIAL AND METHODS: See page nos. 16-37

RESULTS:

- **Body and organ weights:** There is a significant decrease in the body weight of melatonin treated rats as compared to control rats. The relative weight of pancreas, liver, spleen and testes increased significantly while those of kidneys decreased significantly in the hypermelatonemic rats (Figure and Table; 7.1, 7.4, and 7.5).
- **Serum glucose and insulin levels:** Whereas, the serum glucose level decreased significantly, there is a significant increase in the serum insulin level of the melatonin treated animals as compared to the control animals (Figure and Table; 7 6).
- **Histological observations:** The islets of hypermelatonemic rats seem to be larger in size with less compactly packed cells. However there is significantly more number of B cells compared to controls. B cell neogenesis by transdifferentiation from acinar cells seems to be a distinct feature (Plate; 7 and 8)

Figure 7.1: Body weight of young adult rats on 60th day subjected to neonatal melatonin treatment:

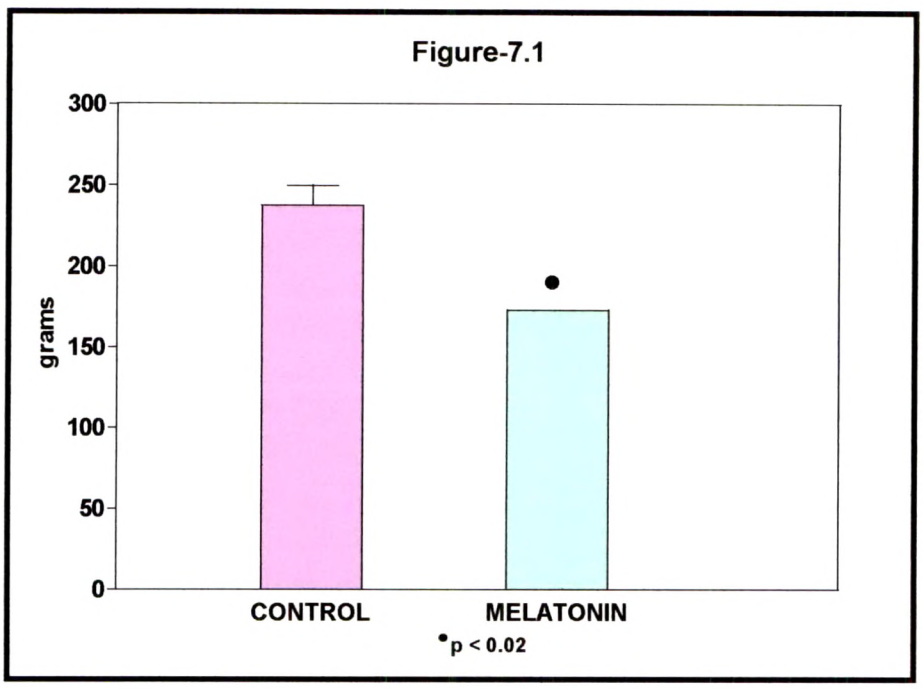


Table 7.1: Body weight of young adult rats on 60th day subjected to neonatal melatonin treatment:

	CONTROL	MELATONIN
BODY WEIGHT	237.25 ±12.38	173.00• ±0.02

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

Figure 7.2: Absolute weights of pancreas and adrenals of young adult rats on 60th day subjected to neonatal melatonin treatment:

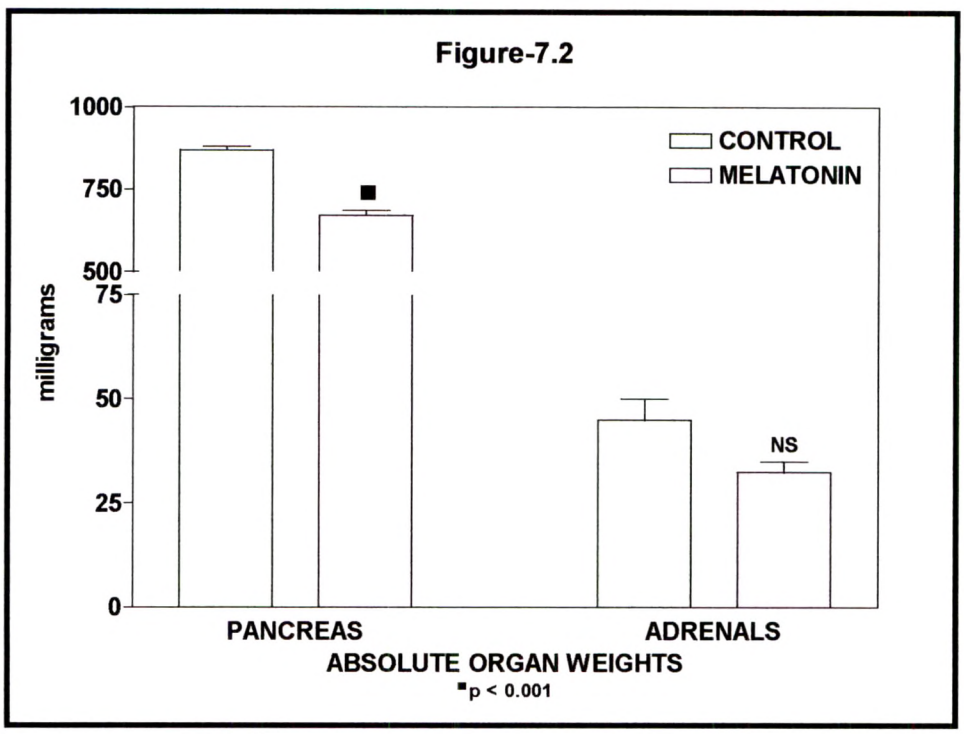


Table 7.2: Absolute weights of pancreas and adrenals of young adult rats on 60th day subjected to neonatal melatonin treatment:

	PANCREAS	ADRENALS
CONTROL	790.00 ±10.02	45.00 ±5.01
MELATONIN	672.25■ ±14.78	32.5NS ±2.5

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

Figure 7.3: Absolute weights of spleen, liver, testes and kidneys of young adult rats on 60th day subjected to neonatal melatonin treatment:

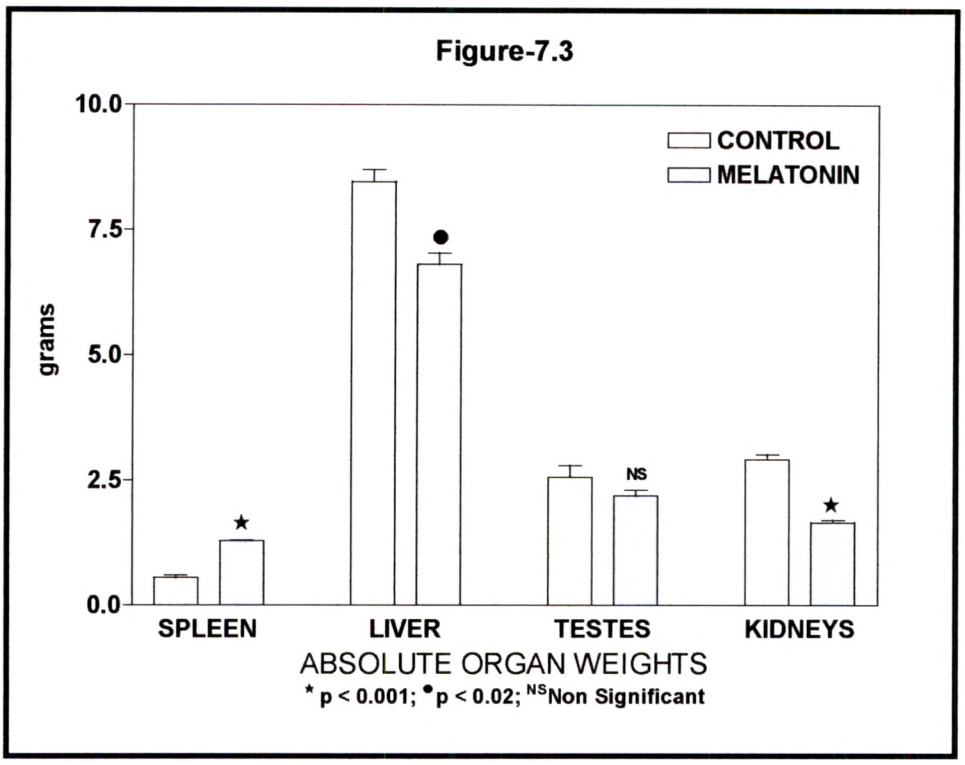


Table 7.3: Absolute weights of spleen, liver, testes and kidneys of young adult rats on 60th day subjected to neonatal melatonin treatment:

	SPLEEN	LIVER	TESTES	KIDNEYS
CONTROL	0.55 ±0.05	8.46 ±0.24	2.56 ±0.24	2.92 ±0.10
MELATONIN	1.29* ±0.014	6.81• ±0.23	2.19NS ±0.11	1.66* ±0.049

Values are expressed as mean ± SEM, * p < 0.001; p < 0.02
NS Non Significant

Figure 7.4: Relative weights of adrenals, pancreas and spleen of young adult rats on 60th day subjected to neonatal melatonin treatment:

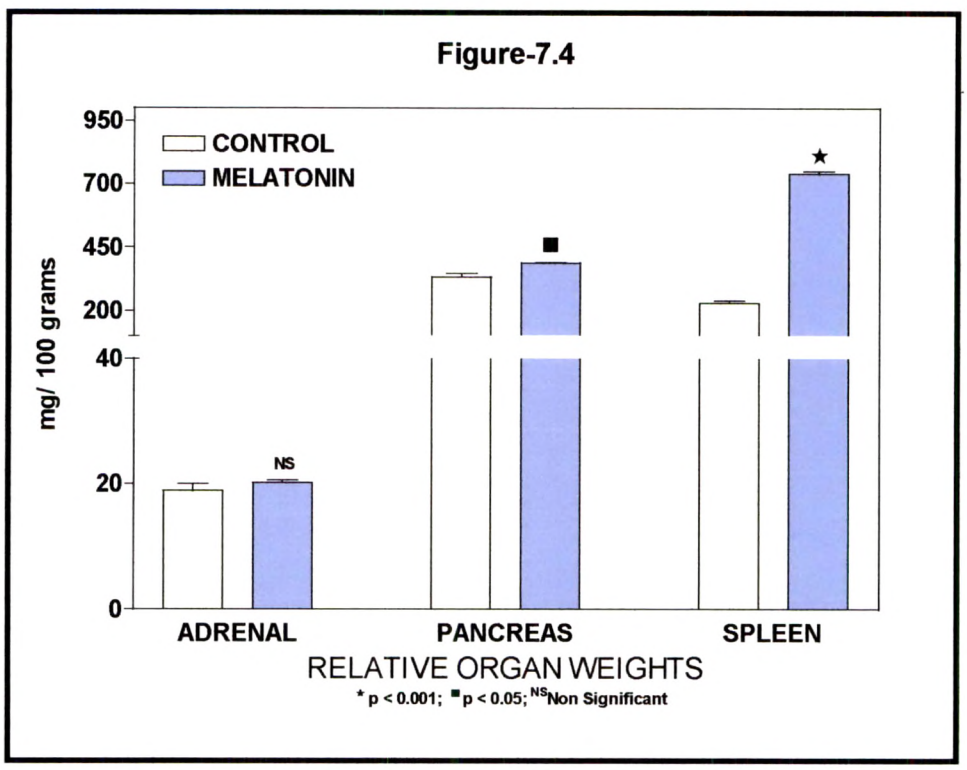


Table 7.4: Relative weights of adrenals, pancreas and spleen of young adult rats on 60th day subjected to neonatal melatonin treatment:

	ADRENAL	PANCREAS	SPLEEN
CONTROL	18.90 ±1.12	333.66 ±13.19	231.35 ±0.042
MELATONIN	20.23 ±0.34	388.55■ ±1.79	740.00* ±9.9

Values are expressed as mean ± SEM, * p < 0.001; ■p < 0.05; NS Non Significant

Figure 7.5: Relative weights of liver, testes and kidneys of young adult rats on 60th day subjected to neonatal melatonin treatment:

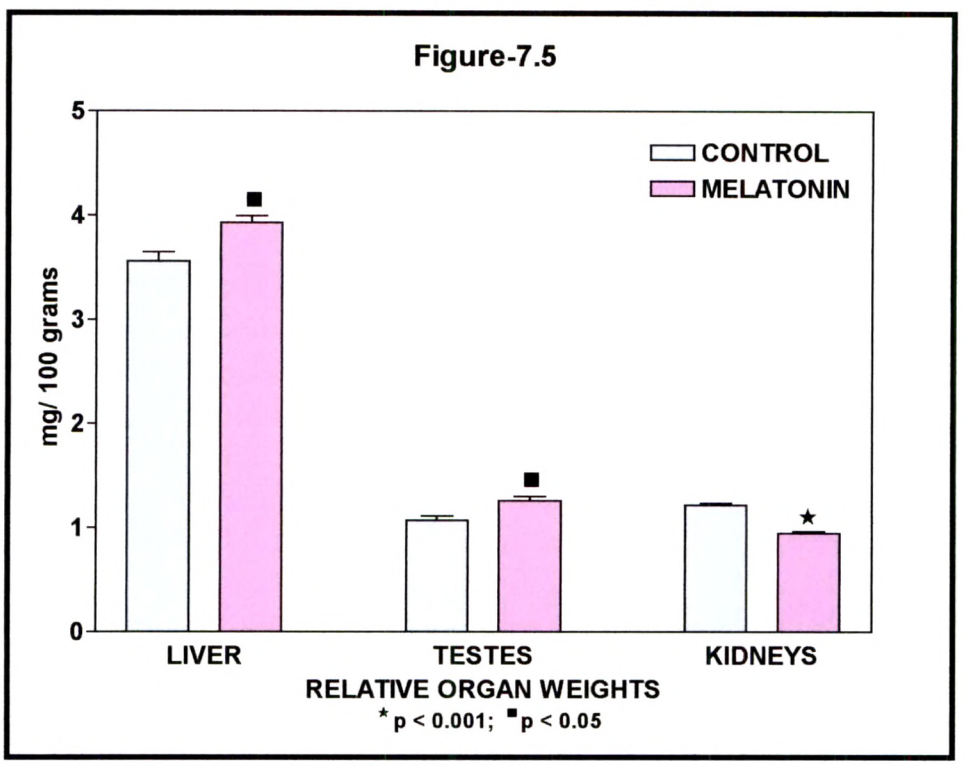


Table 7.5: Relative weights of liver, testes and kidneys of young adult rats on 60th day subjected to neonatal melatonin treatment:

	LIVER	TESTES	KIDNEYS
CONTROL	3.56 ±0.085	1.07 ±0.042	1.22 ±0.014
MELATONIN	3.93■ ±0.064	1.26■ ±0.04	0.95* ±0.014

Values are expressed as mean ± SEM, * p < 0.001; ■p < 0.05

Figure 7.6: Serum insulin and glucose levels of young adult rats on 60th day subjected to neonatal melatonin treatment:

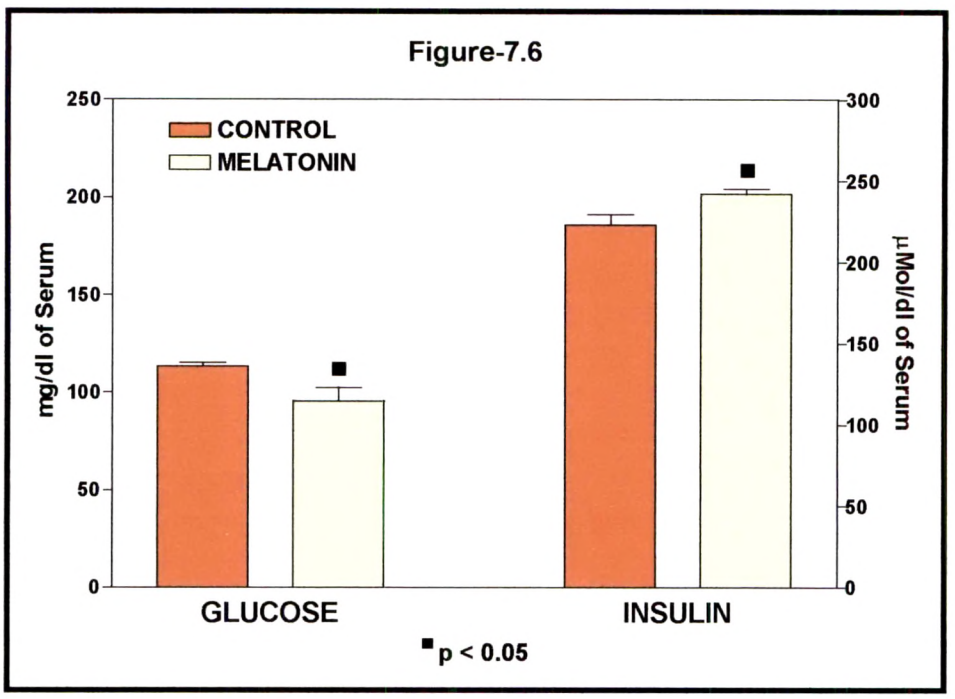


Table 7.6: Serum insulin and glucose levels of young adult rats on 60th day subjected to neonatal melatonin treatment:

	INSULIN	GLUCOSE
CONTROL	185.85 ±5.26	113.15 ±1.92
MELATONIN	201.66■ ±2.59	95.57■ ±6.74

Values are expressed as mean ± SEM, ■p < 0.05

Figure 7.7: Glycogen phosphorylase activity in liver and muscle of young adult rats on 60th day subjected to neonatal melatonin treatment:

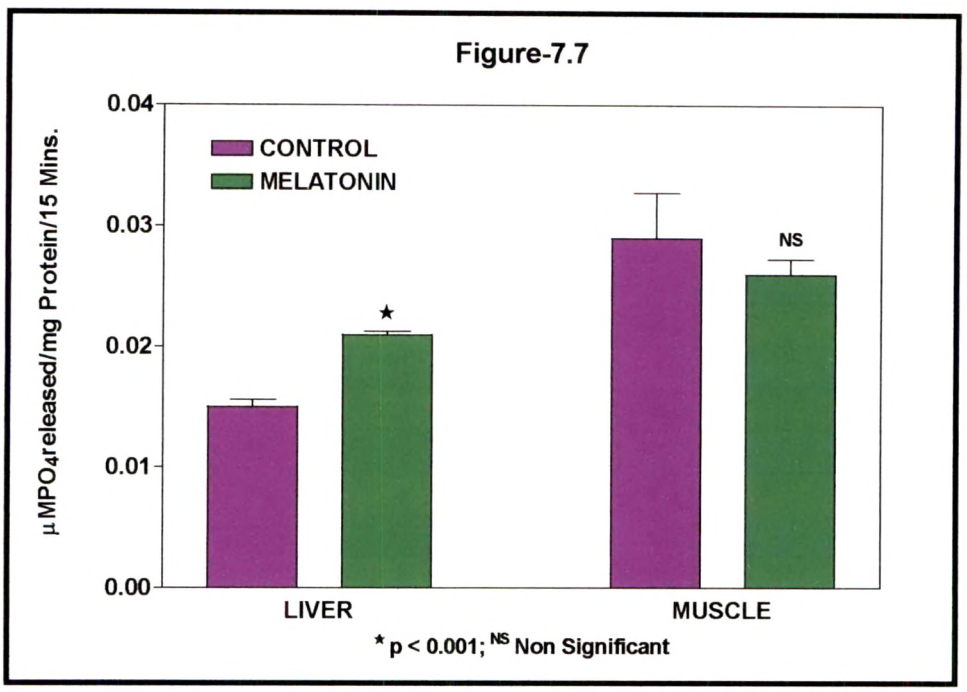


Table 7.7: Glycogen phosphorylase activity in liver and muscle of young adult rats on 60th day subjected to neonatal melatonin treatment:

	LIVER	MUSCLE
CONTROL	0.015 ±0.0006	0.029 ±0.0037
MELATONIN	0.021* ±0.00028	0.026NS ±0.0012

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

Figure 7.8: Glucose-6-phosphatase activity in the liver of young adult rats on 60th day subjected to neonatal melatonin treatment:

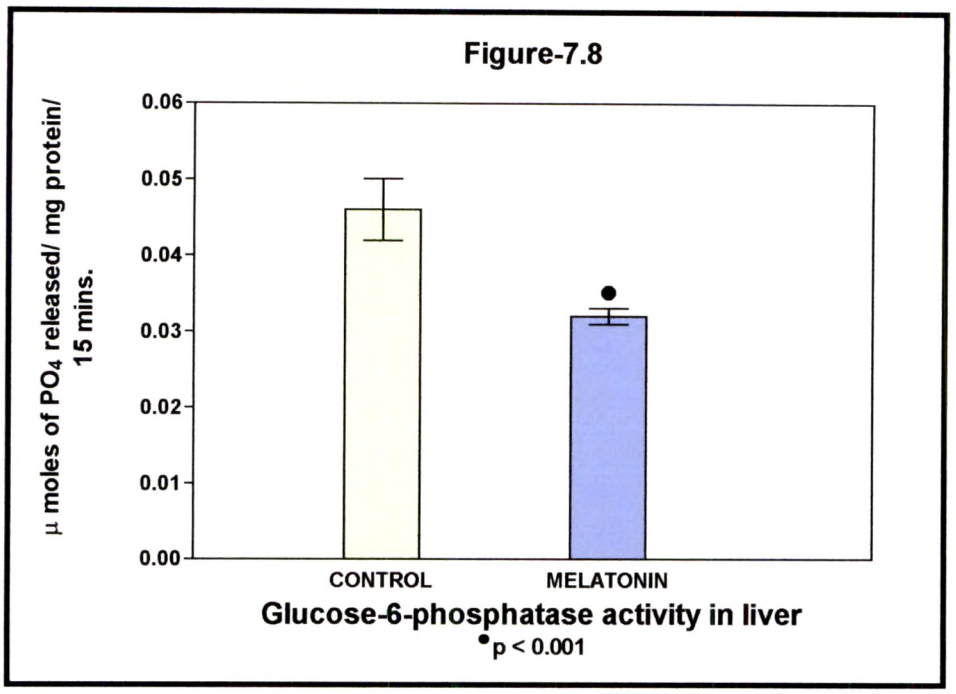


Table 7.8: Glucose-6-phosphatase activity in the liver of young adult rats on 60th day subjected to neonatal melatonin treatment:

	CONTROL	MELATONIN
GLUCOSE-6-PHOPHATASE	0.046 ±0.0041	0.032• ±0.0010

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

Figure 7.9: Glycogen synthetase activity in liver and muscle of young adult rats on 60th day subjected to neonatal melatonin treatment:

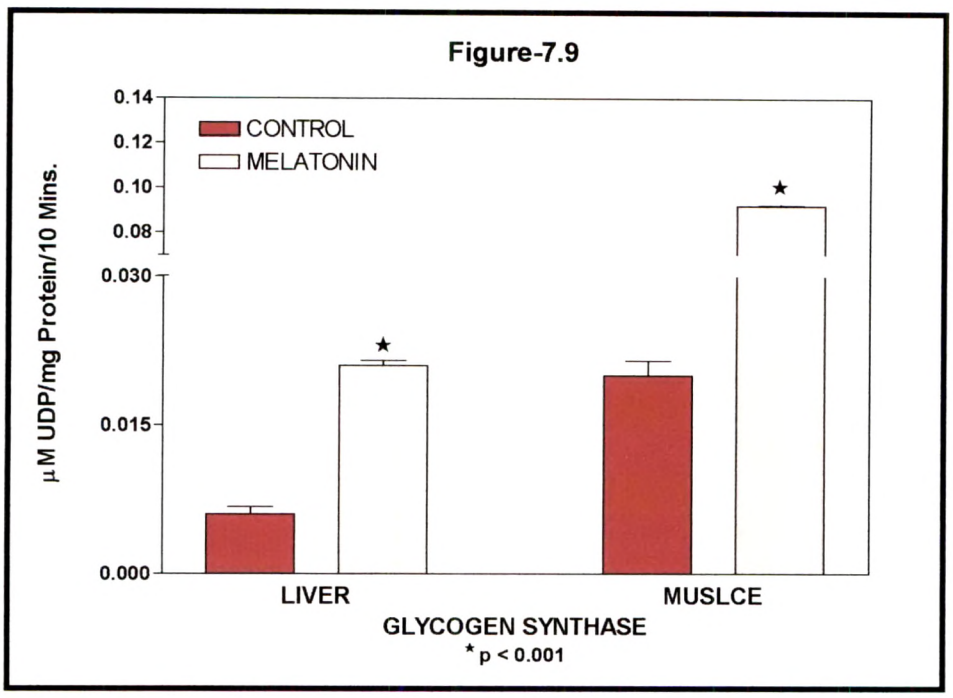


Table 7.9: Glycogen synthetase activity in liver and muscle of young adult rats on 60th day subjected to neonatal melatonin treatment:

	LIVER	MUSCLE
CONTROL	0.006 ±0.00075	0.020 ±0.0015
MELATONIN	0.021* ±0.00047	0.092* ±0.00042

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

Figure 7.10: Hepatic and muscle glycogen content of the young adult rats on 60th day subjected to neonatal melatonin treatment:

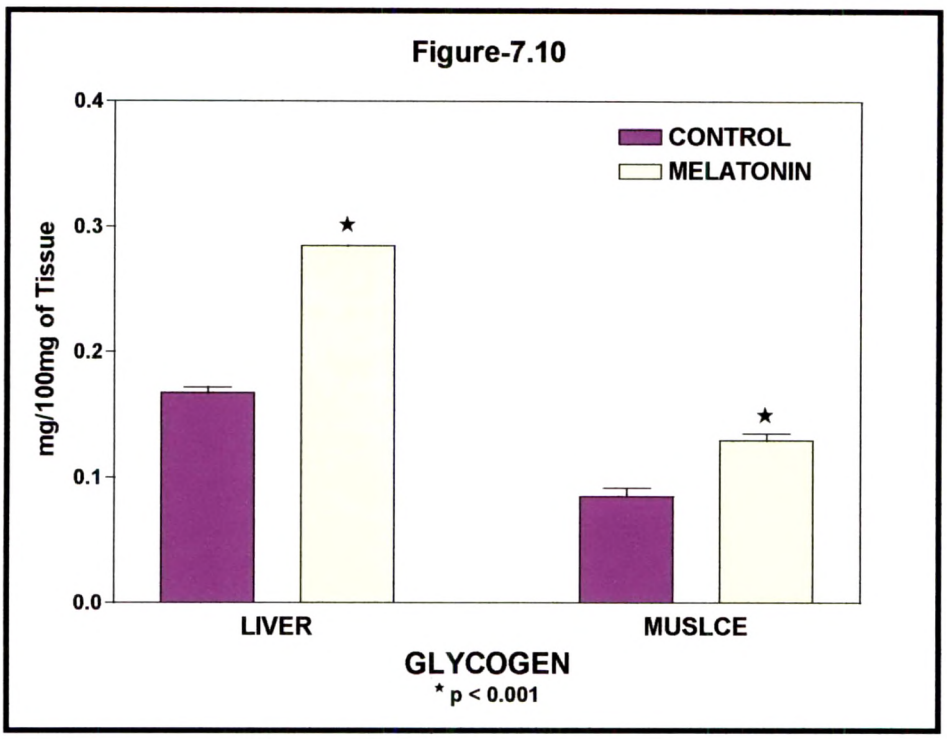


Table 7.10: Hepatic and muscle glycogen content of young adult rats on 60th day subjected to neonatal melatonin treatment:

	LIVER	MUSCLE
CONTROL	0.1672 ±0.0048	0.0848 ±0.0069
MELATONIN	0.2846* ±0.00041	0.1297* ±0.0055

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

Figure 7.11: Hepatic and muscle protein content of the young adult rats on 60th day subjected to neonatal melatonin treatment:

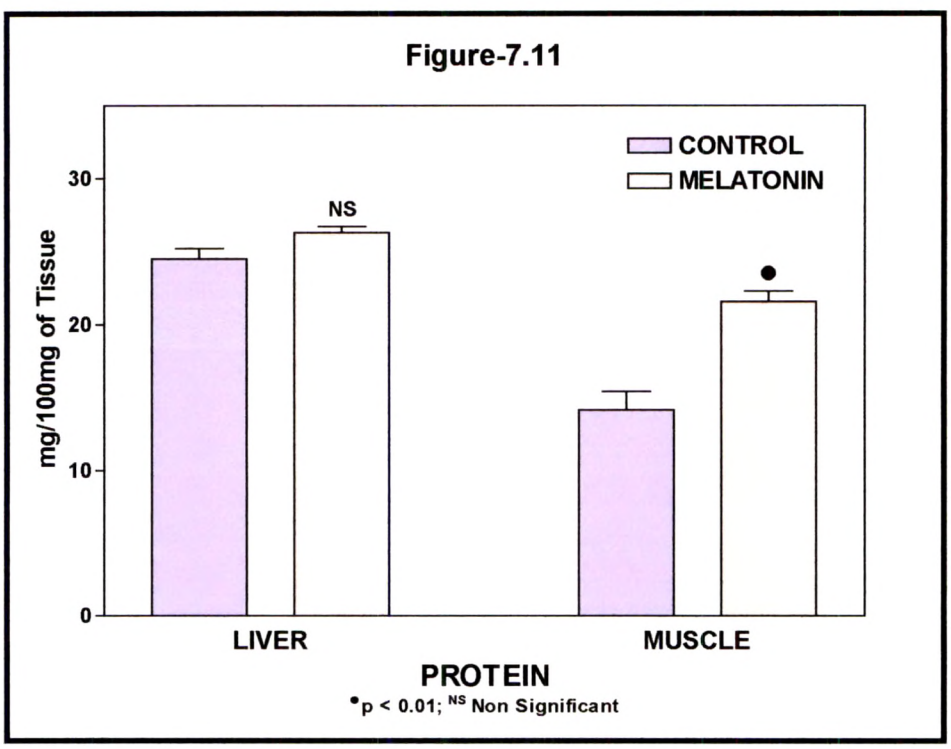


Table 7.11: Hepatic and muscle protein content of the young adult rats on 60th day subjected to neonatal melatonin treatment:

	LIVER	MUSCLE
CONTROL	24.49 ±0.75	14.16 ±1.26
MELATONIN	26.32^{NS} ±0.41	21.54$^{\bullet}$ ±0.72

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

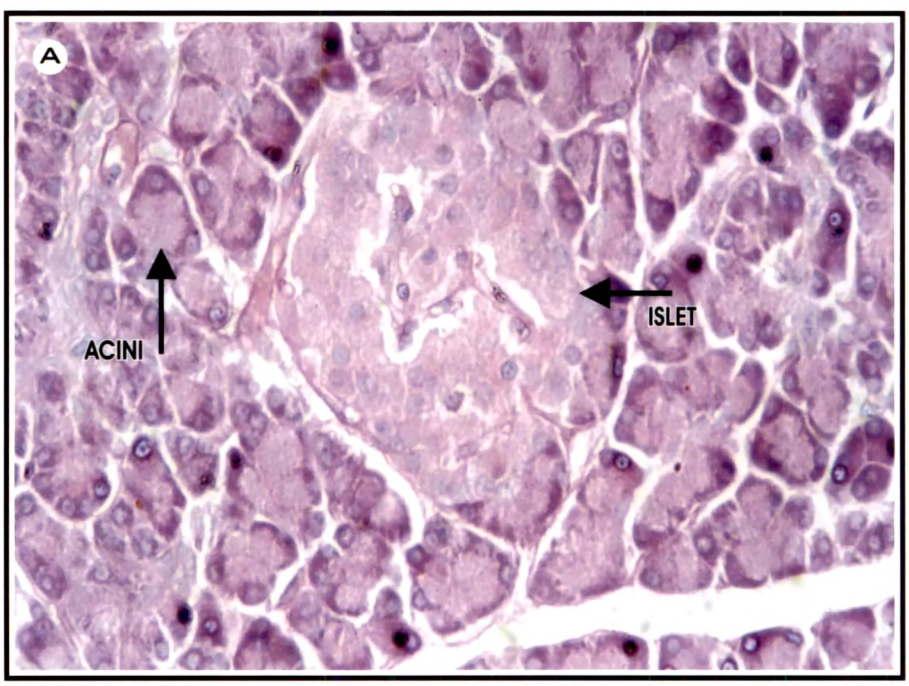
PLATE – 7

Photomicrographs of sections of pancreas – 450 X

FIGURE (A): Transverse section of the pancreas of male control adult (60^{th} day) rats showing islet and pancreatic acini. Note the centrally distributed A cells, peripherally distributed B cells. The acinar and islet cells are loosely packed with a reduced cell number.

FIGURE (B): Transverse section of the pancreas of male hypermelatonemic rats on the 60^{th} day showing islet and pancreatic acini. The A cells can be viewed in the core of the islet area whereas the B cells are prominently seen on the periphery of the islet. The acinar and islet cells are loosely packed, the number of B is increased while the number of A cells is decreased

PLATE - 7



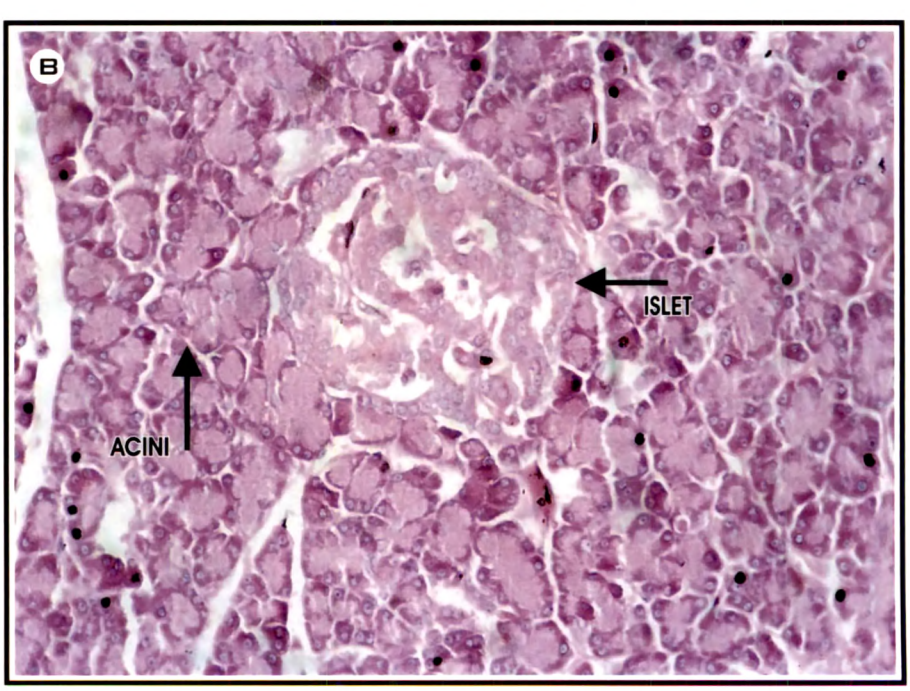


PLATE – 8

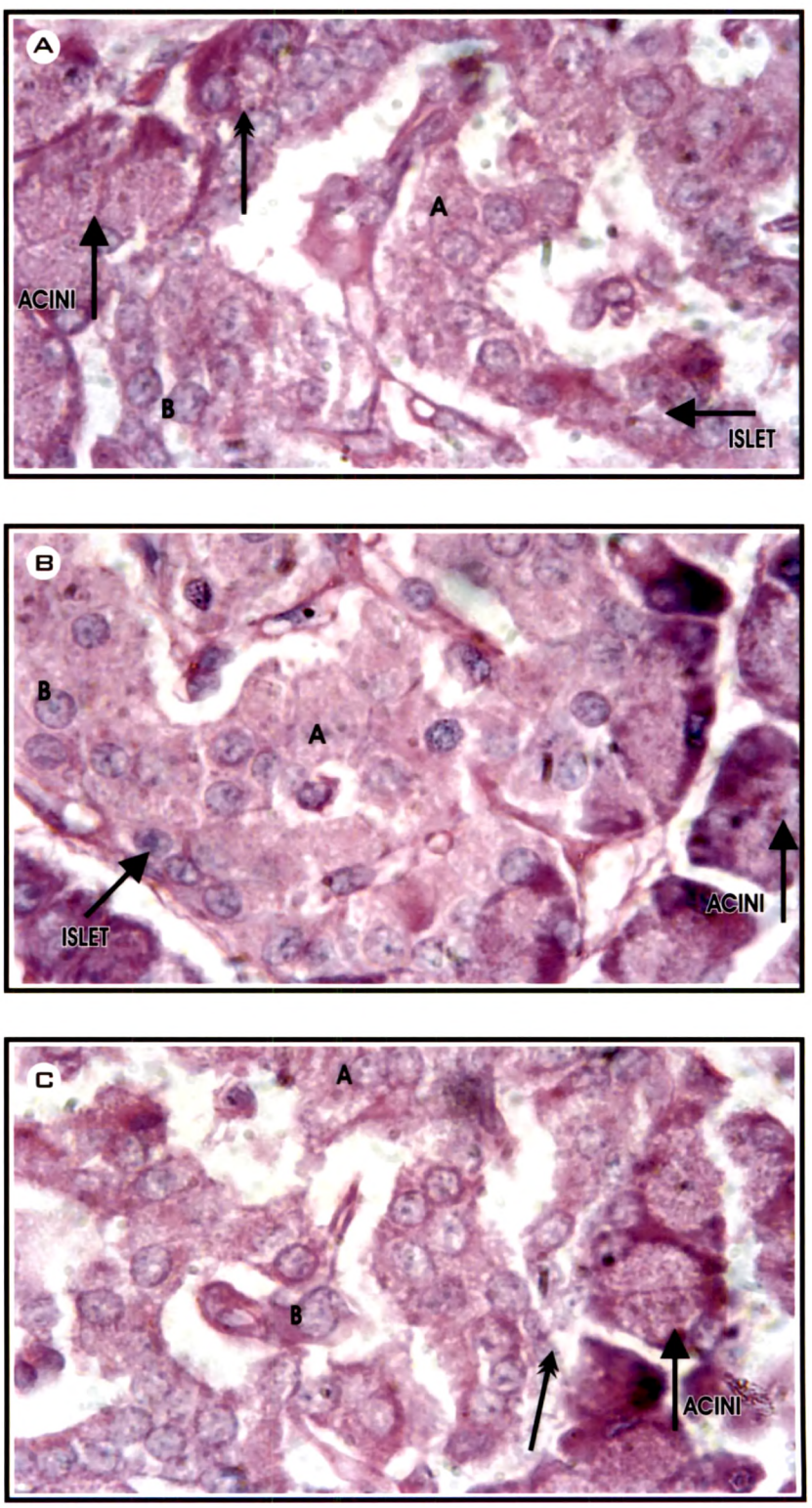
Photomicrographs of sections of pancreas – 1000 X

FIGURE (A): Transverse section of the pancreas of male hypermelatonemic rats on the 60th day showing islet and pancreatic acini. The islet and acinar cells are loosely packed with empty spaces in between. There is an increase in the islet and acinar cell number; also, the transdifferentiating cells (double headed arrow) are clearly visible

FIGURE (B): Transverse section of the pancreas of male control adult (60th day) rats showing islet and pancreatic acini. Note the centrally distributed A cells, peripherally distributed B cells with almost an equal number of A and B cells.

FIGURE (C): Transverse section of the pancreas of male hypermelatonemic rats on the 60th day showing islet and pancreatic acini. The acinar and islet cells are loosely packed. The A cells can be viewed in the core of the islet area whereas the B cells are prominently seen on the periphery of the islet. Note the transdifferentiating cells (double headed arrow).

PLATE - 8



- **Hepatic glycogen content and the activities of glycogen synthetase, glycogen phosphorylase and glucose-6-phosphatase:** The melatonin treated rats showed a significant increase in the hepatic glycogen content and the activities of glycogen synthetase and glycogen phosphorylase, while the activity of glucose-6-phosphatase decreased significantly in the melatonin treated rats (Figure and Table, 7.7, 7.8, 7.9 and 7.10).
- **Muscle glycogen content and the activities of glycogen synthetase and glycogen phosphorylase:** The muscle glycogen content and the activity of glycogen synthetase increased significantly in the hypermelatonemic rats whereas the activity of glycogen phosphorylase decreased marginally in the melatonin treated rats (Figure and Table; 7.7, 7.9 and 7.10).
- **Hepatic and muscle protein content:** The muscle protein content increased significantly while the hepatic protein content showed a marginal increase in the melatonin treated rats (Figure and Table; 7.11).

DISCUSSION: Neonatal stage being a sensitive phase whence many neuroendocrine axes are gradually getting established any imbalance as an excess or deficiency of a particular hormone can have significant effect on the overall neuroendocrine status and consequent long term effects on adult homeostasis. Melatonin being a hormone with central and peripheral effects can be considered as one such hormone whose imbalance in the neonatal period could have a profound influence on adult homeostasis. The present study which has evaluated the effects

clearly manifested in the muscle of control animals. Since there is no significant change in the muscle glycogen content or tissue protein contents of hypermelatonemic animals not only a decreased anabolic influence but even a reduced insulin resistance may be presumed. The significantly higher muscle glycogen and protein content of the hypermelatonemic animals is therefore a consequence of the higher levels attained at the pubertal age itself. Clearly, transient neonatal hypermelatonemia has no significant effect on adult carbohydrate homeostasis except for higher tissue glycogen and protein contents which are a consequence of significantly high levels attained at the pubertal stage. There is nevertheless a tendency for hyperinsulinemia with a 68% increase from the pubertal level as against only 32% increase in the control animals. The higher insulin level in hypermelatonemic rats is substantiated by the relatively higher number of B cells, many of which are generated by transdifferentiation of acinar cells and increased B:A cell ratio, thereby providing histo-numerical evidence to the same end (Plate; 7 and 8).

Overall it can be concluded that there is similar degree of glycogenolysis in both control and experimental animals indicating utilization for energy purposes and the hypermelatonemic animals show a higher tissue load of metabolites essentially due to the increase recorded in the pubertal age. The hyperinsulinemic status of hypermelatonemic animals may have to be evaluated in terms of possible insulin resistance at later stages.

The body weight of hypermelatonemic animals is significantly low with significantly reduced relative weight of pancreas, liver, testes, kidney and adrenals and only spleen shows a significant increase, which may be related with increased immune functions in these animals.

SUMMARY:

Earlier studies have shown significant higher insulin sensitivity with hyperinsulinemia and stimulated tissue glycogenesis in the weaning period and persistent higher glycogenic effect even in the pubertal period due to transient pre-weaning hypermelatonemia (Chapter 1 & 4). In the present investigation, long term consequences of neonatal hypermelatonemia have been assessed in the mature stage (60 days) in terms of tissue glycogen and protein load, glycemic and insulinemic status and activities of enzymes related to carbohydrate metabolism. 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 60^{th} day. There is a significant decrease in body weight and relative weight of kidneys of melatonin treated animals. The relative weight of pancreas, liver and spleen increased significantly. The serum insulin level increased while, the serum glucose level decreased significantly in the experimental rats. Whereas the hepatic and muscle glycogen contents and the activity of glycogen synthetase increased significantly in the experimental rats, the activity of glucose-6-phosphatase decreased significantly in the muscle of the experimental rats. The activity of glycogen phosphorylase increased significantly in

the liver while remained unaltered in the muscle of experimental rats. The muscle protein content increased significantly while the hepatic protein content remained unaltered in the melatonin treated rats The islets of hypermelatonemic rats seem to be larger in size with less compactly packed cells. Overall it can be concluded from the present study that there is similar degree of glycogenolysis in both control and experimental animals indicating utilization for energy purposes and the hypermelatonemic rats show a higher tissue load of metabolites essentially due to the increase recorded in the pubertal age. The hyperinsulinemic status of hypermelatonemic rats may have to be evaluated in terms of possible insulin resistance at later stages.