CHAPTER – 7

DECREASED HEPATIC GLYCOGEN CONTENT BUT INCREASED MUSCLE GLYCOGEN, TISSUE PROTEIN AND SERUM GLUCOSE LEVEL WITH HYPOINSULINEMIA AS A LONG TERM EFFECTS OF NEONATAL MELATONIN ANTAGONISM IN POST PUBERTAL RATS.

INTRODUCTION:

A wide range of physiological functions is being regulated and modulated by the internally generated melatonin signal (Amstrong, 1989; Carneiro *et al.*, 1991; Cipolla-Neto *et al.*, 1991). An indication for the role of pineal gland and melatonin in the regulation of carbohydrate metabolism had come quite early from studies on humans and rodents (Alcozer *et al.*, 1956; Milcu *et al.*, 1971). It has been demonstrated that pinealectomized rats show decreased hepatic and muscle glycogenesis and an increase in blood pyruvate concentration (Milcu *et al.*, 1971; Mellado *et al*,. 1986). Further, pinealectomy has been shown to increase blood sugar levels in normal as well as alloxan treated rats (Csaba and Barath, 1971). Effects of pinealectomy on many other physiological parameters involved in carbohydrate metabolism have also been demonstrated (Diaz and Blazquez, 1986). In recent times a relationship between melatonin and regulation of carbohydrate

metabolism is under increasing scrutiny (Van Cauter *et al.*, 1989, 1991; Lima *et al.*, 1998; Peschke and Peschke, 1998). Enhanced adipocyte sensitivity to insulin, induced by melatonin, has also been demonstrated (Lima *et al.*, 1994). As a corollary, pinealectomy has been shown to decrease insulin response and manifest a fall in GLUT-4 content in adipose and muscle tissues (Lima *et al.*, 1998). It has also been shown that melatonin suppresses insulin secretion under several experimental conditions (Feldman and Lebovitz, 1972; Peschke and Peschke, 1998; La Fleur, 2001).

Since no studies are available on neonatal hypo or hypermelatonemia in terms of immediate or long term effects on carbohydrate or lipid metabolism, such studies have been initiated in this laboratory. Accordingly, rat neonates subjected to functional hypomelatonemia by receptor antagonism had shown significantly increased tissue glycogen contents with hyperinsulinemia as immediate effect in the weaning period (Chapter – 1). Further, significantly decreased tissue glycogen contents coupled with hyperinsulinemia and hyperglycemia and decreased insulin sensitivity were recorded as long term effects of neonatal melatonin antagonism in the pubertal stage (Chapter 1, 2, 4 & 5). In continuation, the present study tries to evaluate the long term effects of neonatal melatonin antagonism on carbohydrate metabolism, tissue protein contents and serum insulin and glucose levels in the post pubertal sexually mature rats.

MATERIAL AND METHODS: See page numbers 18-38

RESULTS:

- **Body and organ weights:** The body weight of luzindole treated animals showed a significant increase. Whereas the relative weights of liver, kidney and adrenals decreased significantly in the experimental animals, the relative weight of pancreas and testes did not show any significant alteration. The relative weight of spleen increased significantly in the experimental rats (Figure and Table; 7.1, 7.4, 7.5).
- **Serum glucose and insulin level:** Serum glucose level increased significantly while the serum insulin level decreased significantly in the luzindole treated rats as compared to control rats (Figure and Table; 7.6)
- **Hepatic glycogen content and the activities of glycogen synthetase, glycogen phosphorylase and glucose-6-phosphatase:** There is a significant decrease in the hepatic glycogen content of the luzindole treated animals. Whereas, the activity of glycogen synthetase increased significantly the activitiy of glucose-6-phosphatase decreased in the luzindole treated rats. The glycogen phosphorylase activity remained unaltered in the liver of he experimental rats (Figure and Table; 7.7, 7.8, 7.9, 7.10)

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

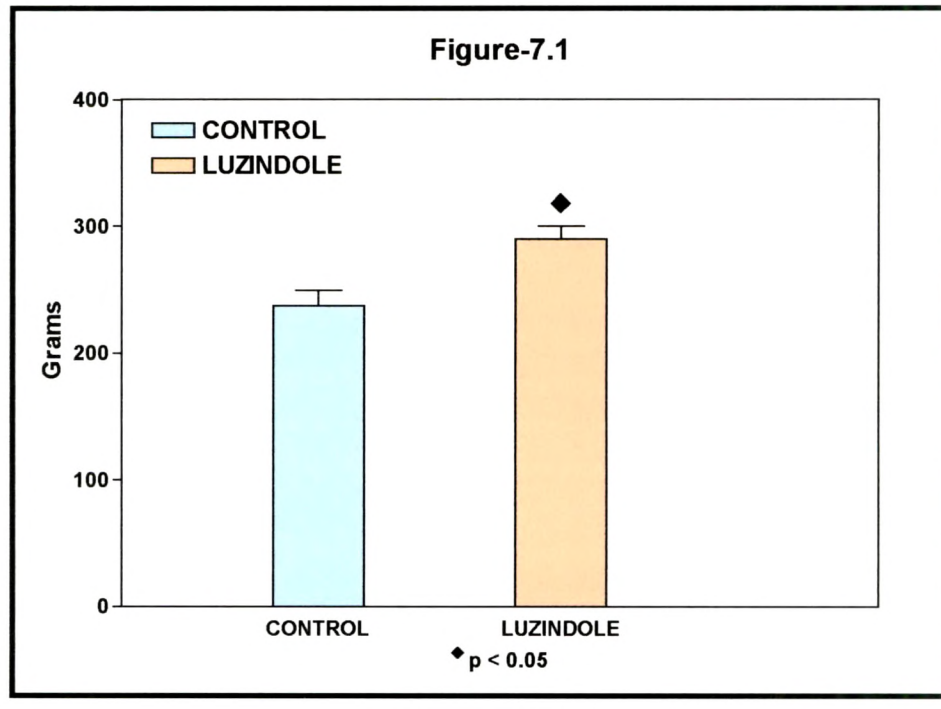


Table 7.1: Body weight of adult rats on 60th day subjected to neonatal luzindole treatment:

	CONTROL	LUZINDOLE
BODY WEIGHT	237.25 ±12.38	290.00◆ ±10.25

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

Figure 7.2: Absolute weight of pancreas and adrenals of adult rats on 60th day subjected to neonatal luzindole treatment:

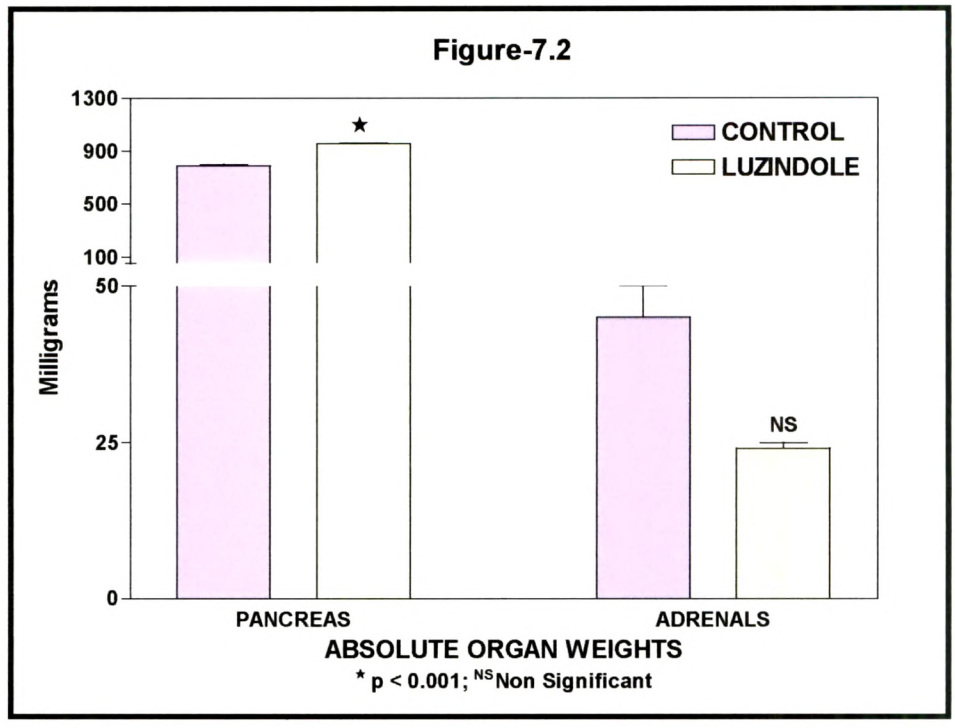


Table 7.2: Absolute weight of pancreas and adrenals of adult rats on 60th day subjected to neonatal luzindole treatment:

	PANCREAS	ADRENALS
CONTROL	790.00 ±10.02	45.00 ±5.01
LUZINDOLE	955.00* ±6.40	24.00NS ±0.90

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

Figure 7.3: Absolute weight of spleen, liver, testes and kidney of adult rats on 60th day subjected to neonatal luzindole treatment:

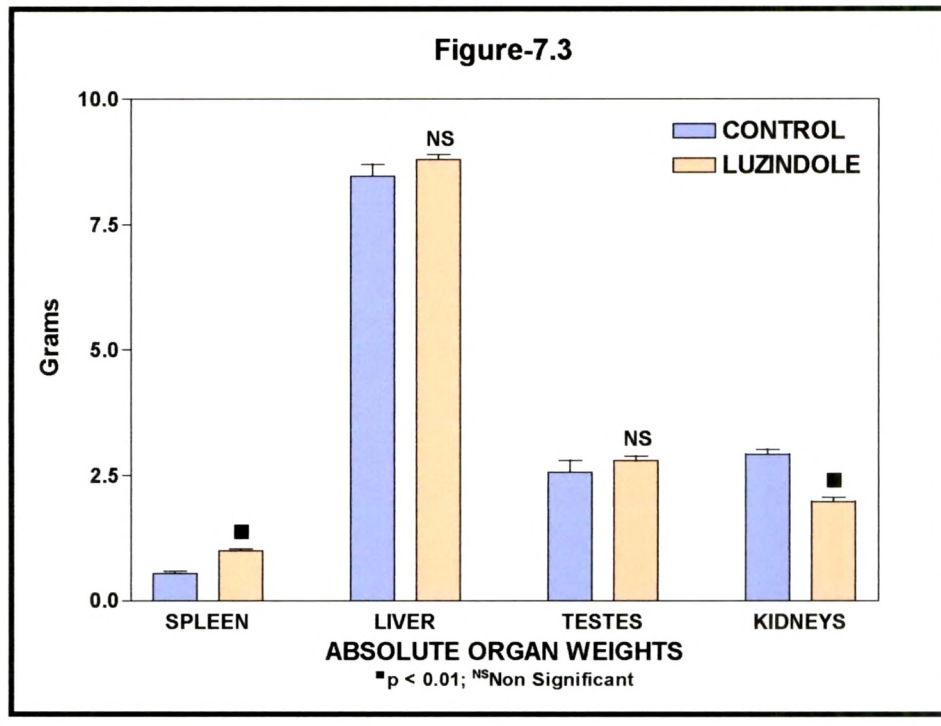


Table 7.3: Absolute weight of spleen, liver, testes and kidney of adult rats on 60th day subjected to neonatal luzindole treatment:

	SPLEEN	LIVER	TESTES	KIDNEYS
CONTROL	0.55 ±0.05	8.46 ±0.24	2.56 ±0.24	2.92 ±0.10
LUZINDOLE	1.00■ ±0.039	8.79NS ±0.099	2.79NS ±0.095	1.98■ ±0.087

Values are expressed as mean ± SEM, ■p < 0.01; NSNon Significant

Figure 7.4: Relative weight of adrenals, pancreas, spleen of adult rats on 60th day subjected to neonatal luzindole treatment:

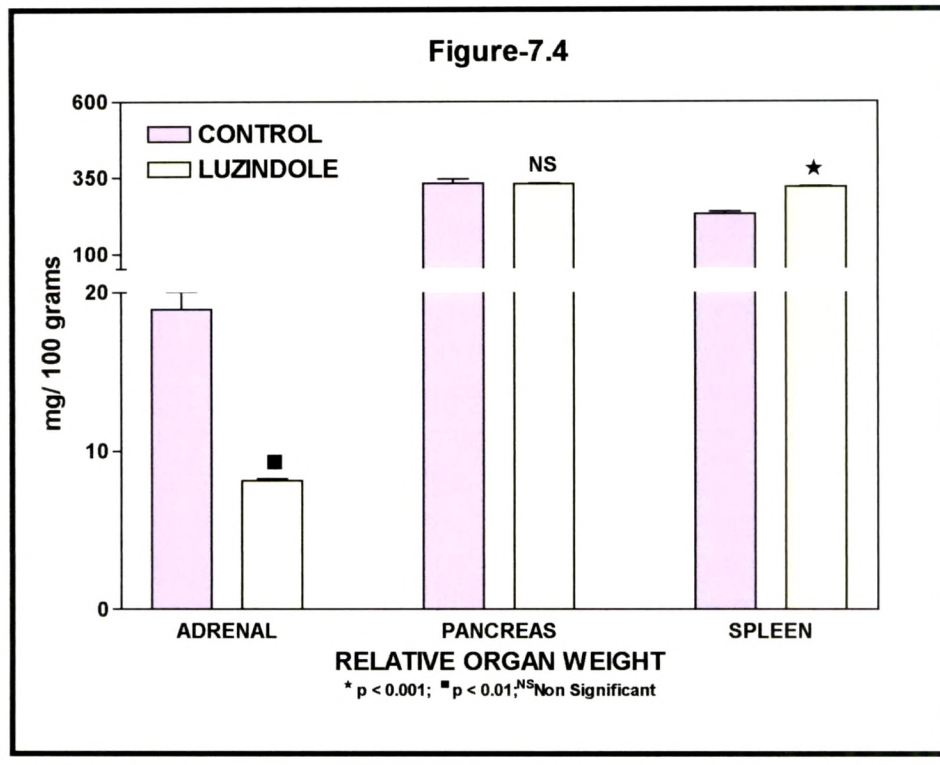


Table 7.4: Relative weight of adrenals, pancreas, spleen of adult rats on 60th day subjected to neonatal luzindole treatment:

	ADRENAL	PANCREAS	SPLEEN
CONTROL	18.90 ±1.12	333.66 ±13.19	231.35 ±0.042
LUZINDOLE	8.11■ ±0.14	330.00NS ±1.40	320.00* ±1.90

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

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

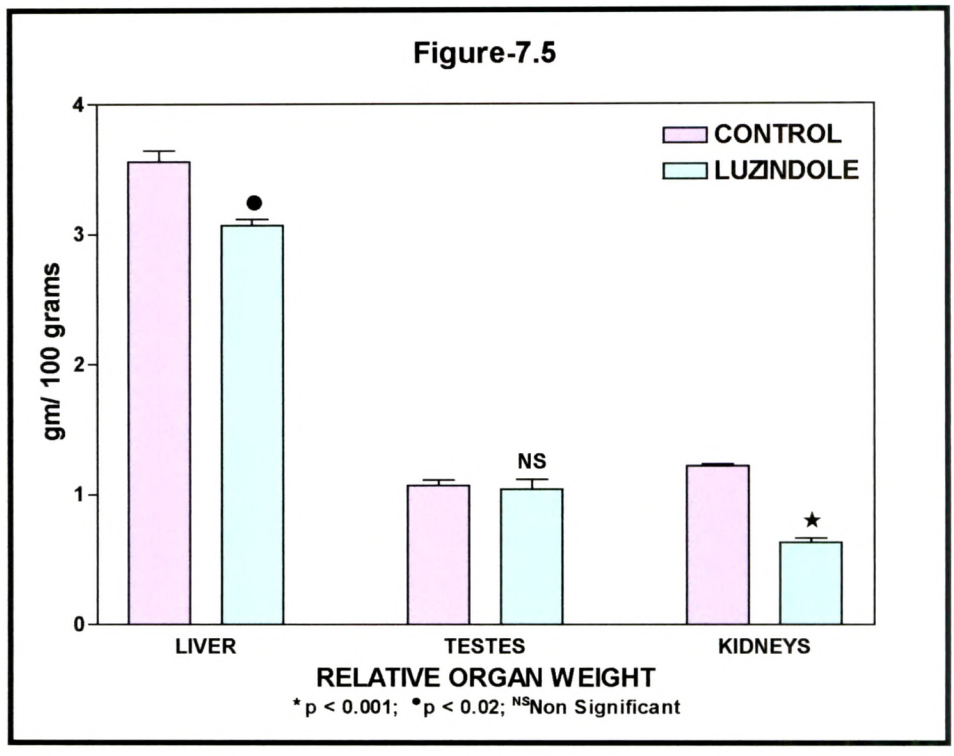


Table 7.5: Relative weight of liver, testes and kidneys adult rats on 60th day subjected to neonatal luzindole treatment:

	LIVER	TESTES	KIDNEYS
CONTROL	3.56 ±0.085	1.07 ±0.042	1.22 ±0.014
LUZINDOLE	3.07• ±0.044	1.04NS ±0.078	0.63* ±0.039

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

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

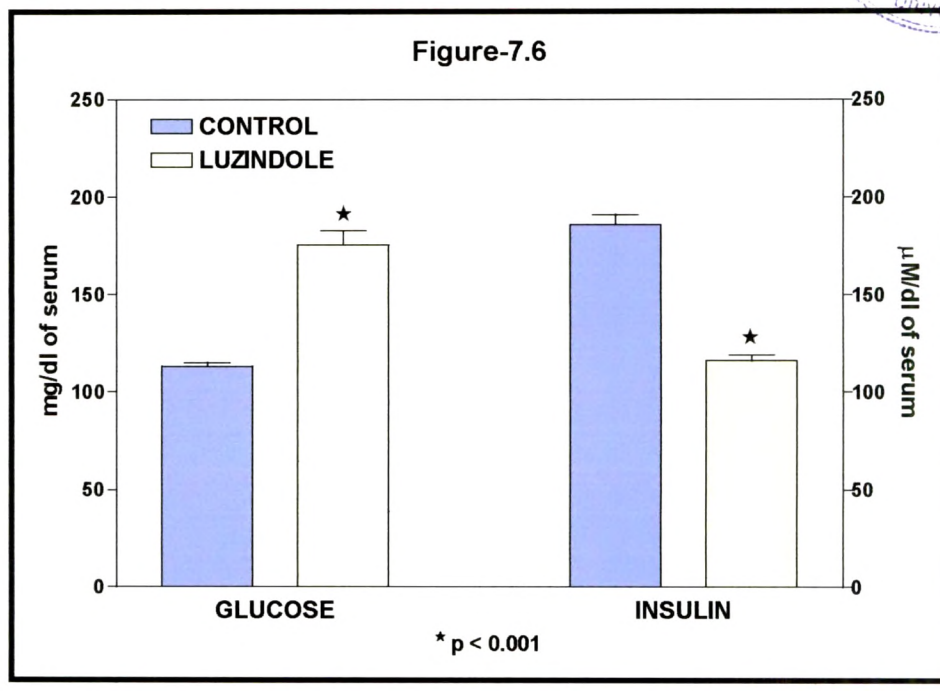


Table 7.6: Serum insulin and glucose levels of adult rats on 60th day subjected to neonatal luzindole treatment:

	INSULIN	GLUCOSE
CONTROL	185.85 ±5.259	113.15 ±1.921
LUZINDOLE	116.09* ±2.9235	175.53* ±7.225

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

Figure 7.7: Activity of glycogen phosphorylase in liver and muscle of adult rats on 60th day subjected to neonatal luzindole treatment:

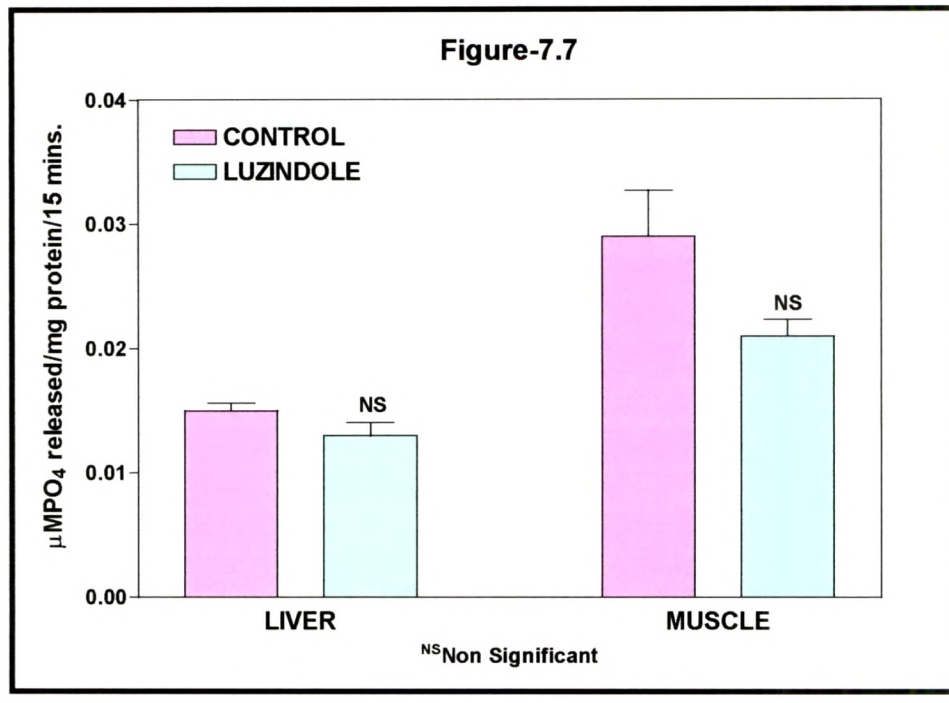


Table 7.7: Activity of glycogen phosphorylase in liver and muscle of adult rats on 60th day subjected to neonatal luzindole treatment:

	LIVER	MUSCLE
CONTROL	0.015 ±0.0006	0.029 ±0.0037
LUZINDOLE	0.013[NS] ±0.00105	0.021[NS] ±0.0013

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

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

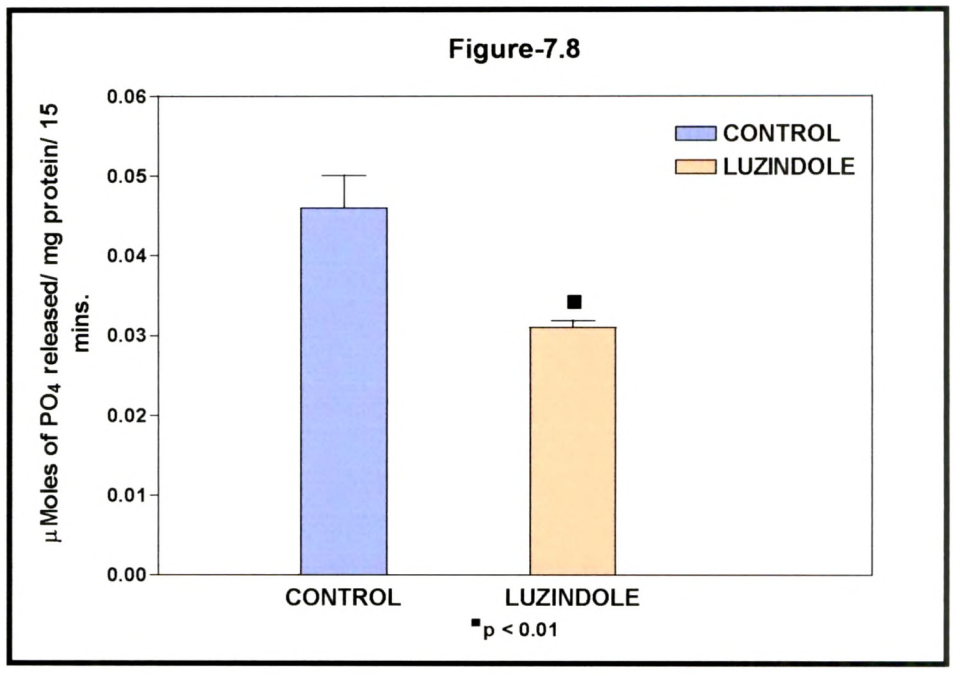


Table 7.8: Glucose-6-phosphatase activity in the liver of adult rats on 60th day subjected to neonatal luzindole treatment:

	CONTROL	LUZINDOLE
GLUCOSE-6-PHOPHATASE	0.046 ±0.0041	0.031■ ±0.0009

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

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

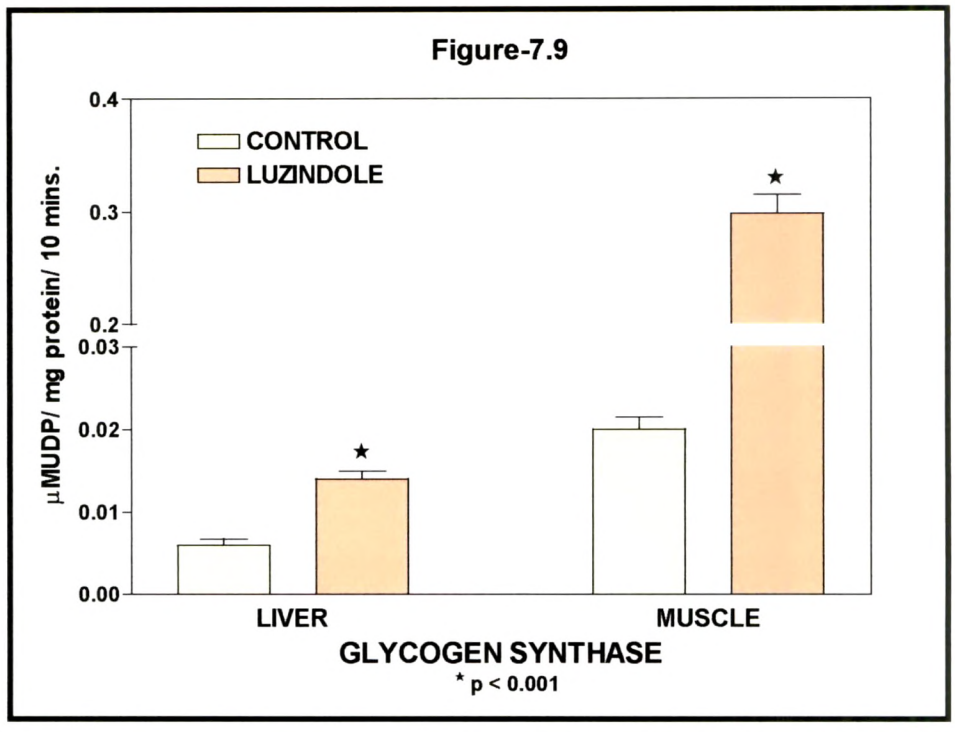


Table 7.9: Glycogen synthetase activity in liver and muscle of adult rats on 60th day subjected to neonatal luzindole treatment:

	LIVER	MUSCLE
CONTROL	0.006 ±0.00075	0.020 ±0.0015
LUZINDOLE	0.014 ±0.00091	0.298 ±0.017

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

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

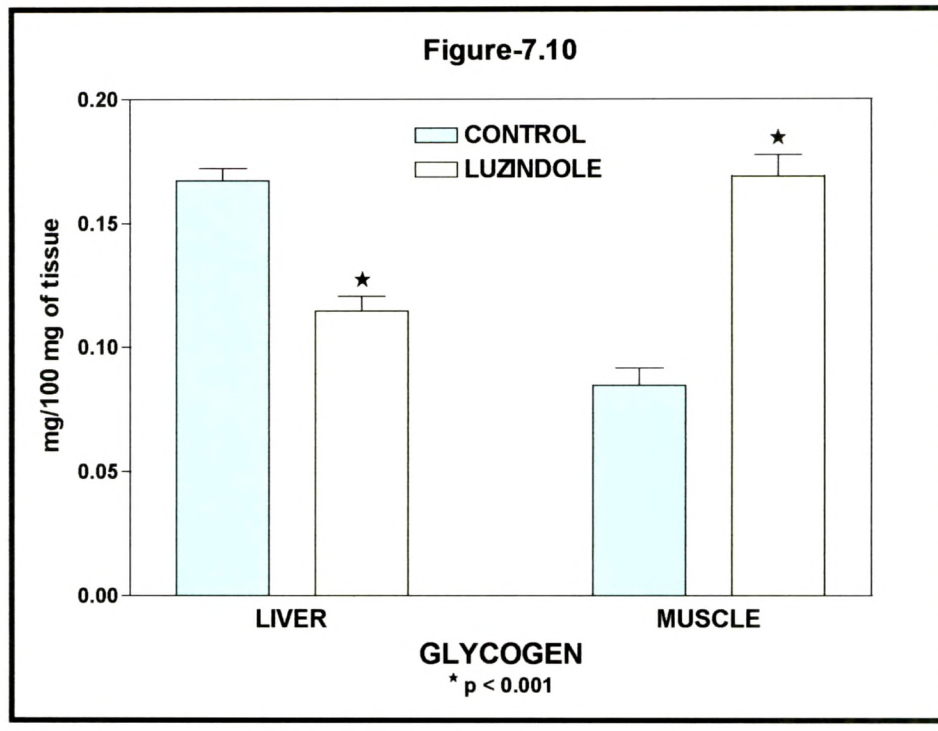


Table 7.10: Hepatic and muscle glycogen content of the adult rats on 60th day subjected to neonatal luzindole treatment:

	LIVER	MUSCLE
CONTROL	0.17 ±0.0048	0.085 ±0.0069
LUZINDOLE	0.1147 ±0.0058	0.17 ±0.0085

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

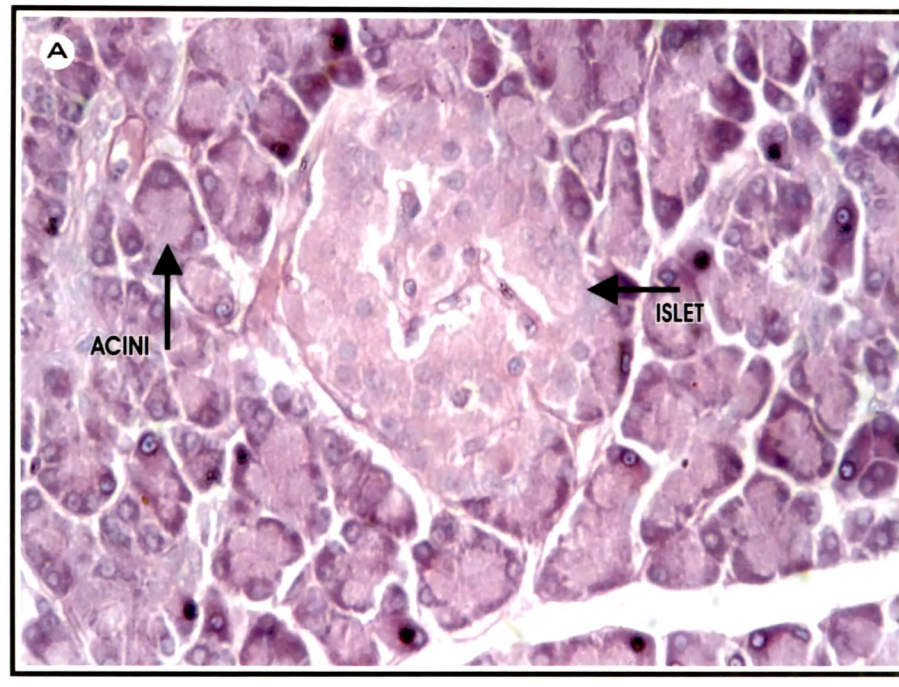
PLATE – 9

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 increased B cells as compared to A cells.

FIGURE (B): Transverse section of the pancreas of male luzindole treated rats on the 60^{th} day showing islet and pancreatic acini. There is an increase in the islet size and islet cell number, with an increased B:A cell ratio. Note the islet periphery showing acinar cells transdifferentiating (double headed arrow) into islet cells.

PLATE - 9



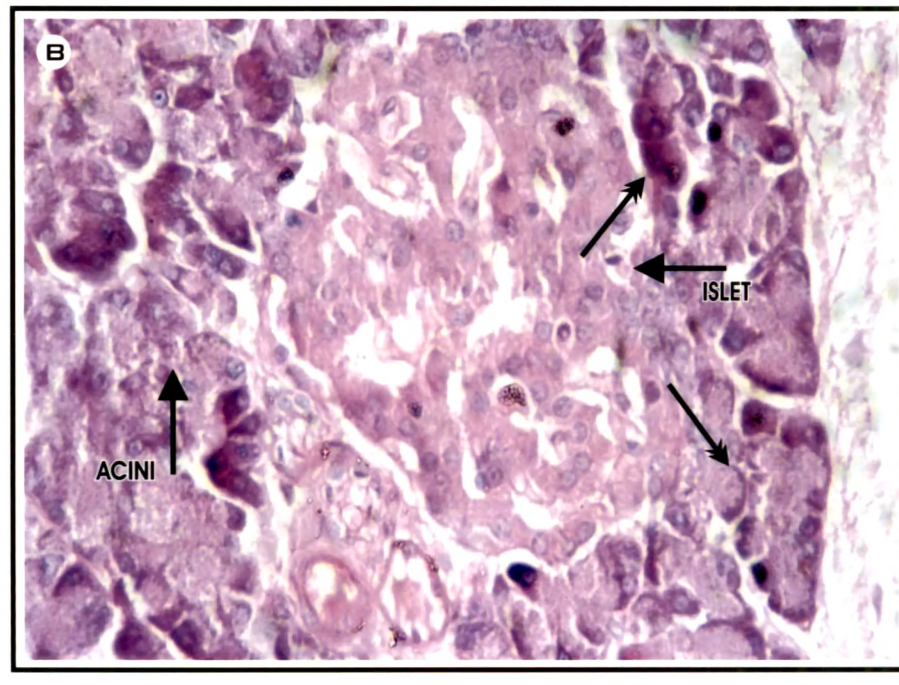


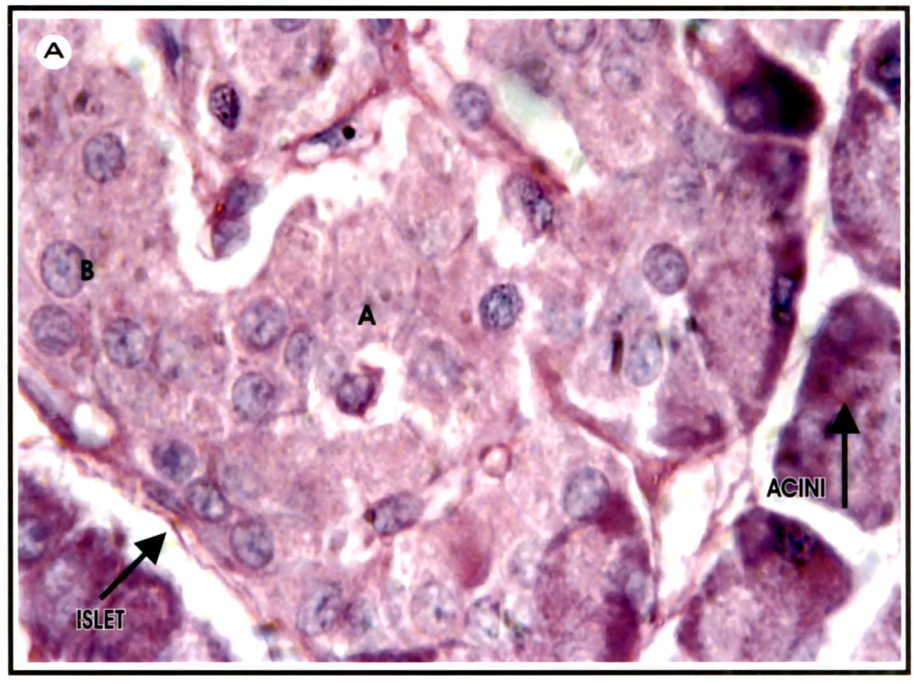
PLATE – 10

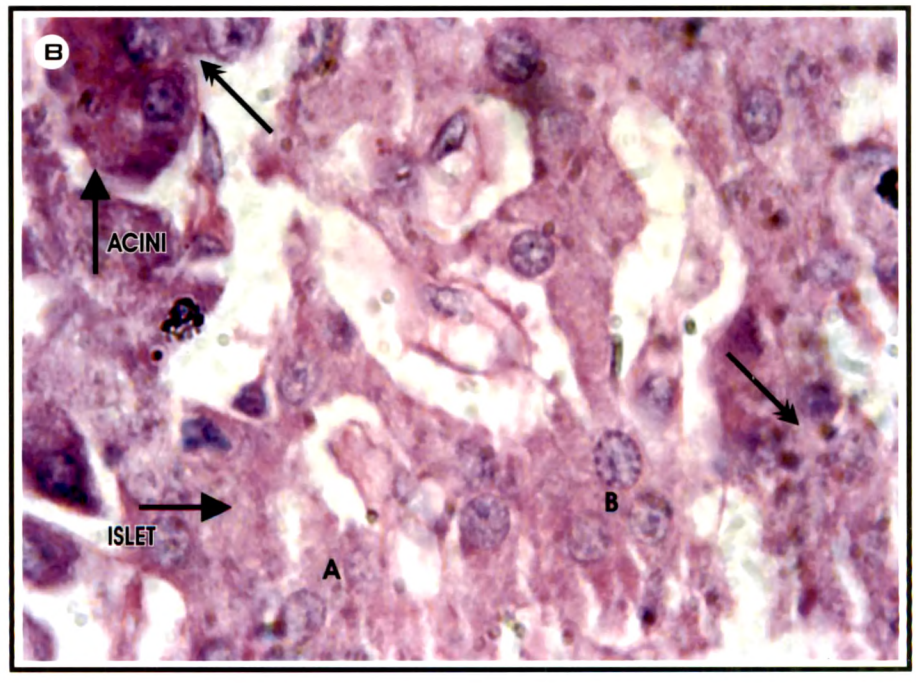
Photomicrographs of sections of pancreas – 1000 X

FIGURE (A): Transverse section of the pancreas of male control adult (60^{th} day) rats showing islet and pancreatic acini. Note the increased B cells as compared to A cells.

FIGURE (B): Transverse section of the pancreas of male luzindole treated rats on the 60^{th} day showing islet and pancreatic acini. There is an increase in the islet size and islet cell number, with an increased B:A cell ratio. Note the islet periphery showing acinar cells transdifferentiating (double headed arrow) into islet cells.

PLATE - 10





- **Muscle glycogen content and the activities of glycogen synthetase and glycogen phosphorylase:** Luzindole treatment significantly increased the muscle glycogen content and the activity of glycogen synthetase but had no significant alteration in the activity of glycogen phosphorylase in the experimental rats (Figure and Table; 7.7, 7.9, 7.10)

- **Hepatic and muscle protein content:** Both hepatic and muscle protein contents increased significantly in the luzindole treated rats as compared to the control rats (Figure and Table; 7.11).

- **Histological observations:** The pancreatic islets which are relatively larger in luzindole treated rats also show a relatively greater B:A cell ratio compared to the control rats (Plate; 9, 10).

DISCUSSION:

The results of the present study show differential effects on hepatic and muscle glycogen load but increased tissue protein and serum glucose levels with hypoinsulinemia as a long term effect of neonatal melatonin antagonism. An age related decrease in hepatic glycogen content and increase in muscle glycogen content are seen in both control and experimental animals from pubertal to the adult stage but the degree of decrease in the liver and the degree of increase in the muscle are significantly greater in the experimental animals. Obviously, the actions of the regulatory *milieu* pervading the post pubertal period are potentiated by neonatal functional hypomelatonemia. Whereas the decrease in the hepatic glycogen contents of control rats is explicable

in terms of the increased phosphorylase activity and decreased synthetase: phosphorylase activity ratio (Fig. and Tab.; 7.7, 7.9), the significantly higher glycogen depletion seen in the experimental rats need an alternative explanation as there is neither an increase in phosphorylase activity nor a change in the synthetase: phosphorylase activity ratio from the pubertal period. Since the experimental rats show hypoinsulinemia and hyperglycemia(Fig. and Tab. 7.6), it is likely that there is decreased insulin sensitivity and reduced glucose uptake by the liver and hence observed decrease in the glycogen content. A reverse situation seems to be operative for muscle glycogen content. Whereas, the significantly increased muscle glycogen content in the experimental animals relative to the pubertal period is explicable in terms of the decreased phosphorylase activity and increased synthetase activity with a significantly high synthetase: phosphorylase activity ratio, the same explanation does not support the relatively less but significant increase in the muscle glycogen content of control rats as the synthetase: phosphorylase activity ratio remains unchanged and at the significant low level characteristic of the pubertal period. This could be essentially due to increased insulin sensitivity and as such a higher insulin sensitivity and increased glucose uptake have been recorded (Chapter 8). The significantly increased glucose-6-phosphatase activity in the control rats from the pubertal period and the observed decrease in blood glucose level suggest increased carbohydrate utilization (Fig. and Tab.; 7.8). In contrast the unchanged glucose-6-phosphatase activity and hyperglycemia seen in the

experimental rats relative to the pubertal period suggest decreased carbohydrate utilization and energy generation. Neonatal melatonin antagonism seems to have a definite influence on pancreatic islet functions. This is suggested by the age related decrease in the insulin level from the neonatal to adult stage as against an age related increase in serum insulin level in control animals (Chapter 1 & 4). Melatonin may have an inhibitory influence on insulin secretion in the neonatal period as the weaning level of insulin in melatonin treated rats was significantly low (Jani, 2004), while melatonin antagonism results significantly higher insulin level (Chapter 1). A permanent effect on pancreatic homeostasis in term of insulin release is presumably as neonatal hypermelatonemic rats depict gradual but significant increase in serum insulin level from weaning to adult stage much more than the controls (Jani, 2004), while neonatal functional hypomelatonemia results in gradual but significant decrease in serum insulin level from weaning to adult stage (Chapter 1 & 4). This presumed inhibitory influence of melatonin in neonates on insulin secretion is in very much of contrast of stimulatory role of melatonin in insulin release from pancreatic islet in adults and is well corroborated by the histological observations showing increased B:A cell ratio and relatively larger islet size in the luzindole treated rats (Plate, 9, 10). A generalized protein anabolic influence is noticeable in control animals as the tissue protein contents are significantly increased from the pubertal period. Neonatal functional hypomelatonemia also seems to exert a significant carbohydrate and protein anabolic influence in the muscle of adult rats

as both the tissue load of glycogen and protein is significantly higher than the controls.

Overall it can be concluded that neonatal functional hypomelatonemia has significant long term effects in terms of serum insulin and glucose levels as well as on carbohydrate and protein metabolism quite different to those of control animals.

SUMMARY:

Previous studies on neonatal melatonin antagonism in the pre-weaning period on pubertal carbohydrate homeostasis revealed significantly decreased tissue glycogen contents coupled with hyperinsulinemia and hyperglycemia and, decreased insulin sensitiviy. In continuation, the present study tries to evaluate the long term effects of neonatal melatonin antagonism on carbohydrate metabolism, tissue protein contents and serum insulin and glucose levels in the post pubertal sexually mature rats. 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 60^{th} day. The body weight of luzindole treated rats increased significantly. Whereas the, relative weight of liver, kidney and adrenals decreased significantly, the, relative weight of spleen increased. The relative weight of pancreas and testes showed no significant alteration as compared to control rats. The hepatic glycogen content decreased significantly in the experimental rats whereas, the muscle glycogen content increased significantly. The glycogen synthetase activity

increased significantly in the liver and muscle of luzindole treated rats while the glycogen phosphorylase activity decreased. The glucose-6-phosphatase activity decreased significantly in the liver of experimental rats. The serum glucose level increased significantly while the serum insulin level decreased significantly in the experimental rats.. Both hepatic and muscle protein contents increased significantly in the luzindole treated rats. The pancreatic islets of luzindole treated rats are relatively larger and also show a relatively greater B:A cell ratio as compared to control rats. The findings of the present study reveal that neonatal functional hypomelatonemia has significant long term effects in terms of serum insulin and glucose levels as well as on carbohydrate and protein metabolism quite different to those of control animals.