CHAPTER – 8

RESISTANCE TO *IN VITRO* GLUCOSE UPTAKE BY ADULT TISSUES OF NEONATAL HYPERMELATONEMIC RATS AND INCREASED SENSITIVITY TO MELATONIN AND ACETYLCHOLINE BY TISSUES OF NORMAL RATS

INTRODUCTION:

Regulation of glucose metabolism and insulin action involves the integrated effect of the central nervous system, endocrine signals on pancreatic cells, and insulin-dependent and insulin-independent glucose utilization by the liver and peripheral tissues. Also, melatonin has been known to influence the functions of liver and muscle through specific receptors (Pang *et al*, 1993). Melatonin also influences the activities of a number of enzymes (Frehn *et al.*, 1974; Becham *et al.*, 1989; Popov *et al.*, 1990; Walsh *et al.*, 1994; Woodward and Fisher, 1996) and affects carbohydrate metabolism (Ramachandran, 2002). With regard to carbohydrate metabolism both hyper and hypoglycemic effects of melatonin has 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. Melatonin has been shown to influence the plasma insulin level (Diaz and Blazquez, 1986), insulin secretion (Bailey *et al.*, 1974) and even possibly insulin action (Frankel and Strandberg, 1991). Previous study has shown that continuous melatonin administration for the entire duration of pre-weaning period of rat neonates leads to hypoinsulinemia (Chapter 1) and increased insulin sensitivity of liver and muscle in the weaning stage supported by higher glucose uptake by these tissues *in vitro* (Chapter 2). However, there is an age related increase in resistance to glucose uptake, though less marked in the hypermelatonemic rats (Chapter 5). It has also been demonstrated that circadian changes in the sensitivity of glucose utilization by skeletal muscle to insulin (Leighton *et al.*, 1988) and glucose transport into skeletal muscle and in particular its sensitivity and responsiveness to insulin diminishes progressively during early development and adolescence (Goodman *et al.*, 1983)

Based on the above observations and previous studies it was thought pertinent to evaluate the long term influence of neonatal melatonin treatment on *in vitro* uptake of glucose by liver and muscle slices. To this end rat neonates have been treated with melatonin from day 1 to day 21 and assessed on day 60.

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

RESULTS:

LIVER SLICES:

- **Uptake in presence of insulin, acetylcholine and melatonin:** The liver slices of control rats showed uptake in presence of all the three agents with no significant difference by either of them. The liver slices of hypermelatonemic rats showed uptake with all the three agents in the order of insulin>melatonin>acetylcholine. Though insulin induced uptake was not significantly different from the control liver slices, acetylcholine and melatonin induced glucose uptake was significantly decreased in that order in the experimental liver slices (Figure and Table; 8.1, 8.2, 8.5, 8.6).
- **Uptake by combinations of insulin, acetylcholine and melatonin:** The uptake promoted by M+Ac was significantly higher (almost double) than that by I+Ac, M+I or M+Ac+I or by any of the agents individually in the liver slices of the control rats. While, the uptake promoted by I+Ac and M+I was similar that, of M+Ac+I was increased as compared to all other agents individually or in combinations except for M+Ac. In the liver slices of hypermelatonemic rats the uptake promoted by I+Ac increased significantly as compared to that of control slices and all other agents singly or in combinations in the experimental rats. Whereas, the uptake promoted by M+I was greater than that of M+Ac and M+Ac+I it was almost equal to that promoted by insulin alone in the liver slices of experimental rats. As compared to control liver slices the uptake promoted by M+Ac and M+Ac+I significantly decreased in the experimental liver

slices while, that by I+Ac and M+I increased significantly (Figure and Table; 8.1, 8 2, 8 5, 8.6).

- **Uptake by combinations of insulin, acetylcholine and melatonin in presence of luzindole:** In the control liver slices the uptake promoted by luzindole was greater than that by insulin or acetylcholine alone or by the combinations of I+Ac, L+I and L+Ac. The combination of L+Ac+I induced glucose uptake by control liver slices was significantly higher than by all the agents alone or by any of the combinations The liver slices of hypermelatonemic rats showed maximum glucose uptake with luzindole as compared to its combinations with insulin or acetylcholine or all of them together but was significantly decreased as compared to the liver slices of control rats. There was no significant difference in glucose uptake promoted by L+I, L+Ac or L+Ac+I in the liver slices of experimental rats but was less than that of the liver slices of control rats with same combinations (Figure and Table; 8 3, 8 4, 8 5, 8.6)

Table and Figure: 8.1

Bonferroni's Multiple Comparison Test Control Groups

	H vs J	H vs K	H vs L	H vs N	H vs O	H vs P	J vs K	J vs L	J vs N	J vs O	J vs P
p	NS	NS	NS	NS	*	NS	NS	NS	NS	*	NS
	K vs L	K vs N	K vs O	K vs P	L vs N	L vs O	L vs P	N vs O	N vs P	O vs P	
p	NS	NS	*	NS	NS	*	NS	*	NS	*	

Bonferroni's Multiple Comparison Test Melatonin Groups

	S vs T	S vs U	S vs V	S vs W	S vs X	S vs Y	T vs U	T vs V	T vs W	T vs X	T vs Y
p	*	⊙	*	NS	*	NS	*	NS	*	NS	NS
	U vs V	U vs W	U vs X	U vs Y	V vs W	V vs X	V vs Y	W vs X	W vs Y	X vs Y	
p	*	⊙	*	*	*	NS	NS	*	NS	NS	

*p<0.001; ■P<0.01; ⊙P<0.05; NSNon Significant

Figure 8.1: Glucose uptake at 10 minutes by liver slices of adult rats on 60th day with combinations of insulin, acetylcholine and melatonin subjected to neonatal melatonin treatment:

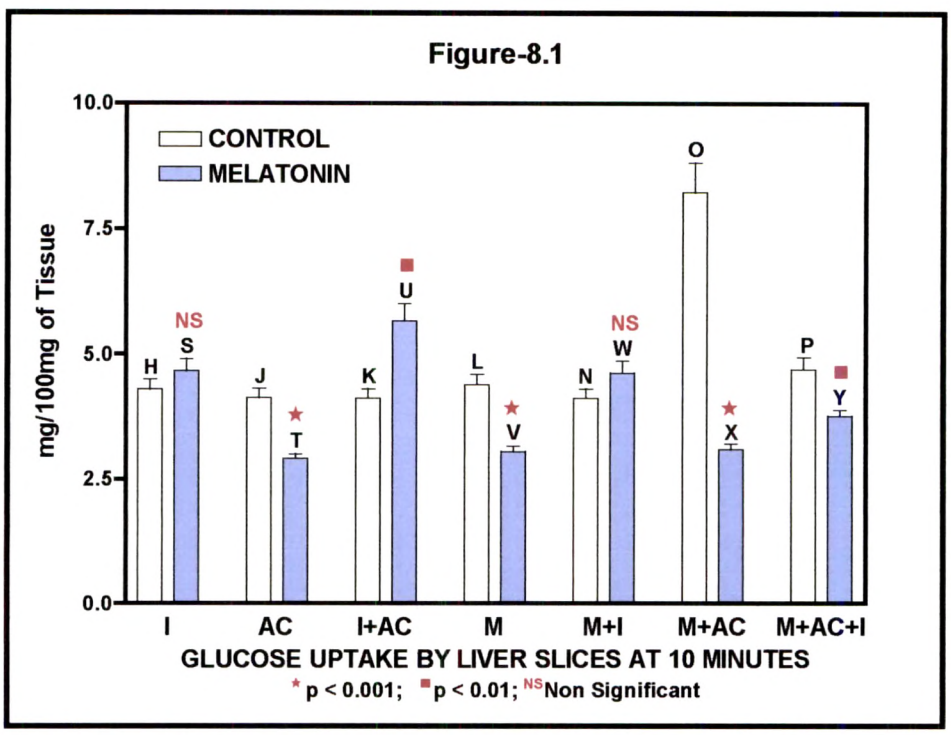

Table 8.1: Glucose uptake at 10 minutes by liver slices of adult rats on 60th day with combinations of insulin, acetylcholine and melatonin subjected to neonatal melatonin treatment:

	I	AC	I+AC	M	M+I	M+AC	M+AC+I
CONTROL	4.29$^{(H)}$ ±0.20	4.12$^{(J)}$ ±0.19	4.11$^{(K)}$ ±0.19	4.38$^{(L)}$ ±0.21	4.11$^{(N)}$ ±0.19	8.22$^{(O)}$ ±0.60	4.69$^{(P)}$ ±0.24
MELATONIN	^{NS}4.66$^{(S)}$ ±0.24	*2.91$^{(T)}$ ±0.09	$^{■}$5.66$^{(U)}$ ±0.34	*3.05$^{(V)}$ ±0.10	^{NS}4.62$^{(W)}$ ±0.24	*3.09$^{(X)}$ ±0.11	$^{■}$3.76$^{(Y)}$ ±0.12

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

Table and Figure: 8.2

Bonferroni's Multiple Comparison Test Control Groups

	H vs J	H vs K	H vs L	H vs N	H vs O	H vs P	J vs K	J vs L	J vs N	J vs O	J vs P
p	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
	K vs L	K vs N	K vs O	K vs P	L vs N	L vs O	L vs P	N vs O	N vs P	O vs P	
p	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	

Bonferroni's Multiple Comparison Test Melatonin Groups

	S vs T	S vs U	S vs V	S vs W	S vs X	S vs Y	T vs U	T vs V	T vs W	T vs X	T vs Y
p	NS	NS	⊙	✶	NS	✶	NS	■	■	NS	✶
	U vs V	U vs W	U vs X	U vs Y	V vs W	V vs X	V vs Y	W vs X	W vs Y	X vs Y	
p	■	■	NS	✶	✶	✶	✶	NS	NS	■	

*p<0.001; ■P<0.01; ⊙P<0.05; NSNon Significant

Figure 8.2: Glucose uptake at 90 minutes by liver slices of adult rats on 60th day with combinations of insulin, acetylcholine and melatonin subjected to neonatal melatonin treatment:

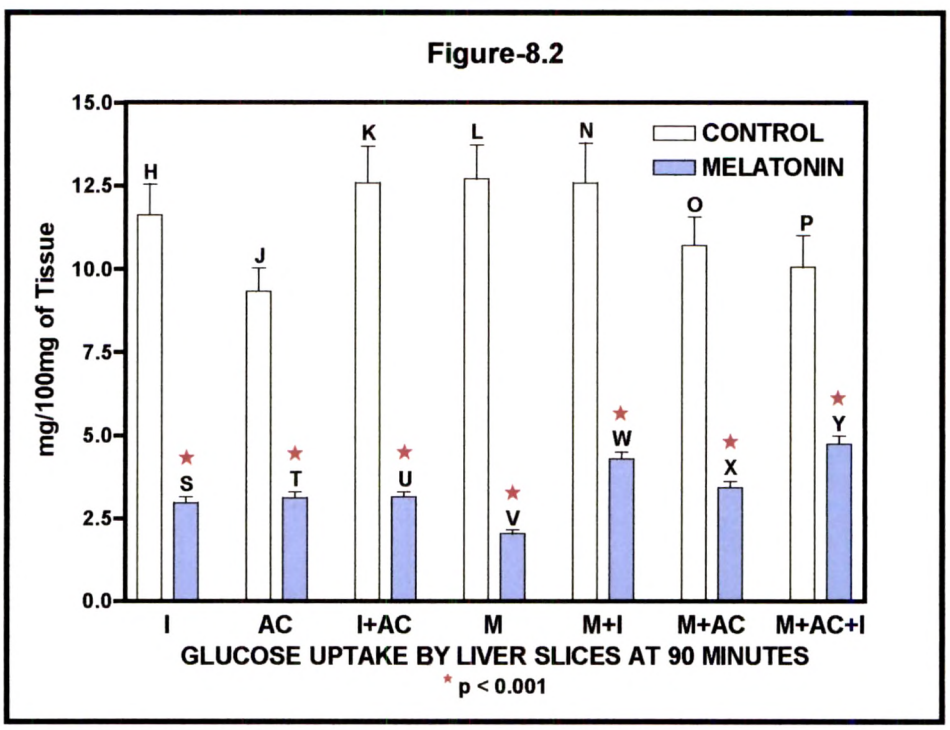

Table 8.2: Glucose uptake at 90 minutes by liver slices of adult rats on 60th day with combinations of insulin, acetylcholine and melatonin subjected to neonatal melatonin treatment:

	I	AC	I+AC	M	M+I	M+AC	M+AC+I
CONTROL	11.61$^{(H)}$ ±0.94	9.32$^{(J)}$ ±0.71	12.59$^{(K)}$ ±1.09	12.70$^{(L)}$ ±1.03	12.59$^{(N)}$ ±1.20	10.71$^{(O)}$ ±0.85	10.06$^{(P)}$ ±0.94
MELATONIN	*2.97$^{(S)}$ ±0.18	*3.12$^{(T)}$ ±0.17	*3.15$^{(U)}$ ±0.15	*2.03$^{(V)}$ ±0.11	*4.29$^{(W)}$ ±0.20	*3.42$^{(X)}$ ±0.19	*4.73$^{(Y)}$ ±0.25

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

Table 8.3

Bonferroni's Multiple Comparison Test Control Groups

	H vs J	H vs K	H vs N	H vs O	H vs P	H vs Q	J vs K	J vs N	J vs O	J vs P	J vs Q
p	NS	NS	NS	NS	NS	■	NS	NS	NS	NS	✶
	K vs N	K vs O	K vs P	K vs Q	N vs O	N vs P	N vs Q	O vs P	O vs Q	P vs Q	
p	NS	NS	NS	✶	⊙	NS	⊙	NS	✶	✶	

Bonferroni's Multiple Comparison Test Melatonin Groups

	S vs T	S vs U	S vs V	S vs W	S vs X	S vs Y	T vs U	T vs V	T vs W	T vs X	T vs Y
p	✶	⊙	■	✶	✶	✶	✶	NS	NS	NS	NS
	U vs V	U vs W	U vs X	U vs Y	V vs W	V vs X	V vs Y	W vs X	W vs Y	X vs Y	
p	✶	✶	✶	✶	⊙	NS	NS	NS	NS	NS	

*p<0.001; ⊙P<0.05; NSNon Significant

Figure 8.3: Glucose uptake at 10 minutes by liver slices of adult rats on 60th day with combinations of insulin, acetylcholine and luzindole subjected to neonatal melatonin treatment:

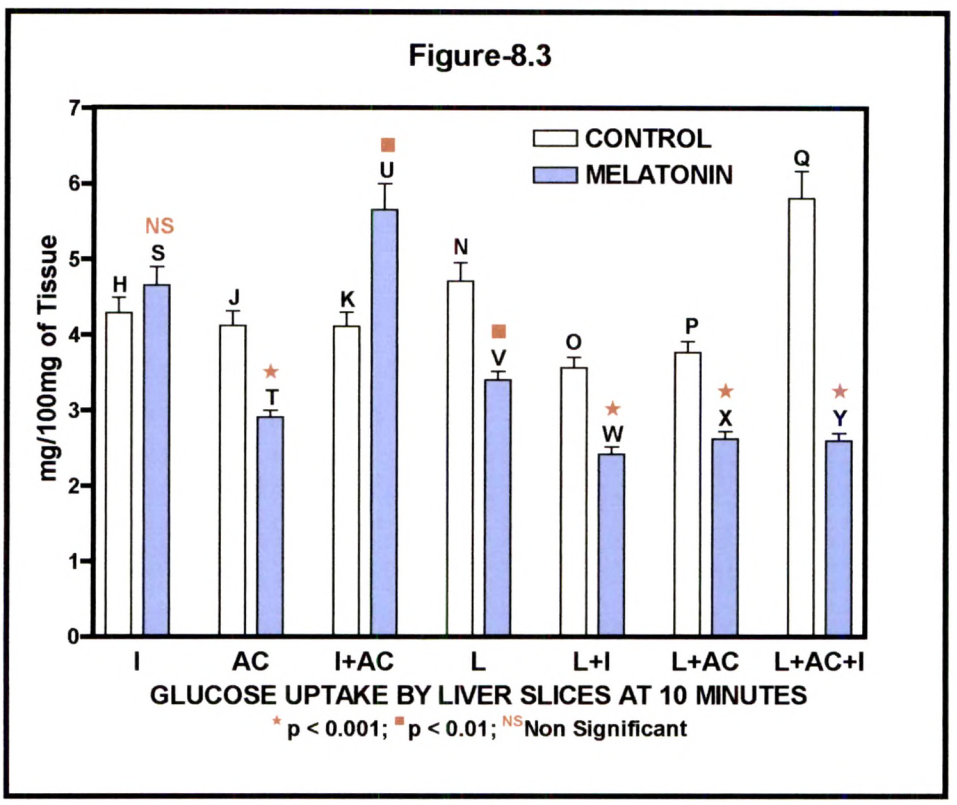

Figure 8.3: Glucose uptake at 10 minutes by liver slices of adult rats on 60th day with combinations of insulin, acetylcholine and luzindole subjected to neonatal melatonin treatment:

	I	AC	I+AC	L	L+I	L+AC	L+AC+I
CONTROL	4.29(H) ±0.20	4.12(J) ±0.19	4.11(K) ±0.19	4.71(N) ±0.25	3.57(O) ±0.13	3.77(P) ±0.15	5.81(Q) ±0.36
MELATONIN	NS4.66(S) ±0.24	*2.91(T) ±0.09	■5.66(U) ±0.34	■3.40(V) ±0.12	*2.42(W) ±0.092	*2.62(X) ±0.10	*2.60(Y) ±0.099

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

Table 8.4

Bonferroni's Multiple Comparison Test Control Groups

	H vs J	H vs K	H vs N	H vs O	H vs P	H vs Q	J vs K	J vs N	J vs O	J vs P	J vs Q
p	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
	K vs N	K vs O	K vs P	K vs Q	N vs O	N vs P	N vs Q	O vs P	O vs Q	P vs Q	
p	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	

Bonferroni's Multiple Comparison Test Melatonin Groups

	S vs T	S vs U	S vs V	S vs W	S vs X	S vs Y	T vs U	T vs V	T vs W	T vs X	T vs Y
p	NS	NS	■	✶	⊙	✶	NS	■	✶	⊙	✶
	U vs V	U vs W	U vs X	U vs Y	V vs W	V vs X	V vs Y	W vs X	W vs Y	X vs Y	
p	✶	■	✶	⊙	✶	■	NS	NS	⊙	NS	

*p<0.001; ⊙P<0.05; NSNon Significant

Figure 8.4: Glucose uptake at 90 minutes by liver slices of adult rats on 60th day with combinations of insulin, acetylcholine and luzindole subjected to neonatal melatonin treatment:

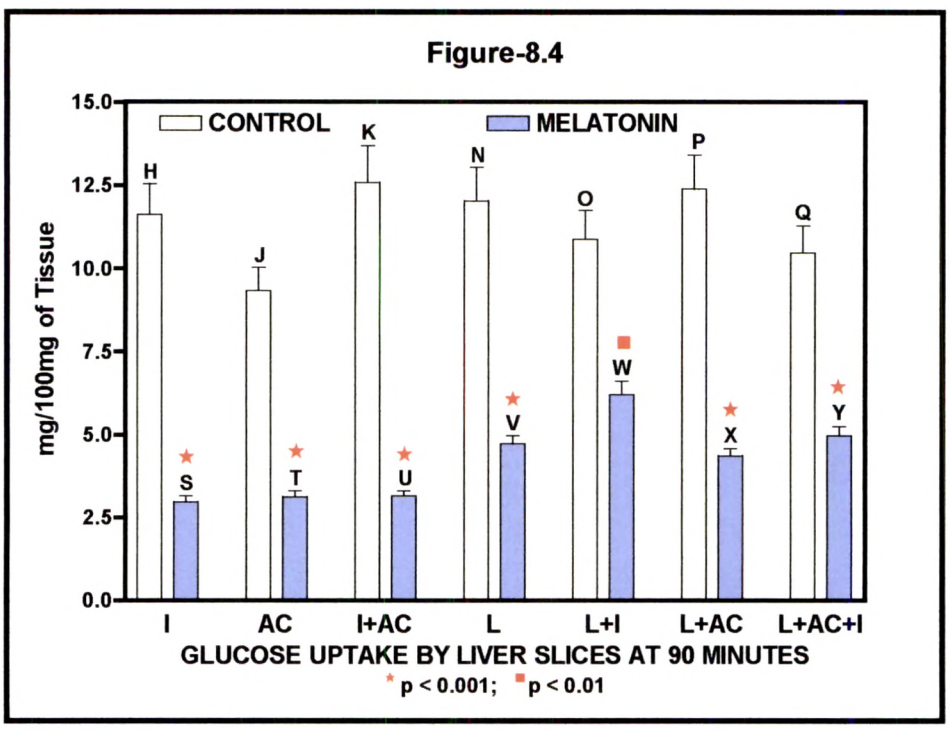

Table 8.4: Glucose uptake at 90 minutes by liver slices of adult rats on 60th day with combinations of insulin, acetylcholine and luzindole subjected to neonatal melatonin treatment:

	I	AC	I+AC	L	L+I	L+AC	L+AC+I
CONTROL	11.61$^{(H)}$ ±0.94	9.32$^{(J)}$ ±0.71	12.59$^{(K)}$ ±1.09	12.03$^{(N)}$ ±1.01	10.88$^{(O)}$ ±0.86	12.38$^{(P)}$ ±1.03	10.47$^{(Q)}$ ±0.82
MELATONIN	*2.97$^{(S)}$ ±0.18	*3.12$^{(T)}$ ±0.17	*3.15$^{(U)}$ ±0.15	*4.71$^{(V)}$ ±0.25	■6.20$^{(W)}$ ±0.40	*4.36$^{(X)}$ ±0.21	*4.96$^{(Y)}$ ±0.27

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

Table 8.5

Bonferroni's Multiple Comparison Test Control Groups

	A vs B	A vs C	A vs D	A vs E	A vs F	A vs G	A vs H	B vs C	B vs D	B vs E	B vs F	B vs G	B vs H	C vs D
p	NS	✶	NS	NS	NS	NS	NS	✶	NS	NS	NS	NS	⊙	✶
	C vs E	C vs F	C vs G	C vs H	D vs E	D vs F	D vs G	D vs H	E vs F	E vs G	E vs H	F vs G	F vs H	G vs H
p	✶	✶	✶	✶	NS	NS	NS	NS	NS	NS	NS	NS	✶	■

Bonferroni's Multiple Comparison Test Melatonin Groups

	S vs T	S vs U	S vs V	SvsW	S vs X	S vs Y	S vs Z	T vs U	T vs V	TvsW	T vs X	T vs Y	T vs Z	U vs V
p	✶	NS	⊙	NS	NS	NS	NS	✶	■	✶	✶	✶	✶	⊙
	UvsW	U vs X	U vs Y	U vs Z	VvsW	V vs X	V vs Y	V vs Z	WvsX	WvsY	WvsZ	X vs Y	X vs Z	Y vs Z
p	NS	⊙	NS	NS	NS	✶	✶	✶	✶	■	■	NS	NS	NS

*p<0.001; ⊙P<0.05; NSNon Significant

Figure 8.5: Glucose uptake at 10 minutes by liver slices of adult rats on 60th day with combinations of insulin, acetylcholine, melatonin and luzindole subjected to neonatal melatonin treatment:

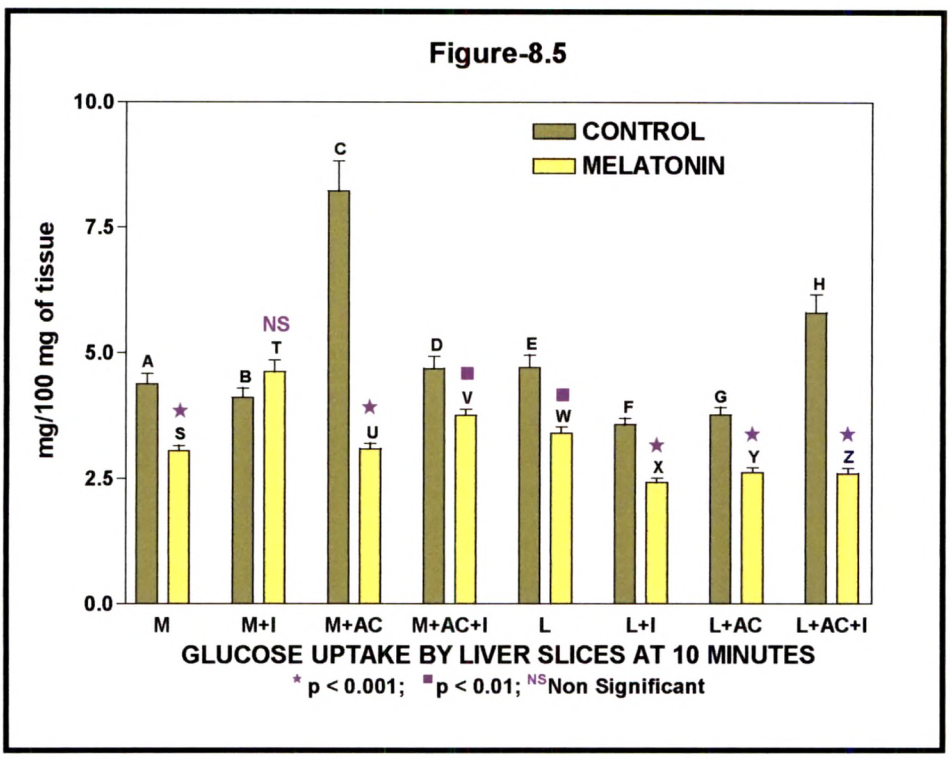

Table 8.5: Glucose uptake at 10 minutes by liver slices of adult rats on 60th day with combinations of insulin, acetylcholine, melatonin and luzindole subjected to neonatal melatonin treatment:

	M	M+I	M+AC	M+AC+I	L	L+I	L+AC	L+AC+I
CONTROL	4.38(A) ±0.21	4.11(B) ±0.19	8.22(C) ±0.60	4.69(D) ±0.24	4.71(E) ±0.25	3.57(F) ±0.13	3.77(G) ±0.15	5.81(H) ±0.36
MELATONIN	*3.05(S) ±0.10	NS4.62(T) ±0.24	*3.09(U) ±0.11	■3.76(V) ±0.12	■3.40(W) ±0.12	*2.42(X) ±0.092	*2.62(Y) ±0.10	*2.60(Z) ±0.099

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

Table 8.6

Bonferroni's Multiple Comparison Test Control Groups

	A vs B	A vs C	A vs D	A vs E	A vs F	A vs G	A vs H	B vs C	B vs D	B vs E	B vs F	B vs G	B vs H	C vs D
p	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
	C vs E	C vs F	C vs G	C vs H	D vs E	D vs F	D vs G	D vs H	E vs F	E vs G	E vs H	F vs G	F vs H	G vs H
p	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS

Bonferroni's Multiple Comparison Test Melatonin Groups

	S vs T	S vs U	S vs V	SvsW	S vs X	S vs Y	S vs Z	T vs U	T vs V	TvsW	T vs X	T vs Y	T vs Z	U vs V
p	✶	⊙	✶	✶	✶	✶	✶	NS	NS	NS	✶	NS	NS	⊙
	UvsW	U vs X	U vs Y	U vs Z	VvsW	V vs X	V vs Y	V vs Z	WvsX	WvsY	WvsZ	X vs Y	X vs Z	Y vs Z
p	⊙	✶	NS	■	NS	■	NS	NS	■	NS	NS	✶	⊙	NS

*p<0.001; ⊙P<0.05; NSNon Significant

Figure 8.6: Glucose uptake at 90 minutes by liver slices of adult rats on 60th day with combinations of insulin, acetylcholine, melatonin and luzindole subjected to neonatal melatonin treatment:

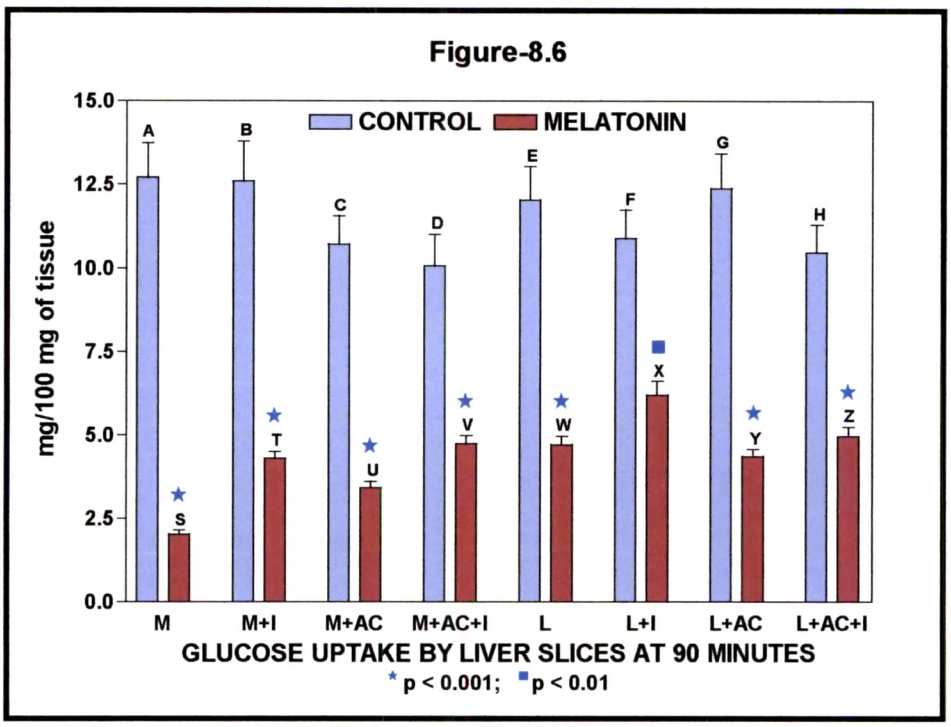

Table 8.6: Glucose uptake at 90 minutes by liver slices of adult rats on 60th day with combinations of insulin, acetylcholine, melatonin and luzindole subjected to neonatal melatonin treatment:

	M	M+I	M+AC	M+AC+I	L	L+I	L+AC	L+AC+I
CONTROL	12.70(A) ±1.03	12.59(B) ±1.20	10.71(C) ±0.85	10.06(D) ±0.94	12.03(E) ±1.01	10.88(F) ±0.86	12.38(G) ±1.03	10.47(H) ±0.82
MELATONIN	*2.03(S) ±0.11	*4.29(T) ±0.20	*3.42(U) ±0.19	*4.73(V) ±0.25	*4.71(W) ±0.25	■6.20(X) ±0.40	*4.36(Y) ±0.21	*4.96(Z) ±0.27

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

Table and Figure: 8.7

Bonferroni's Multiple Comparison Test Control Groups

	H vs J	H vs K	H vs L	H vs N	H vs O	H vs P	J vs K	J vs L	J vs N	J vs O	J vs P
p	NS	NS	*	*	⊙	*	NS	*	⊙	NS	■
	K vs L	K vs N	K vs O	K vs P	L vs N	L vs O	L vs P	N vs O	N vs P	O vs P	
p	*	*	NS	*	⊙	*	NS	NS	NS	■	

Bonferroni's Multiple Comparison Test Melatonin Groups

	S vs T	S vs U	S vs V	S vs W	S vs X	S vs Y	T vs U	T vs V	T vs W	T vs X	T vs Y
p	NS	NS	*	*	⊙	*	NS	*	*	NS	*
	U vs V	U vs W	U vs X	U vs Y	V vs W	V vs X	V vs Y	W vs X	W vs Y	X vs Y	
p	*	*	NS	*	*	*	*	*	NS	⊙	

*p<0.001; ■P<0.01; ⊙P<0.05; NSNon Significant

Figure 8.7: Glucose uptake at 10 minutes by muscle slices of adult rats on 60th day with insulin, acetylcholine and melatonin subjected to neonatal melatonin treatment:

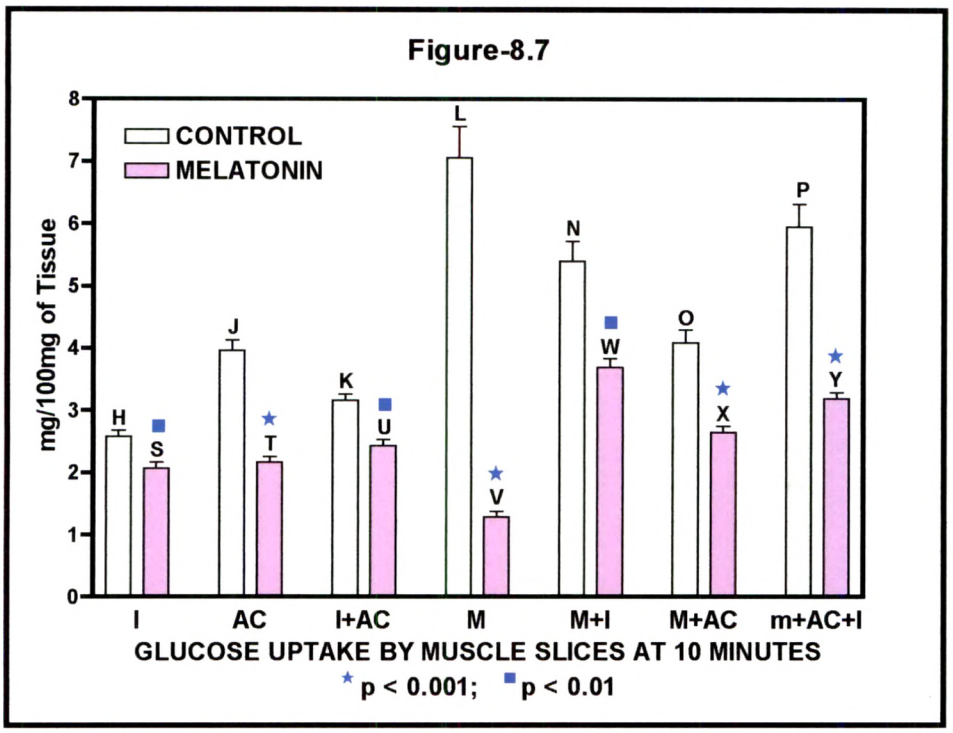

Figure 8.7: Glucose uptake at 10 minutes by muscle slices of adult rats on 60th day with combinations of insulin, acetylcholine and melatonin subjected to neonatal melatonin treatment:

	I	AC	I+AC	M	M+I	M+AC	M+AC+I
CONTROL	2.58$^{(H)}$ ±0.099	3.96$^{(J)}$ ±0.17	3.16$^{(K)}$ ±0.10	7.06$^{(L)}$ ±0.50	5.40$^{(N)}$ ±0.32	4.09$^{(O)}$ ±0.20	5.95$^{(P)}$ ±0.37
MELATONIN	■2.07$^{(S)}$ ±0.094	★2.16$^{(T)}$ ±0.096	■2.43$^{(U)}$ ±0.098	★1.29$^{(V)}$ ±0.080	■3.69$^{(W)}$ ±0.14	★2.65$^{(X)}$ ±0.099	★3.19$^{(Y)}$ ±0.10

Values are expressed as mean ± SEM, ★p < 0.001; ■ p < 0.01

Table and Figure: 8.8

Bonferroni's Multiple Comparison Test Control Groups

	H vs J	H vs K	H vs L	H vs N	H vs O	H vs P	J vs K	J vs L	J vs N	J vs O	J vs P
p	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
	K vs L	K vs N	K vs O	K vs P	L vs N	L vs O	L vs P	N vs O	N vs P	O vs P	
p	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	

Bonferroni's Multiple Comparison Test Melatonin Groups

	S vs T	S vs U	S vs V	S vs W	S vs X	S vs Y	T vs U	T vs V	T vs W	T vs X	T vs Y
p	NS	*	⊙	NS	■	⊙	NS	NS	*	*	NS
	U vs V	U vs W	U vs X	U vs Y	V vs W	V vs X	V vs Y	W vs X	W vs Y	X vs Y	
p	*	NS	*	NS	*	*	*	*	*	*	

*p<0.001; ■P<0.01; ⊙P<0.05; NSNon Significant

Figure 8.8: Glucose uptake at 90 minutes by muscle slices of adult rats on 60th day with combinations of insulin, acetylcholine and melatonin subjected to neonatal melatonin treatment:

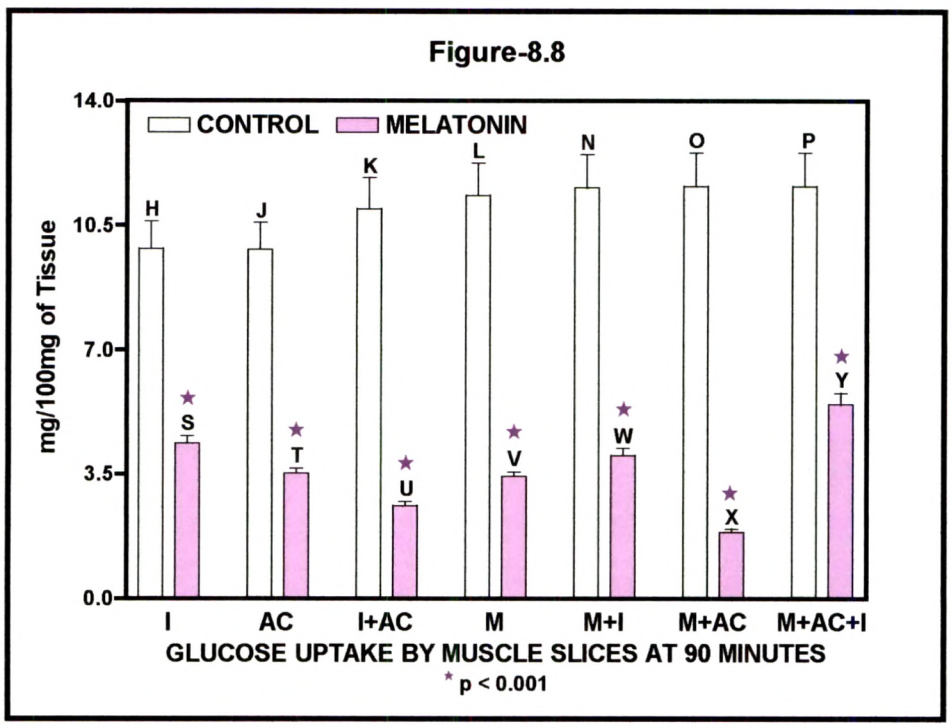

Figure 8.8: Glucose uptake at 90 minutes by muscle slices of adult rats on 60th day with combinations of insulin, acetylcholine and melatonin subjected to neonatal melatonin treatment:

	I	AC	I+AC	M	M+I	M+AC	M+AC+I
CONTROL	9.85(H) ±0.76	9.82(J) ±0.76	10.97(K) ±0.87	11.35(L) ±0.91	11.56(N) ±0.93	11.61(O) ±0.94	11.61(P) ±0.94
MELATONIN	*4.37(S) ±0.21	*3.53(T) ±0.13	*2.62(U) ±0.099	*3.44(V) ±0.12	*4.03(W) ±0.20	*1.87(X) ±0.086	*5.47(Y) ±0.32

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

Table 8.9

Bonferroni's Multiple Comparison Test Control Groups

	H vs J	H vs K	H vs N	H vs O	H vs P	H vs Q	J vs K	J vs N	J vs O	J vs P	J vs Q
p	*	⊙	NS	NS	*	■	*	*	*	*	*
	K vs N	K vs O	K vs P	K vs Q	N vs O	N vs P	N vs Q	O vs P	O vs Q	P vs Q	
p	NS	⊙	*	*	NS	*	*	*	■	NS	

Bonferroni's Multiple Comparison Test Melatonin Groups

	S vs T	S vs U	S vs V	S vs W	S vs X	S vs Y	T vs U	T vs V	T vs W	T vs X	T vs Y
p	NS	NS	■	*	■	NS	NS	*	*	⊙	NS
	U vs V	U vs W	U vs X	U vs Y	V vs W	V vs X	V vs Y	W vs X	W vs Y	X vs Y	
p	*	*	NS	NS	*	■	*	*	*	*	

*p<0.001; ■P<0.01; ⊙P<0.05; NSNon Significant

Figure 8.9: Glucose uptake at 10 minutes by muscle slices of adult rats on 60th day with combinations of insulin, acetylcholine and luzindole subjected to neonatal melatonin treatment:

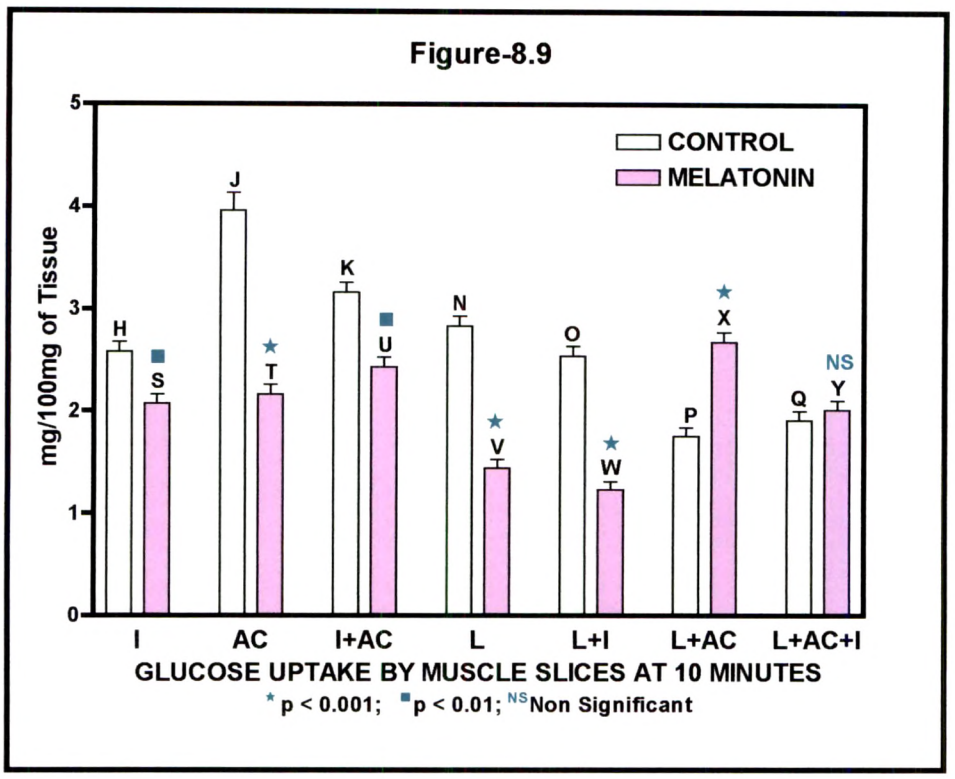

Table 8.9: Glucose uptake at 10 minutes by muscle slices of adult rats on 60th day with combinations of insulin, acetylcholine and luzindole subjected to neonatal melatonin treatment:

	I	AC	I+AC	L	L+I	L+AC	L+AC+I
CONTROL	2.58$^{(H)}$ ±0.099	3.96$^{(J)}$ ±0.17	3.16$^{(K)}$ ±0.10	2.83$^{(N)}$ ±0.096	2.54$^{(O)}$ ±0.093	1.75$^{(P)}$ ±0.085	1.91$^{(Q)}$ ±0.087
MELATONIN	■2.07$^{(S)}$ ±0.094	*2.16$^{(T)}$ ±0.096	■2.43$^{(U)}$ ±0.098	*1.44$^{(V)}$ ±0.082	*1.23$^{(W)}$ ±0.080	*2.68$^{(X)}$ ±0.094	NS2.01$^{(Y)}$ ±0.089

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

Table 8.10

Bonferroni's Multiple Comparison Test Control Groups

	H vs J	H vs K	H vs N	H vs O	H vs P	H vs Q	J vs K	J vs N	J vs O	J vs P	J vs Q
p	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
	K vs N	K vs O	K vs P	K vs Q	N vs O	N vs P	N vs Q	O vs P	O vs Q	P vs Q	
p	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	

Bonferroni's Multiple Comparison Test Melatonin Groups

	S vs T	S vs U	S vs V	S vs W	S vs X	S vs Y	T vs U	T vs V	T vs W	T vs X	T vs Y
p	⊙	✶	NS	■	✶	NS	⊙	■	NS	✶	NS
	U vs V	U vs W	U vs X	U vs Y	V vs W	V vs X	V vs Y	W vs X	W vs Y	X vs Y	
p	✶	NS	NS	✶	■	✶	NS	✶	⊙	✶	

*p<0.001; ■P<0.01; ⊙P<0.05; NSNon Significant

Figure 8.10: Glucose uptake at 90 minutes by muscle slices of adult rats on 60th day with combinations of insulin, acetylcholine and luzindole subjected to neonatal melatonin treatment:

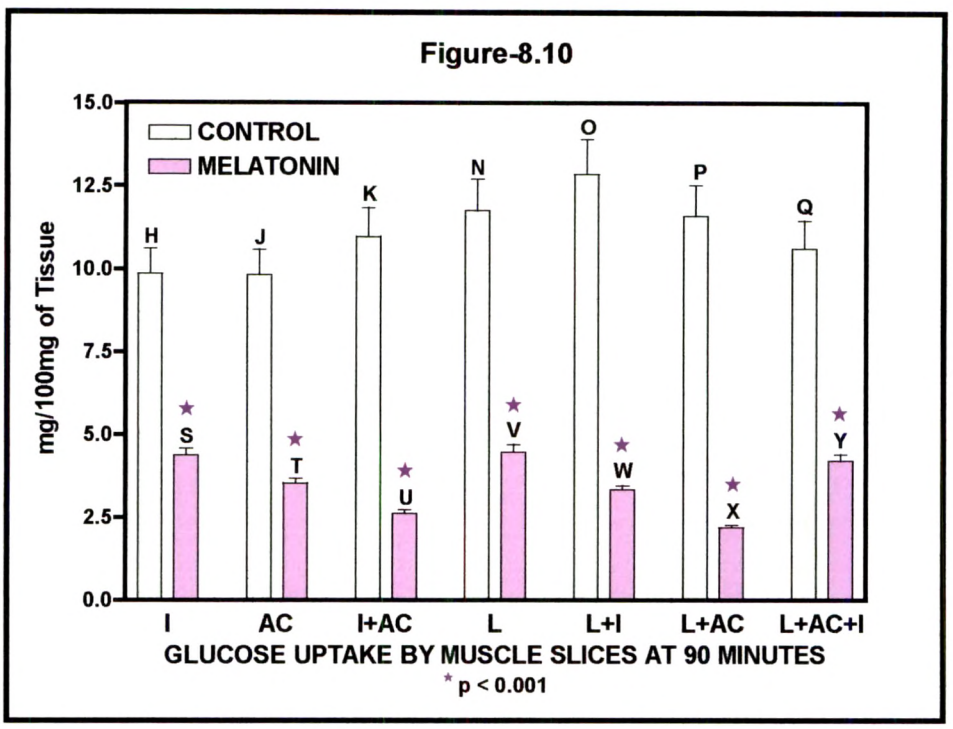

Table 8.10: Glucose uptake at 90 minutes by muscle slices of adult rats on 60th day with combinations of insulin, acetylcholine and luzindole subjected to neonatal melatonin treatment:

	I	AC	I+AC	L	L+I	L+AC	L+AC+I
CONTROL	9.85(H) ±0.76	9.82(J) ±0.76	10.97(K) ±0.87	11.75(N) ±0.95	12.85(O) ±1.06	11.59(P) ±0.93	10.61(Q) ±0.84
MELATONIN	*4.37(S) ±0.21	*3.53(T) ±0.13	*2.62(U) ±0.099	*4.47(V) ±0.22	*3.34(W) ±0.11	*2.18(X) ±0.089	*4.20(Y) ±0.20

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

Table 8.11

Bonferroni's Multiple Comparison Test Control Groups

	A vs B	A vs C	A vs D	A vs E	A vs F	A vs G	A vs H	B vs C	B vs D	B vs E	B vs F	B vs G	B vs H	C vs D
p	■	✶	NS	✶	✶	✶	✶	NS	NS	✶	✶	✶	✶	■
	C vs E	C vs F	C vs G	C vs H	D vs E	D vs F	D vs G	D vs H	E vs F	E vs G	E vs H	F vs G	F vs H	G vs H
p	NS	⊙	✶	✶	✶	✶	✶	✶	NS	NS	NS	NS	NS	NS

Bonferroni's Multiple Comparison Test Melatonin Groups

	S vs T	S vs U	S vs V	SvsW	S vs X	S vs Y	S vs Z	T vs U	T vs V	TvsW	T vs X	T vs Y	T vs Z	U vs V
p	✶	✶	✶	NS	NS	✶	✶	✶	⊙	✶	✶	✶	✶	⊙
	UvsW	U vs X	U vs Y	U vs Z	VvsW	V vs X	V vs Y	V vs Z	WvsX	WvsY	WvsZ	X vs Y	X vs Z	Y vs Z
p	✶	✶	NS	■	✶	✶	⊙	✶	NS	✶	⊙	✶	✶	■

*p<0.001; ⊙P<0.05; NSNon Significant

Figure 8.11: Glucose uptake at 10 minutes by muscle slices of adult rats on 60th day with combinations of insulin, acetylcholine, melatonin and luzindole subjected to neonatal melatonin treatment:

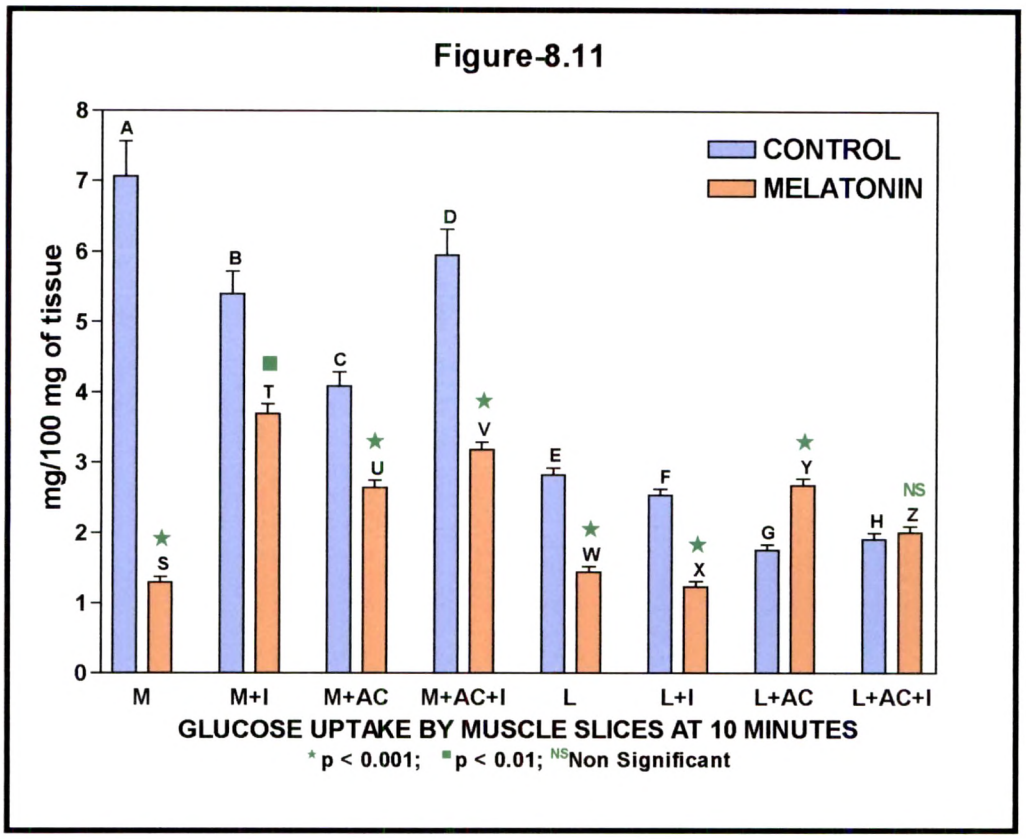

Table 8.11: Glucose uptake at 10 minutes by muscle slices of adult rats on 60th day with combinations of insulin, acetylcholine, melatonin and luzindole subjected to neonatal melatonin treatment:

	M	M+I	M+AC	M+AC+I	L	L+I	L+AC	L+AC+I
CONTROL	7.06 (A) ±0.50	5.40 (B) ±0.32	4.09 (C) ±0.20	5.95 (D) ±0.37	2.83 (E) ±0.096	2.54 (F) ±0.093	1.75 (G) ±0.085	1.91 (H) ±0.087
MELATONIN	* 1.29 (S) ±0.08	■ 3.69 (T) ±0.14	* 2.65 (U) ±0.09	* 3.19 (V) ±0.10	* 1.44 (W) ±0.082	* 1.23 (X) ±0.08	* 2.68 (Y) ±0.094	NS 2.01 (Z) ±0.089

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

Table 8.12

Bonferroni's Multiple Comparison Test Control Groups

	A vs B	A vs C	A vs D	A vs E	A vs F	A vs G	A vs H	B vs C	B vs D	B vs E	B vs F	B vs G	B vs H	C vs D
p	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
	C vs E	C vs F	C vs G	C vs H	D vs E	D vs F	D vs G	D vs H	E vs F	E vs G	E vs H	F vs G	F vs H	G vs H
p	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS

Bonferroni's Multiple Comparison Test Melatonin Groups

	S vs T	S vs U	S vs V	SvsW	S vs X	S vs Y	S vs Z	T vs U	T vs V	TvsW	T vs X	T vs Y	T vs Z	U vs V
p	NS	✶	✶	⊙	NS	■	NS	✶	✶	NS	NS	✶	NS	✶
	UvsW	U vs X	U vs Y	U vs Z	VvsW	V vs X	V vs Y	V vs Z	WvsX	WvsY	WvsZ	X vs Y	X vs Z	Y vs Z
p	✶	✶	NS	✶	⊙	✶	✶	■	■	✶	NS	■	NS	✶

*p<0.001; ⊙P<0.05; NSNon Significant

Figure 8.12: Glucose uptake at 90 minutes by muscle slices of adult rats on 60th day with combinations of insulin, acetylcholine, melatonin and luzindole subjected to neonatal melatonin treatment:

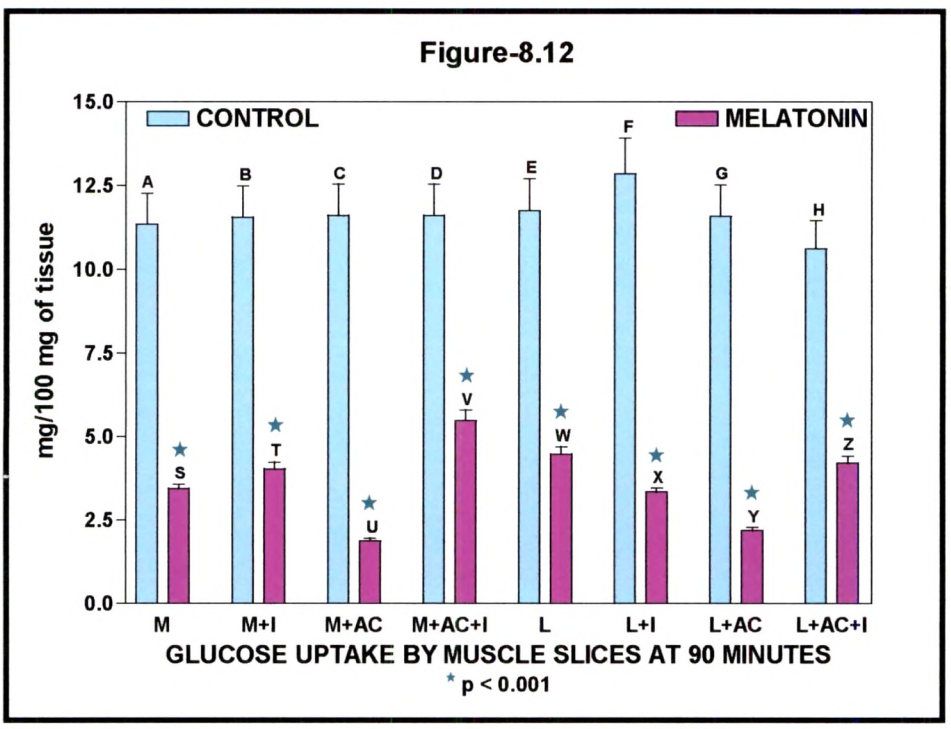

Table 8.12: Glucose uptake at 90 minutes by muscle slices of adult rats on 60th day with combinations of insulin, acetylcholine, melatonin and luzindole subjected to neonatal melatonin treatment:

	M	M+I	M+AC	M+AC+I	L	L+I	L+AC	L+AC+I
CONTROL	11.35$^{(A)}$ ±0.91	11.56$^{(B)}$ ±0.93	11.61$^{(C)}$ ±0.94	11.61$^{(D)}$ ±0.94	11.75$^{(E)}$ ±0.95	12.85$^{(F)}$ ±1.06	11.59$^{(G)}$ ±0.93	10.61$^{(H)}$ ±0.84
MELATONIN	*3.44$^{(S)}$ ±0.12	*4.03$^{(T)}$ ±0.20	*1.87$^{(U)}$ ±0.086	*5.47$^{(V)}$ ±0.32	*4.47$^{(W)}$ ±0.22	*3.34$^{(X)}$ ±0.11	*2.18$^{(Y)}$ ±0.089	*4.20$^{(Z)}$ ±0.20

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

MUSCLE SLICES:

- **Uptake in presence of insulin, acetylcholine and melatonin:** The uptake induced by melatonin in the muscle slices of control rats was significantly higher than that by insulin or acetylcholine. In the muscle slices of hypermelatonemic rats melatonin induced glucose uptake decreased significantly as compared to insulin or acetylcholine and also as compared to control liver slices. The glucose uptake promoted by insulin, acetylcholine or melatonin in the experimental rat muscle slices decreased significantly as compared to that of control muscle slices. There was no significant difference in uptake promoted by insulin or acetylcholine in the muscle slices of experimental rats (Figure and Table, 8.7, 8.8,).
- **Uptake by combinations of insulin, acetylcholine and melatonin:** All combinations of the three stimulants did not induce glucose uptake more than that promoted by melatonin alone in the control muscle slices The uptake promoted by I+Ac and M+Ac was almost similar to that of acetylcholine and less than that of M+I and M+Ac+I, the later induced uptake was higher than that of all the combinations in the muscle slices of control rats. The uptake induced by all the combinations in the muscle slices of hypermelatonemic rats decreased significantly as compared to that of control muscle slices. The uptake promoted by the combinations was in the order M+I>M+Ac+I>M+Ac>I+Ac and was also greater by all the

agents alone in the experimental muscle slices (Figure and Table; 8.7, 8.8, 8.11, 8.12).

- **Uptake by combinations of insulin, acetylcholine and melatonin in presence of luzindole:** The muscle slices of control animals showed glucose uptake in presence of luzindole which was greater than that by insulin alone and by the combinations of L+I, L+Ac and L+Ac+I. Whereas, glucose uptake by control muscle slices in presence of L+Ac was minimal as compared to all other combinations and individual agents, the uptake promoted by L+I was similar to that of insulin alone. Glucose uptake induced by luzindole in the muscle slices of hypermelatonemic rats was greater than that by L+I and reduced as compared to the control muscle slices. While, L+I showed significantly increased glucose uptake than by any other combination or individual agent, the glucose uptake by L+Ac+I was reduced significantly as compared to I+Ac in the experimental muscle slices and increased as compared to the control muscle slices. The uptake promoted by L+Ac increased significantly in the experimental muscle slices that by L+I decreased significantly (Figure and Table; 8.9, 8.10, 8.11, 8.12).

DISCUSSION:

Previous studies on glucose uptake in the weaning and pubertal ages had shown the significantly higher glucose uptake in the former age and significantly lower glucose uptake at the later age (Chapter 2 & 5). The present study in young rats showed relatively increased glucose

uptake compared to the pubertal age but less than the weaning age. Though this pattern of age dependent glucose uptake is similar in both control and hypermelatonemic animals, there is nevertheless a difference in degree of uptake, with hypermelatonemic rats showing higher uptake in weaning period and lower uptake in pubertal period. In the present study on young rats the hypermelatonemic rat tissues show a generalized reduction in glucose uptake relative to controls at 10 minutes. This is in contrast to an observation by M. N. Goodman who recorded progressively decreasing sensitivity and responsiveness to insulin induced glucose transport during early development and adolescence (i.e. 3-5 weeks and 5-16 weeks). In the present study we have recorded an increased sensitivity to stimulators of uptake and increased glucose uptake at the young age of 60 days relative to pubertal age of 45 days. Apparently post pubertal period is marked by reversal of the decreased sensitivity manifested in the pubertal period. Though the hypermelatonemic rat tissue slices show almost similar glucose uptake at 10 minutes, the uptake on a long term basis at 90 minutes was significantly higher in the control animals with only a marginal increase in the hypermelatonemic rats. This would suggest a definite resistance to glucose uptake in hypermelatonemic rats. A combination of melatonin and acetylcholine seems to have a potentiated effect in the control liver slices as the uptake at 10 minutes was significantly higher than insulin and melatonin as well as all combinations. The hypermelatonemic liver slices show significantly reduced acetylcholine sensitivity as marked by low and unchanged

uptake at 10 or 90 minutes suggesting a reduced parasympathetic tone (Fig. and Tab.; 8.1, 8.2). Though luzindole promotes glucose uptake to the same extent as melatonin, luzindole and combinations of luzindole show similar significantly high glucose uptake at 90 minutes in control animals not manifested in the experimental animals (Fig. and Tab.; 8.5. 8.6). The only noticeable feature with respect to glucose uptake by muscle is the significantly high melatonin induced uptake at 10 minutes relative to insulin and acetylcholine this is reflected in the higher uptake promoted by combinations of melatonin relative to insulin and acetylcholine (Fig. and Tab., 8.7). Though the glucose uptake promoted by luzindole or combinations of luzindole in the muscle of control animals at 10 minutes is relatively lower than that promoted by melatonin or combinations of melatonin, the long term uptake at 90 minutes is as high and similar to those promoted by insulin, acetylcholine, melatonin or there combinations (Fig. and Tab.; 8.11, 8.12) A comparison of the glucose uptake by tissues of weaning rats (Chapter 2) and young rats (present chapter) indicates a higher glucose uptake at 10 minutes with lower intake at 90 minutes in the former and a reversed lower intake at 10 minutes and higher intake at 90 minutes at later. This may be considered as an indication of an age related increased resistance to insulin as well as other uptake agents which may be related with the observations of Goodman *et al*, (1983). In a recent study an interaction of age and diet has been shown for increasing insulin resistance and reduced glucose uptake in the gastrocnemius muscle (Pagliassotti *et al.*, 2000). The observed higher

glucose uptake by control tissues relative to hypermelatonemic tissues along with the observed depletion of tissue glycogen contents (Chapter 7) may be related to observed lipid content increase (Chapter 9). The decreased glucose uptake shown by hypermelatonemic tissues at both 10 and 90 minutes with concurrent glycogenolysis (Chapter 7) may be again in this context be related to decreased lipid contents. These observations tend to suggest channelisation of increased glucose taken up by the tissues towards lipid deposition in control animals as a function of post pubertal maturity which is nullified by neonatal hypermelatonemia. This could suggest a long term effect of neonatal hypermelatonemia on subtly altered metabolic channelisation and metabolic homeostasis which need to be studied in greater detail. Apparently there could be an age dependent difference in the partitioning of carbohydrates among tissues and intra cellular pathways.

SUMMARY:

Previous studies have shown that continuous melatonin administration for the entire duration of the weaning period of rat neonates leads to hypoinsulinemia (Chapter 1) and increased insulin sensitivity of liver and muscle in the weaning stage supported by higher glucose uptake by these tissues *in vitro* (Chapter 2). There is an age related increase in resistance to glucose uptake, though less marked in the hypermelatonemic rats (Chapter 5). Based on the above observations it was thought pertinent to evaluate the long term influence of neonatal melatonin treatment on *in vitro* uptake of glucose by liver and muscle

slices. 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. Melatonin and Acetylcholine induced glucose uptake decreased significantly in the experimental rat liver slices. The uptake promoted by M+Ac and M+Ac+I decreased significantly in the experimental rat liver slices while, that by I+Ac and M+I increased significantly. However Luzindole and its combinations showed a significantly decreased glucose uptake in the liver slices of experimental rats. The glucose uptake promoted by I, Ac, M and their combinations in the muscle slices of melatonin treated rats decreased significantly. Glucose uptake induced by Luzindole and its combinations decreased significantly in the muscle slices of experimental rats. These observations tend to suggest channelisation of increased glucose taken up by the tissues towards lipid deposition in control animals as a function of post pubertal maturity which is nullified by neonatal hypermelatonemia. This could suggest a long term effect of neonatal hypermelatonemia on subtly altered metabolic channelisation and metabolic homeostasis which need to be studied in greater detail. Apparently there could be an age dependent difference in the partitioning of carbohydrate reserves among tissues and intra cellular pathways.