CHAPTER – 5

NEONATAL HYPERMELATONEMIA INCREASES GLUCOSE UPTAKE BUT DECREASES GLUCOSE OXIDATION BY LIVER AND MUSCLE IN THE PUBERTAL PERIOD: AN *IN VITRO* STUDY.

INTRODUCTION:

Involvement of melatonin in modulating intermediary metabolism is gaining increasing validation (Mustonen *et al.*, 2002). In seasonal mammals its role in the annual changes in body weight, adiposity and food intake has already been related with the pattern of melatonin secretion (Wade and Bartness, 1984) The identification of melatonin receptors not only in the brain but also in many systemic organs suggest both central as well as peripheral mode of action of the hormone (Accuna-Castroveijo *et al* , 1994; Song *et al.*, 1995; Shima *et al.*, 1997; Williams *et al.*, 1997; Martin *et al.*, 1998; Van Cauter E., 1998). Though the mechanism of action of melatonin on energy and intermediary metabolism is not clear, the presence of its receptors in liver, muscle and adipose tissue seems to suggest a definite role for melatonin in modulating the metabolic functions of these organs. This possibility has been validated by the reported influence of melatonin on blood glucose and tissue glycogen contents in various species and

they were providing more support for its role on glycemic status and carbohydrate homeostasis in vertebrates (Ramachandran, 2002). Though relatively less well studied, even the effects of melatonin on lipid metabolism are under scrutiny (de Vlaming *et al.*, 1974). Some of the studies in this direction have shown the efficacy of pineal extracts to lower serum, hepatic, adrenal and testicular cholesterol levels. In one such study on rabbits, pineal extracts could decrease serum cholesterol, biliary cholesterol and serum phospholipids (Esquifino *et al.*, 1997). The cholesterol lowering effect of melatonin has been considered a potent long term influence of melatonin which could not only decrease plasma cholesterol level but also prevent fatty liver development in genetic hypercholestolemic rats (Aayoma *et al.*, 1988). Melatonin has also been shown to prevent hyperlipidemia caused by hyper glucocorticoids in rats (Aayoma *et al.*, 1988) or by cholesterol rich feed (Mori *et al.*, 1984). During the course of this study involving neonatal hypermelatonemia, significant hyperinsulinemia and hypoglycemia along with tissue glycogenic effect but with reduced tissue sensitivity to insulin and other agents promoting glucose uptake have been seen in the weaning period (Chapter 1&2). Concurrently, neonatal hypermelatonemic status has also been shown to lower tissue lipid and cholesterol contents but increase serum lipid fractions in the weaning period (Chapter 3). Evaluation of the long term influence of neonatal hypermelatonemia on pubertal metabolic strategy has revealed higher serum insulin level and potentiated glycogenic and protein anabolic influence, even more than in the weaning stage

(Chapter 4). Parallel studies being conducted with luzindole an MT_2 melatonin receptor blocker have also provided confirmatory evidence towards above observations Adi, 2004. As a follow up of the above observations on persistent glycogenic effect and the hyperinsulinemic and hyperglycemic status in the pubertal period compared to the weaning period, present studies on *in vitro* uptake of glucose by liver and muscle slices in presence of various uptake promoting agents singly or in combinations. C^{14} glucose oxidations by liver and muscle slices have been undertaken.

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

RESULTS:

GLUCOSE UPTAKE:

Liver Slices:

- **Uptake in presence of insulin, acetylcholine and melatonin:** The liver slices of control rats showed glucose uptake by all the three agents in the order melatonin>acetylcholine>insulin. The liver slices of hypermelatonemic rats also showed uptake with all the three in the order insulin=melatonin>acetylcholine. Though acetylcholine induced glucose uptake was significantly reduced as compared to the control liver slices, both insulin and melatonin promoted uptake was significantly higher in the liver slices of hypermelatonemic rat liver slices (Figure and Table; 5.1)
- **Uptake by combinations of insulin, acetylcholine and melatonin:** All combinations of the three stimulants promoted

glucose uptake in the control liver slices. I+Ac and M+Ac showed uptake similar to that by acetylcholine or melatonin alone while, M+Ac+I induced uptake was slightly greater than the above two combinations and that of M+I was significantly greater than all other combinations as well as by that of all the stimulants alone. The liver slices of hypermelatonemic rats showed significantly greater uptake with M+I as compared to I+Ac, M+Ac or M+Ac+I though less than that of control liver slices. In the liver slices of hypermelatonemic rats the glucose uptake promoted by I+Ac, M+Ac and M+Ac+I was significantly greater than that of acetylcholine alone (Figure and Table; 5.1, 5.3)

- **Uptake by insulin, acetylcholine and melatonin in presence of luzindole:** In the control liver slices even luzindole promoted uptake though it was significantly less than insulin or acetylcholine. Whereas, the combinations of L+I and L+Ac showed uptake similar to that of acetylcholine or I+Ac, the combination of all the three significantly increased glucose uptake in the control liver slices by any other combination or any of the agents individually. The liver slices of hypermelatonemic rats showed significantly increased glucose uptake by luzindole than by acetylcholine alone or by L+Ac. Whereas, the combination of L+I significantly increased the uptake promoted by liver slices of hypermelatonemic rats than by any other combination or by any of the stimulant alone, L+Ac showed

significantly decreased uptake than all of the combinations or by any of the stimulant alone in the hypermelatonemic rat liver slices (Figure and Table; 5.2, 5.3)

Muscle Slices:

- **Uptake in presence of insulin, acetylcholine and melatonin:** Control muscle slices showed maximal uptake in presence of melatonin and minimal uptake with acetylcholine. The muscle slices of hypermelatonemic rats showed no significant difference in uptake promoted by insulin or melatonin, but was higher than that of acetylcholine by both of them. The uptake promoted by insulin and acetylcholine was significantly greater than that of control slices while, that of melatonin was decreased in the experimental slices (Figure and Table; 5.4)
- **Uptake with combinations of insulin, acetylcholine and melatonin:** Except for M+I which showed significantly higher uptake almost as equal to M+Ac+I, neither M+Ac nor I+Ac showed any further increase in glucose uptake promoted by any of them individually except for melatonin which was nearly equal to that of M+Ac+I in the control liver slices. In the hypermelatonemic rat muscle slices M+Ac+I promoted maximum uptake whereas the uptake promoted by M+I and M+Ac was same and greater than that by I+Ac.

Table and Figure: 5.1

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
	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	

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<0.01; ⊙P<0.05; NSNon Significant

Figure 5.1: Glucose uptake at 10 minutes by liver slices of pubertal rats on 45th day with combinations of insulin, acetylcholine and melatonin subjected to neonatal melatonin treatment:

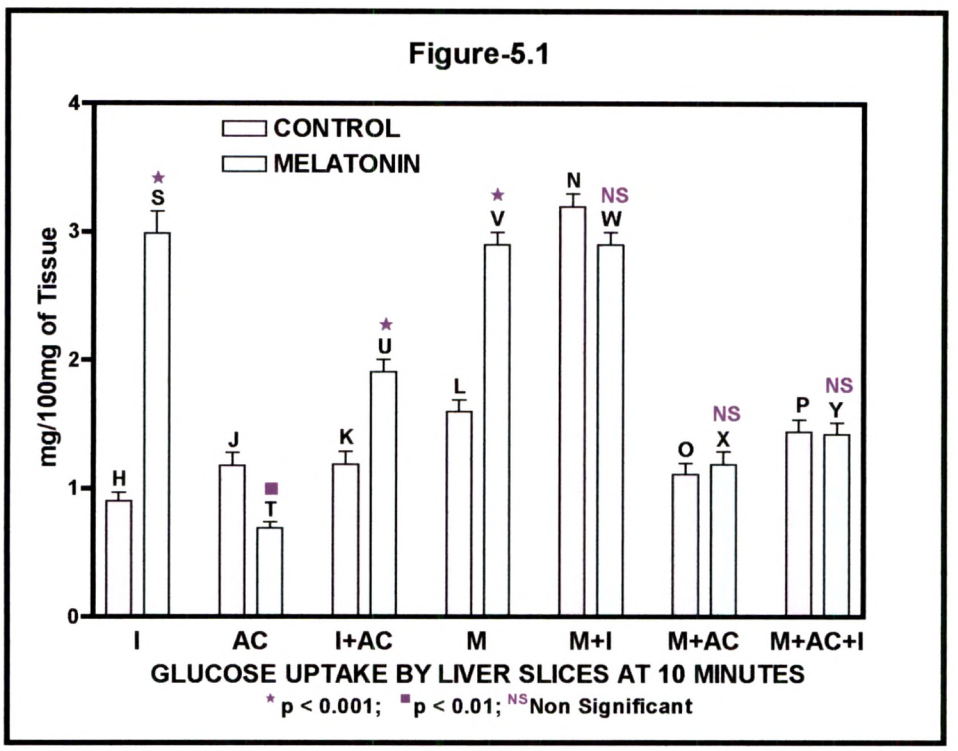

Table 5.1: Glucose uptake at 10 minutes by liver slices of pubertal rats on 45th day with combinations of insulin, acetylcholine and melatonin subjected to neonatal melatonin treatment:

	I	AC	I+AC	M	M+I	M+AC	M+AC+I
CONTROL	0.90$^{(H)}$ ±0.070	1.18$^{(J)}$ ±0.099	1.19$^{(K)}$ ±0.098	1.60$^{(L)}$ ±0.091	3.2$^{(N)}$ ±0.10	1.11$^{(O)}$ ±0.089	1.44$^{(P)}$ ±0.094
MELATONIN	*2.99$^{(S)}$ ±0.170	■0.69$^{(T)}$ ±0.047	*1.91$^{(U)}$ ±0.098	*2.9$^{(V)}$ ±0.10	NS2.9$^{(W)}$ ±0.099	NS1.19$^{(X)}$ ±0.096	NS1.42$^{(Y)}$ ±0.093

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

Table 5.2

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	✶
	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	✶
	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	✶	✶	⊙	✶	✶	■	✶	✶	✶	

*p<0.001; ⊙P<0.05; NSNon Significant

Figure 5.2: Glucose uptake at 10 minutes by liver slices of pubertal rats on 45th day with combinations of insulin, acetylcholine and luzindole subjected to neonatal melatonin treatment:

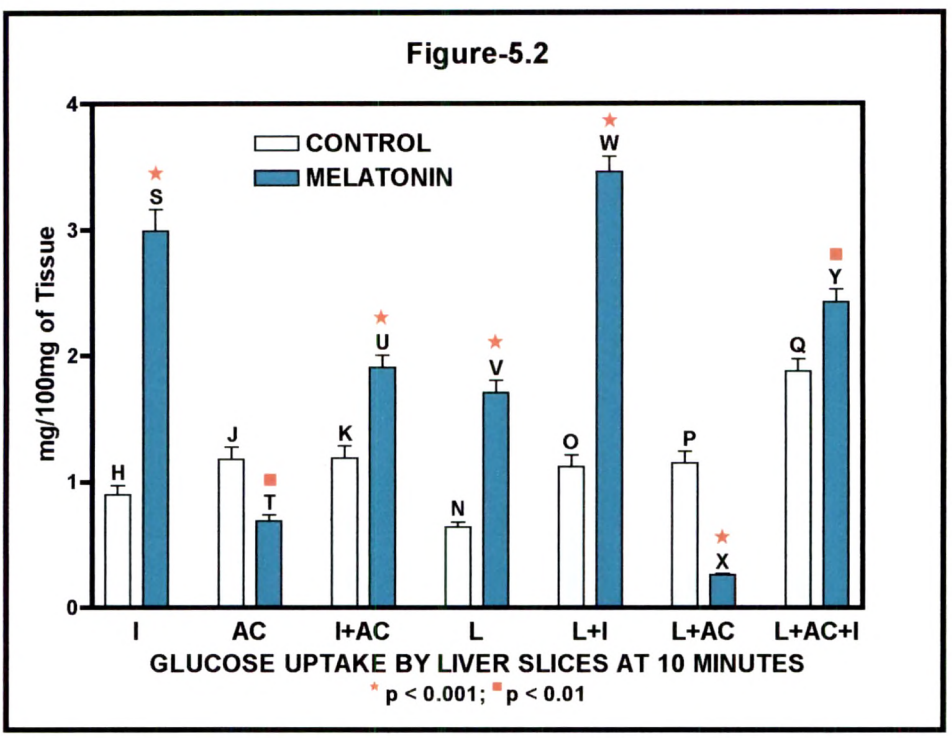

Table 5.2: Glucose uptake at 10 minutes by liver slices of pubertal rats on 45th day with combinations of insulin, acetylcholine and luzindole subjected to neonatal melatonin treatment:

	I	AC	I+AC	L	L+I	L+AC	L+AC+I
CONTROL	0.90 (H) ±0.070	1.18 (J) ±0.099	1.19 (K) ±0.098	0.64 (N) ±0.042	1.12 (O) ±0.098	1.15 (P) ±0.098	1.88 (Q) ±0.11
MELATONIN	*2.99 (S) ±0.170	■0.69 (T) ±0.047	*1.91 (U) ±0.098	*1.71 (V) ±0.099	*3.46 (W) ±0.12	*0.26 (X) ±0.009	■2.43 (Y) ±0.11

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

Table 5.3

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
	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	*	*

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
	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	*	*	*	*	*	*	*	*	*

*p<0.001; ⊙P<0.05; NSNon Significant

Figure 5.3: Glucose uptake at 10 minutes by liver slices of pubertal rats on 45th day with combinations of insulin, acetylcholine, melatonin and luzindole subjected to neonatal melatonin treatment:

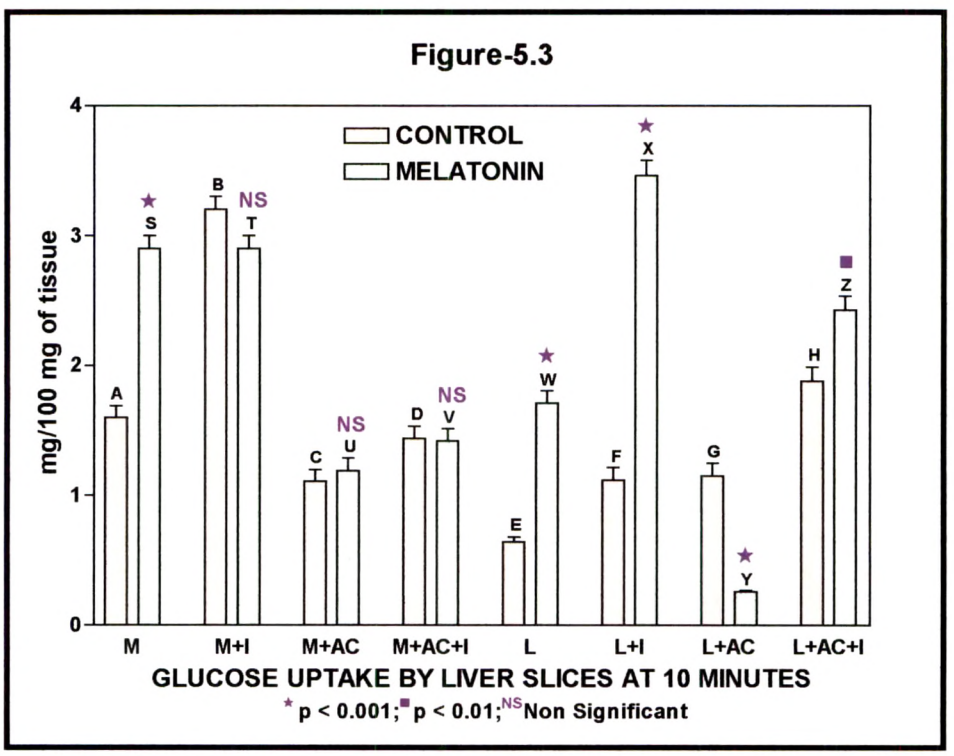

Table 5.3: Glucose uptake at 10 minutes by liver slices of pubertal rats on 45th day with combinations of insulin, acetylcholine, melatonin and luzindole subjected to neonatal melatonin treatment:

	M	M+I	M+AC	M+AC+I	L	L+I	L+AC	L+AC+I
CONTROL	1.60 (A) ±0.091	3.20 (B) ±0.10	1.11 (C) ±0.089	1.44 (D) ±0.094	0.64 (E) ±0.042	1.12 (F) ±0.098	1.15 (G) ±0.098	1.88 (H) ±0.11
MELATONIN	* 2.90 (S) ±0.10	NS 2.9 (T) ±0.099	NS 1.19 (U) ±0.096	NS 1.42 (V) ±0.093	* 1.71 (W) ±0.099	* 3.46 (X) ±0.12	* 0.26 (Y) ±0.009	■ 2.43 (Z) ±0.11

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

Table and Figure: 5.4

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	✶	✶	✶	✶	■	✶
	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	⊙	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	■	NS	NS	⊙	

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

Figure 5.4: Glucose uptake at 10 minutes by muscle slices of pubertal rats on 45th day with combinations of insulin, acetylcholine and melatonin subjected to neonatal melatonin treatment:

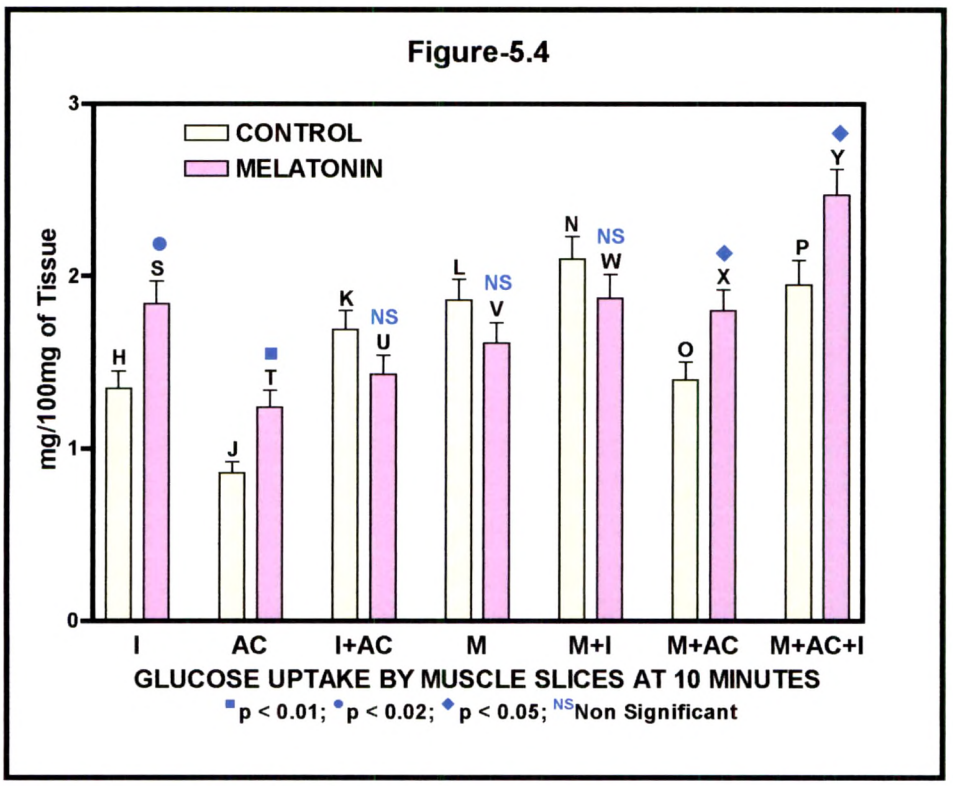

Figure 5.4: Glucose uptake at 10 minutes by muscle slices of pubertal rats on 45th day with combinations of insulin, acetylcholine and melatonin subjected to neonatal melatonin treatment:

	I	AC	I+AC	M	M+I	M+AC	M+AC+I
CONTROL	1.35(H) ±0.099	0.86(J) ±0.064	1.69(K) ±0.11	1.86(L) ±0.12	2.1(N) ±0.13	1.4(O) ±0.10	1.95(P) ±0.14
MELATONIN	•1.84(S) ±0.13	■1.24(T) ±0.10	NS1.43(U) ±0.11	NS1.61(V) ±0.12	NS1.87(W) ±0.14	◆1.8(X) ±0.12	◆2.47(Y) ±0.15

Values are expressed as mean ± SEM, ■ p < 0.01; •p < 0.02; ◆p < 0.05; NSNon Significant

Table 5.5

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	⊙	✶
	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	✶	⊙	✶	✶	✶	✶	

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	■	

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

Figure 5.5: Glucose uptake at 10 minutes by muscle slices of pubertal rats on 4th day with combinations of insulin, acetylcholine and luzindole subjected to neonatal melatonin treatment:

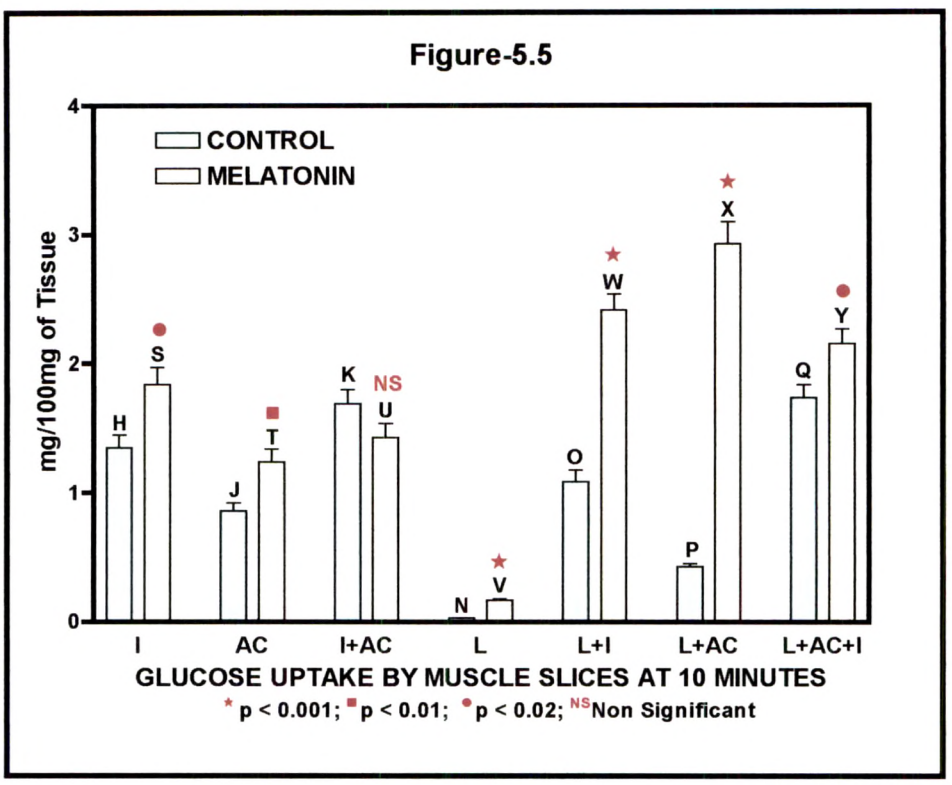

Table 5.5: Glucose uptake at 10 minutes by muscle slices of pubertal rats on 45th day with combinations of insulin, acetylcholine and luzindole subjected to neonatal melatonin treatment:

	I	AC	I+AC	L	L+I	L+AC	L+AC+I
CONTROL	1.35 (H) ±0.099	0.86 (J) ±0.064	1.69 (K) ±0.11	0.03 (N) ±0.0001	1.09 (O) ±0.090	0.43 (P) ±0.021	1.74 (Q) ±0.098
MELATONIN	•1.84 (S) ±0.13	■1.24 (T) ±0.10	NS1.43 (U) ±0.11	*0.17 (V) ±0.0099	*2.42 (W) ±0.12	*2.93 (X) ±0.17	•2.16 (Y) ±0.11

Values are expressed as mean ± SEM, *p < 0.001; ■ p < 0.01; •p < 0.02; NS Non Significant

Table 5.6

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	■
	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	✶	⊙	✶	✶	✶	✶

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	✶	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 5.6: Glucose uptake at 10 minutes by muscle slices of pubertal rats on 45th day with combinations of insulin, acetylcholine, melatonin and luzindole subjected to neonatal melatonin treatment:

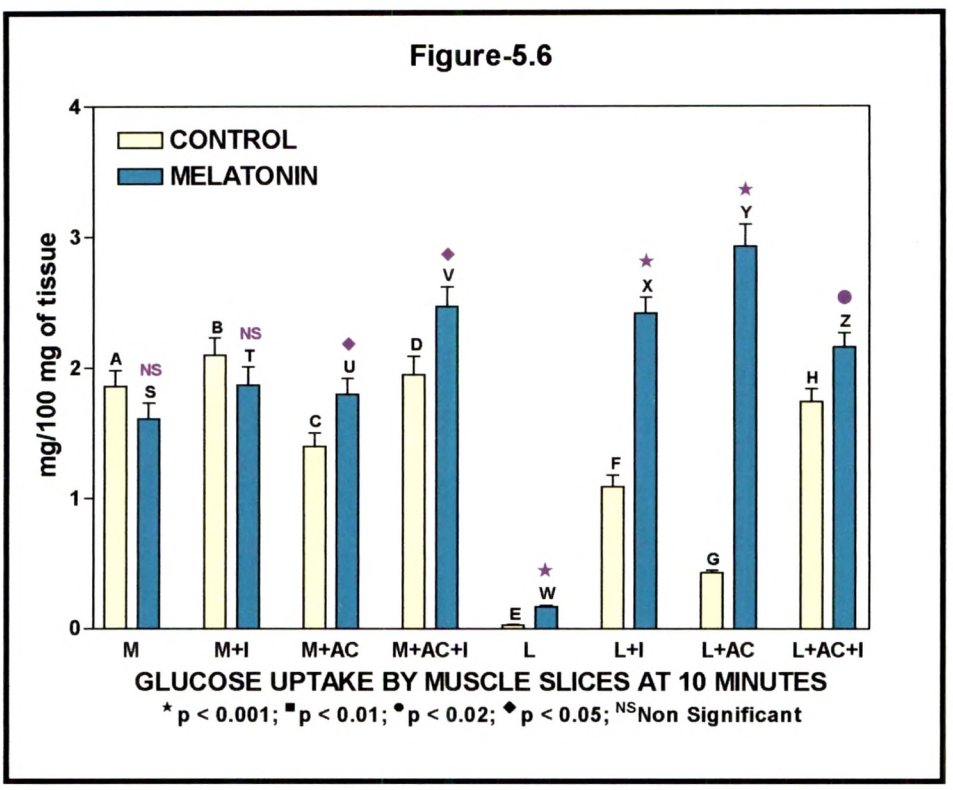

Table 5.6: Glucose uptake at 10 minutes by muscle slices of pubertal rats on 45th day with combinations of insulin, acetylcholine, melatonin and luzindole subjected to neonatal melatonin treatment:

	M	M+I	M+AC	M+AC+I	L	L+I	L+AC	L+AC+I
CONTROL	1.86(A) ±0.12	2.10(B) ±0.13	1.40(C) ±0.10	1.95(D) ±0.14	0.03(E) ±0.0001	1.09(F) ±0.09	0.43(G) ±0.021	1.74(H) ±0.098
MELATONIN	NS1.61(S) ±0.12	NS1.87(T) ±0.14	◆1.80(U) ±0.12	◆2.47(V) ±0.15	*0.17(W) ±0.0099	*2.42(X) ±0.12	*2.93(Y) ±0.17	●2.16(Z) ±0.11

Values are expressed as mean ± SEM, *p < 0.001; ●p < 0.02; ◆p < 0.05; NS Non Significant

Table 5.7

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	⊙	✶	✶	✶	✶	✶	✶	✶	✶	✶	✶	✶	✶
	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	✶	✶

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	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	✶	✶

*p<0.001; ⊙P<0.05; NSNon Significant

Figure 5.7: Glucose oxidation by liver slices of pubertal rats on 45th day with insulin, acetylcholine melatonin and there combinations compared to basal subjected to neonatal melatonin treatment:

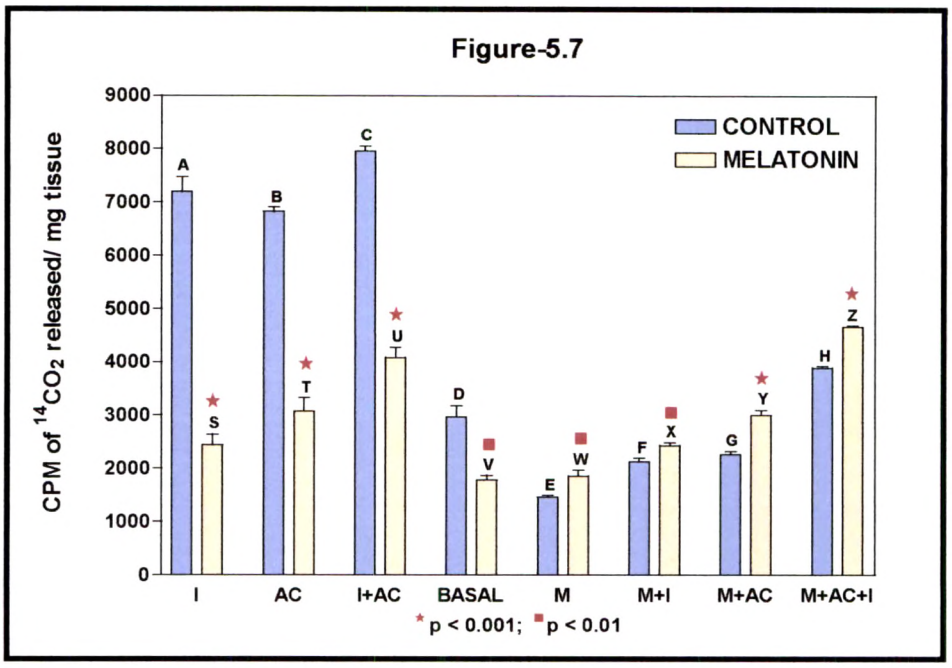

Table 5.7: Glucose oxidation by liver slices of pubertal rats on 45th day with insulin, acetylcholine, melatonin and there combinations compared to basal subjected to neonatal melatonin treatment:

	I	AC	I+AC	BASAL
CONTROL	7195.03(A) ±283.29	6826.72(B) ±83.07	7961.83(C) ±89.40	2975.13(D) ±205.46
MELATONIN	*2441.76(S) ±202.19	*3076.64(T) ±248.95	*4083.41(U) ±195.05	■1785.89(V) ±86.51

	M	M+I	M+AC	M+AC+I
CONTROL	1459.48(E) ±35.51	2127.69(F) ±74.01	2273.86(G) ±48.49	3897.13(H) ±31.94
MELATONIN	■1856.17(W) ±109.60	■2436.84(X) ±47.78	*3005.10(Y) ±97.92	*4663.05(Z) ±27.04

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

Table 5.8

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	✶	✶	✶	✶	✶	⊙	✶	✶	✶	✶	✶	✶
	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	✶

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	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

*p<0.001; ⊙P<0.05; NSNon Significant

Figure 5.8: Glucose oxidation by liver slices of pubertal rats on 45th day with combinations of insulin, acetylcholine and luzindole compared to basal subjected to neonatal melatonin treatment:

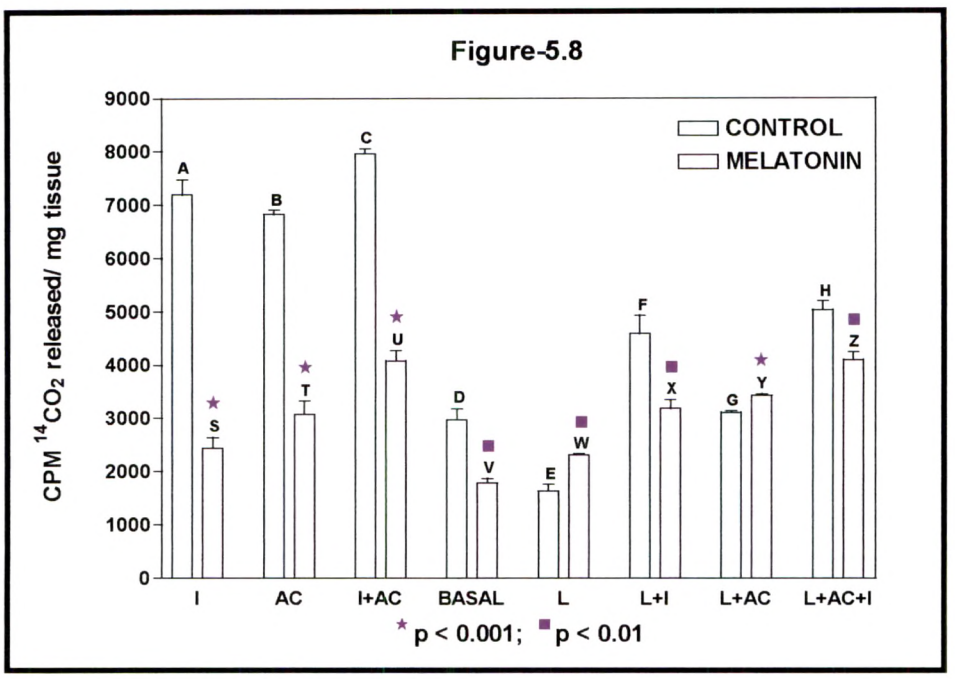

Table 5.8: Glucose oxidation by liver slices of pubertal rats on 45th day with combinations of insulin, acetylcholine and luzindole compared to basal subjected to neonatal melatonin treatment:

	I	AC	I+AC	BASAL
CONTROL	7195.03(A) ±283.29	6826.72(B) ±83.07	7961.83(C) ±89.40	2975.13(D) ±205.46
MELATONIN	*2441.76(S) ±202.19	*3076.64(T) ±248.95	*4083.41(U) ±195.05	■1785.89(V) ±86.51

	L	L+I	L+AC	L+AC+I
CONTROL	1636.96(E) ±124.70	4592.68(F) ±343.05	3113.71(G) ±29.41	5036.82(H) ±166.80
MELATONIN	■2318.10(W) ±22.32	■3184.95(X) ±166.26	*3435.46(Y) ±28.11	■4107.75(Z) ±141.65

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

Table 5.9

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	✶	■	✶	✶
	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	✶

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	✶	✶	✶	✶	⊙	✶	✶	✶	NS	✶	■

*p<0.001; ⊙P<0.05; NSNon Significant

Table 5.9: Glucose oxidation by liver slices of pubertal rats on 45th day with combinations of insulin, acetylcholine melatonin and luzindole subjected to neonatal melatonin treatment:

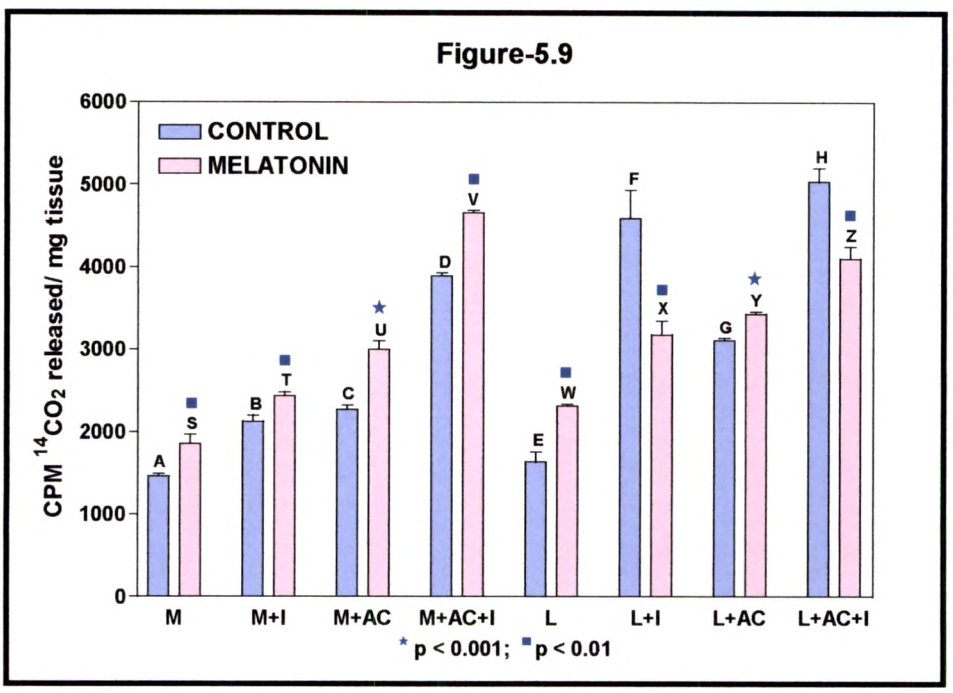

Table 5.9: Glucose oxidation by liver slices of pubertal rats on 45th day with combinations of insulin, acetylcholine melatonin and luzindole subjected to neonatal melatonin treatment:

	M	M+I	M+AC	M+AC+I
CONTROL	1459.48$^{(A)}$ ±35.51	2127.69$^{(B)}$ ±74.01	2273.86$^{(C)}$ ±48.49	3897.13$^{(D)}$ ±31.94
MELATONIN	■1856.17$^{(S)}$ ±109.60	■2436.84$^{(T)}$ ±47.78	*3005.10$^{(U)}$ ±97.92	■4663.05$^{(V)}$ ±27.04

	L	L+I	L+AC	L+AC+I
CONTROL	1636.96$^{(E)}$ ±124.70	4592.68$^{(F)}$ ±343.05	3113.71$^{(G)}$ ±29.41	5036.82$^{(H)}$ ±166.80
MELATONIN	■2318.10$^{(W)}$ ±22.32	■3184.95$^{(X)}$ ±166.26	*3435.46$^{(Y)}$ ±28.11	■4107.75$^{(Z)}$ ±141.65

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

Table 5.10

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	*	*	*	*	*	*	*	*	*	*	*	*	*
	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	*	*	*	*	■	*	*	*	■	*

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	*	*	*	*	*	*	*	*	*	*	*
	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	*	*	*

*p<0.001; ⊙P<0.05; NSNon Significant

Figure 5.10: Glucose oxidation by muscle slices of pubertal rats on 45th day with insulin, acetylcholine melatonin and there combinations compared to basal subjected to neonatal melatonin treatment:

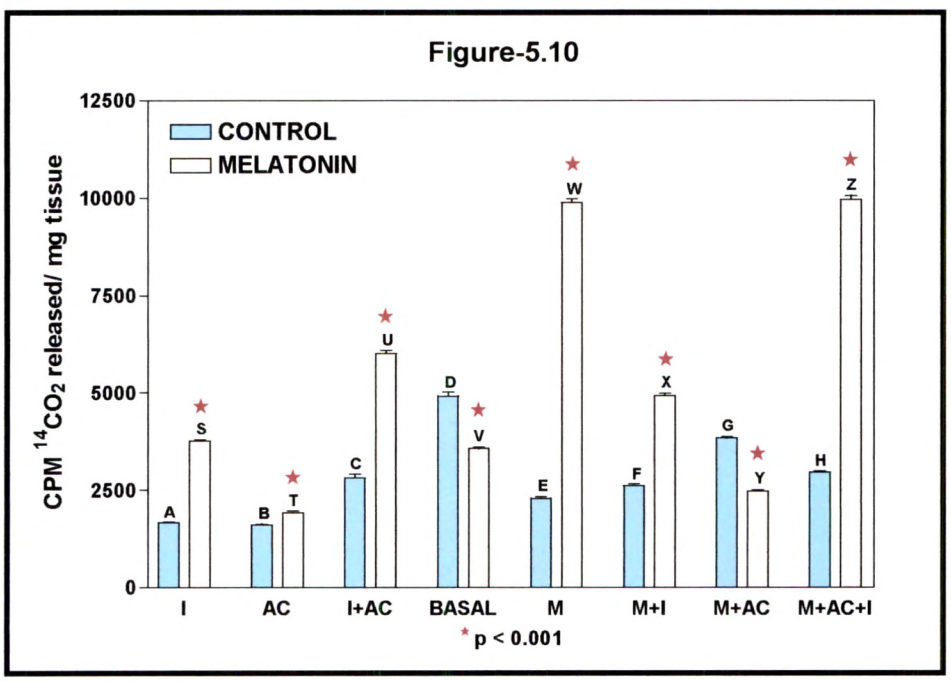

Table 5.10: Glucose oxidation by muscle slices of pubertal rats on 45th day with insulin, acetylcholine melatonin and there combinations compared to basal subjected to neonatal melatonin treatment:

	I	AC	I+AC	BASAL
CONTROL	1660.61(A) ±10.26	1599.55(B) ±25.32	2816.43(C) ±88.49	4912.07(D) ±100.60
MELATONIN	*3759.79(S) ±28.11	*1918.88(T) ±50.32	*6013.96(U) ±75.13	*3560.60(V) ±31.24

	M	M+I	M+AC	M+AC+I
CONTROL	2283.77(E) ±41.05	2605.52(F) ±43.28	3831.69(G) ±29.34	2959.20(H) ±27.98
MELATONIN	*9880.53(W) ±101.11	*4925.80(X) ±60.33	*2467.15(Y) ±23.12	*9961.96(Z) ±99.17

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

Table 5.11

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	*	*	*	*	*	*	*	*	*	*	*	*	*
	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	*	*	*	*	*	*

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	*	*	⊙	*	*	*	*	*	*	*	*	*	*	*
	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	*	*	*	*	*

*p<0.001; ⊙P<0.05; NSNon Significant

Figure 5.11: Glucose oxidation by muscle slices of pubertal rats on 45th day with combinations of insulin, acetylcholine and luzindole compared to basal subjected to neonatal melatonin treatment:

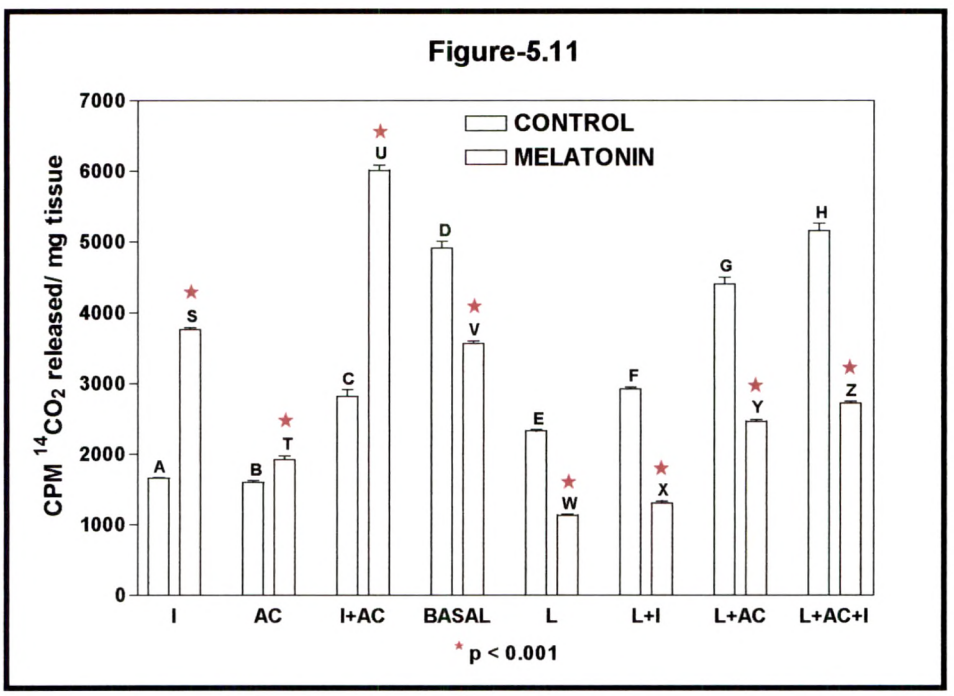

Table 5.11: Glucose oxidation by muscle slices of pubertal rats on 45th day with combinations of insulin, acetylcholine and luzindole compared to basal subjected to neonatal melatonin treatment:

	I	AC	I+AC	BASAL
CONTROL	1660.61(A) ±10.26	1599.55(B) ±25.32	2816.43(C) ±88.49	4912.07(D) ±100.60
MELATONIN	*3759.79(S) ±28.11	*1918.88(T) ±50.32	*6013.96(U) ±75.13	*3560.60(V) ±31.24

	L	L+I	L+AC	L+AC+I
CONTROL	2323.92(E) ±19.87	2920.03(F) ±19.87	4403.01(G) ±95.15	5157.51(H) ±104.58
MELATONIN	*1131.65(W) ±15.14	*1303.35(X) ±28.11	*2462.27(Y) ±24.32	*2719.60(Z) ±26.19

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

Table 5.12: Glucose oxidation by muscle slices of pubertal rats on 45th day with combinations of insulin, acetylcholine melatonin and luzindole subjected to neonatal melatonin treatment:

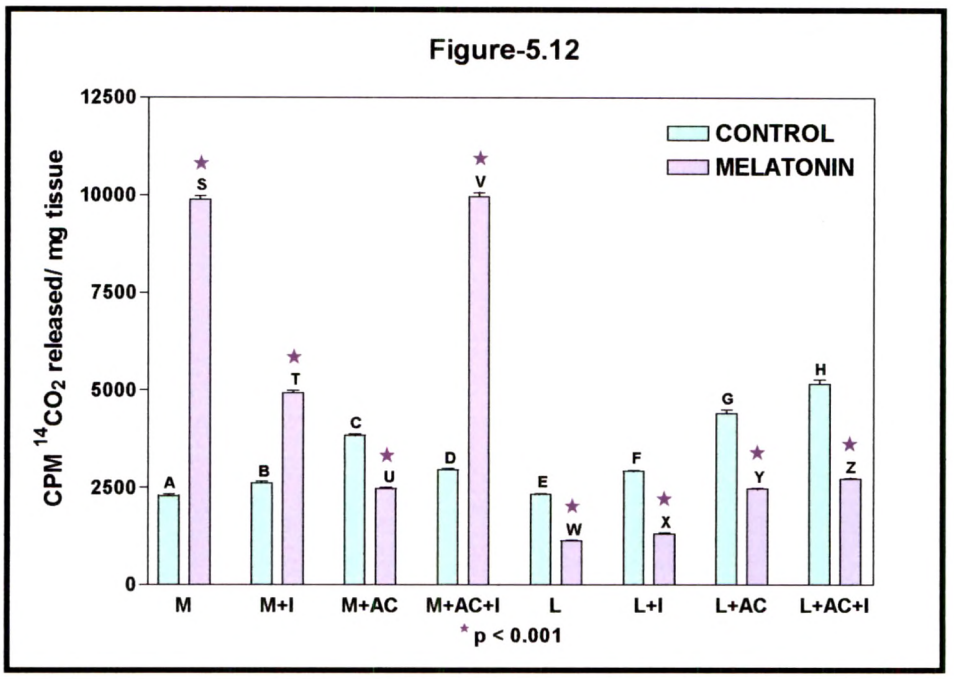

Table 5.12: Glucose oxidation by muscle slices of pubertal rats on 45th day with combinations of insulin, acetylcholine melatonin and luzindole subjected to neonatal melatonin treatment:

	M	M+I	M+AC	M+AC+I
CONTROL	2283.77(A) ±41.05	2605.52(B) ±43.28	3831.69(C) ±29.34	2959.20(D) ±27.98
MELATONIN	*9880.53(S) ±101.11	*4925.80(T) ±60.33	*2467.15(U) ±23.12	*9961.96(V) ±99.17

	L	L+I	L+AC	L+AC+I
CONTROL	2323.92(E) ±19.87	2920.03(F) ±19.87	4403.01(G) ±95.15	5157.51(H) ±104.58
MELATONIN	*1131.65(W) ±15.14	*1303.35(X) ±28.11	*2462.27(Y) ±24.32	*2719.60(Z) ±26.19

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

- As compared to control muscle slices I+Ac and M+I showed decreased uptake while M+Ac and M+Ac+I showed significantly increased uptake, the increase by M+AC+I being greater than any of the combinations or any agent alone both in experimental and control muscle slices (Figure and Table; 5.4, 5.6).
- **Uptake by insulin and acetylcholine in presence of luzindole:** In the control muscle slices the uptake promoted by luzindole was negligible. Whereas, the uptake promoted by L+I was greater than that of acetylcholine or luzindole alone and less than that of insulin alone or combination of I+Ac, the uptake promoted by L+Ac was minimal as compared to all other combinations and stimulants alone except for luzindole. The combination of L+Ac+I significantly increased glucose uptake by control muscle slices than by any other combination or any of the agents alone. In the experimental muscle slices the uptake promoted by luzindole is minimal as compared to all the combinations and stimulants alone but is greater than that of control muscle slices. L+I and L+Ac showed significantly increased uptake which was greater than that by any other combination or individual stimulant in experimental and control muscle slices. The combination of L+Ac+I showed uptake which was less than that by insulin alone and greater than that by acetylcholine or luzindole or the combination of I+Ac in the experimental muscle slices but was significantly decreased as

compared to that of control muscle slices (Figure and Table; 5.5, 5.6)

C^{14} Glucose oxidation by liver slices:

The C^{14} glucose oxidation by liver slices of the experimental rats decreased significantly with I, Ac, I+Ac and Basal state. However the C^{14} glucose oxidation induced by M. M+I, M+Ac and M+Ac+I significantly increased in the liver slices of the hypermelatonemic rats. Whereas the liver slices of experimental rats showed decreased oxidation with L+I, the C^{14} glucose oxidation with L, L+Ac and L+Ac+I was significantly increased in the experimental rats. The C^{14} glucose oxidation was maximum with L+Ac+I and minimum with M in the liver slices of melatonin treated rats. Whereas in the control rat liver slices I+Ac induced C^{14} glucose oxidation was maximum and that induced by M was minimum (Figure and Table; 5.7, 5.8, 5.9)

C^{14} Glucose oxidation by muscle slices:

The melatonin treated rat muscle slices showed significantly decreased C^{14} glucose oxidation in the Basal state and with M+Ac but, but the C^{14} glucose oxidation increased significantly with I, Ac, I+Ac, M, M+I and M+Ac+I. However luzindole and its combinations could induce significantly decreased glucose oxidation in the muscle slices of the melatonin treated rats The C^{14} glucose oxidation was maximum with M and minimum with L in the muscle slices of the experimental rats, whereas in the control slices the C^{14} glucose oxidation was maximum with L+Ac+I and minimum with L (Figure and Table; 5.10, 5.11, 5.12).

DISCUSSION:

Glucose uptake and oxidative potential of control and hypermelatonemic liver and muscle slices have been tested in presence of insulin, acetylcholine, melatonin and luzindole along with combinations of insulin and acetylcholine with both melatonin and luzindole. To avoid confusions and to have better clarity, observations on glucose uptake and glucose oxidation are being discussed separately and valid conclusions drawn thereafter.

Glucose Uptake: There is a generalized age related decrease in the glucose uptake potential of both control and hypermelatonemic liver and muscle slices as the degree of uptake recorded in the present study is significantly less than that seen in the weaning period (Chapter 2). Though this indicates a reduced sensitivity for glucose uptake in the pubertal period, it is relevant to note that the tissues of hypermelatonemic rats are still relatively more sensitive than those of control rats This relatively higher melatonin sensitivity in hypermelatonemic rats is clearly supportive of the higher glycogenic effect recorded in these rats previously (Chapter 4). Another age related change noticeable is the relative insensitivity towards agonist for glucose uptake, whereas the liver of control rats in the weaning stage had shown maximal glucose uptake in the order of insulin>melatonin>acetylcholine, in the present study the order is melatonin>acetylcholine>insulin. However the liver of hypermelatonemic rats show equal potency with both insulin and melatonin and reduced potential with acetylcholine (Fig. and Tab.; 5.1).

This suggests not only an increased ability for melatonin to promote glucose uptake but also potentiated insulin sensitivity due to neonatal hypermelatonemia. The potentiating influence of melatonin on glucose uptake is further emphasized by the presently observed maximal glucose uptake by the combination of melatonin and insulin (100% more than melatonin alone and 3-4 times more than insulin) in the control liver slices. Acetylcholine seems to have a dampening influence on glucose uptake induced by melatonin alone or in combination with insulin. Even in hypermelatonemic liver slices, acetylcholine seems to have a dampening effect on insulin and melatonin promoted glucose uptake as, the degree of glucose uptake in presence of these agents was significantly reduced when in combination with acetylcholine (Fig. and Tab.; 5.1). Though luzindole did not show any significant effect on glucose uptake in control slices, it is interesting to note that luzindole promoted significantly higher glucose uptake in the tissues of hypermelatonemic rats (Fig. and Tab.; 5.2). This effect of luzindole is manifested in the form of maximal glucose uptake by the combination of luzindole and insulin equal to or even more than that with melatonin+insulin (Fig and Tab.; 5.3). But acetylcholine seems to have a nullifying effect as it reduced the uptake promoted by luzindole alone as well as by luzindole in combination with insulin. It is likely that luzindole being a structural analog of melatonin acts through melatonin receptors in a positive way for glucose uptake as against its many negative melatonin receptor blocker effects. It is difficult to explain as to why this action of luzindole on glucose uptake

is manifested only in hypermelatonemic rats and not in control rats and, the underlying molecular mechanisms need to be studied in detail. Glucose uptake by liver slices taken as a whole suggests greater uptake in hypermelatonemic slices in general and the ability of melatonin to promote glucose uptake as well as its potentiating influence on insulin. In general acetylcholine seems to have a dampening effect on melatonin and insulin induced glucose uptake. Further luzindole seems to have potential to increase uptake in hypermelatonemic slices and also potentiates insulin induced glucose uptake like melatonin.

Glucose uptake by both control and hypermelatonemic muscle slices are significantly less compared to the uptake by slices from 22 day old weaning. The control slices showed maximal uptake with melatonin followed by insulin and least by acetylcholine (Fig. and Tab.; 5.4). A synergistic/additive influence could be seen only with melatonin+insulin and melatonin+acetylcholine+insulin suggesting a potentiating action of melatonin on insulin (Fig. and Tab.; 5.4). In contrast the hypermelatonemic muscle slices showed almost similar uptake with insulin, acetylcholine and melatonin, though the insulin induced uptake is significantly greater than in control slices (Fig. and Tab.; 5.4). There is a potentiating influence of melatonin on both acetylcholine and insulin induced uptake but an only marginal increase in uptake occurs when all the three are present together. On a comparative basis, the degree of glucose uptake by either liver or muscle of pubertal animals is 7-10 times less compared to weaning animals. There appears to be

a significant age related decrease in sensitivity to insulin, acetylcholine and melatonin which may be related with the pubertal increase in testosterone and, the male sex hormone is known to have significant effect on insulin sensitivity and thereby contributing to insulin resistance(Seraphim *et al.*, 1997, Lima *et al.*, 1998). An age related increase in insulin resistance and decreased glucose uptake have been reported in male Sprague-Dawley rats (Goodman *et al.*, 1983). Similar to liver, even muscle does not show any significant response to luzindole. However, luzindole seems to potentiate the action of insulin and acetylcholine in hypermelatonemic muscle as in the case of liver and, maximal uptakes are registered in combinations with luzindole (Fig. and Tab.; 5.5). The present observations seem to suggest a role for luzindole in glucose uptake, more so in melatonin primed tissues and the exact significance or molecular mechanisms await future experimental evaluations.

Glucose Oxidation: Though there was no significant difference in the degree of glucose uptake by liver and muscle of control animals, glucose oxidation is significantly higher in muscle compared to liver as observed under basal conditions. An interesting observation is that while the glucose oxidative potential of liver is significantly increased with melatonin, insulin and acetylcholine (Fig. and Tab.; 5.7) independently, there is a significant reduction in glucose oxidation by muscle in presence of these agents (Fig. and Tab.; 5.10). Another notable observation is that while combinations of melatonin with insulin and acetylcholine tend to decrease the potential compared to their

individual capacities in the liver, the combinations tend to increase the potential better than their individual capacities in the muscle though, still significantly less than under basal conditions. Luzindole in general has a depressive influence on glucose oxidation by both liver and muscle but interestingly combinations with insulin and acetylcholine increased the oxidative potential above the basal levels in liver and towards basal level in muscle (Fig. and Tab.; 5.8, 5.11). Apparently luzindole seems to potentiate the oxidative potential of both insulin and acetylcholine in both liver and muscle compared to the ability of melatonin (Fig. and Tab.; 5.9, 5.12).

Neonatal hypermelatonemia seems to have a depressive influence on glucose oxidation potential of both liver and muscle as measured by the C^{14} carbon dioxide released under basal conditions which is significantly less than the control animals. It is remarkable to note that compared to control animals the liver and muscle of hypermelatonemic rats show differential effects in presence of insulin, acetylcholine and melatonin. Whereas melatonin significantly increased glucose oxidation in muscle, it has no significant influence on liver. Whereas insulin does not have any significant influence on oxidative capacity of muscle and acetylcholine significantly decreases the potential, there is increased glucose oxidation in liver in presence of both, more so with acetylcholine. A combination of insulin and acetylcholine has shown greater potential for glucose oxidation by both liver and muscle unlike their differential independent effect. The increased glucose oxidation potential seen with combinations of insulin and acetylcholine with

melatonin in both liver and muscle is similar to the individual capacities of insulin, acetylcholine or even a combination of insulin and acetylcholine. The ability of acetylcholine to decrease the oxidation potential of muscle is emphasized by its ability to decrease melatonin induced oxidation in a combination of melatonin+acetylcholine. Inferably melatonin is not able to potentiate the action of insulin and acetylcholine in liver while, insulin and acetylcholine decrease the ability of melatonin for oxidation in muscle. It is also clear from the present observations that a combination of insulin and acetylcholine has a greater potential for glucose oxidation in both liver and muscle with or without melatonin (Fig. and Tab.; 5.7, 5.10). Luzindole has no significant influence on the oxidative capacity of liver while it decreases the same in the muscle. However in presence of luzindole, insulin and acetylcholine or even a combination of both showed a better capacity of oxidation compared to their individual abilities in the liver while it depressed the oxidative capacity of insulin and acetylcholine or even a combination of both in muscle. These observations on hypermelatonemic rats taken as a whole suggest that while insulin, acetylcholine and a combination of the two have better potential to increase glucose oxidation in the liver, melatonin or a combination of insulin and acetylcholine have a better potential to increase oxidation in the muscle. The observations on *in vitro* glucose oxidation studies taken as a whole tend to indicate that the tissues of hypermelatonemic rats has lowered glucose oxidation potential compared to the tissues of control animals under basal as well as stimulated conditions. Further it

is clear that insulin or acetylcholine or even a combination of the two have a greater potential for glucose oxidation in the liver of both control and hypermelatonemic rats though significantly more in the former. However in the muscle of control rats there is a generalized decrease in the oxidative potential in presence of all stimulants while in the muscle of hypermelatonemic animals melatonin, insulin+acetylcholine and insulin have significantly increased capacity for glucose oxidation in the above order (Fig. and Tab.; 5.7). The generalized decrease in the oxidative potential shown by the tissues of hypermelatonemic rats is correlatable with the significantly greater glycogen, lipid and protein anabolic influence observed in these animals (Chapter 4 & 6). Though there are studies on *in vitro* glucose oxidation by many tissues to understand relative substrate utilization, relative capacities of oxidization by different organs in the same animal as well as on metabolism of glucose in terms of its oxidation or incorporation into carbohydrate and lipid reserves (Goodridge, 1968; Kraft and Johnson, 1972; Turner and Johnson, 1973; Chauhan and Nath, 1978), there are no such studies related to the present line of investigation and this being the only study of this kind, no detailed discussion incorporating relevant observations from literature is possible. Finally it can be concluded from the present observations that there is an age related increase in resistance to glucose uptake though less marked in the hypermelatonemic rats. Concomitantly there is decreased glucose oxidation in hypermelatonemic rats. The relatively greater uptake capacity coupled with reduced glucose oxidation suggests an overall

anabolic *milieu* in the neonatal hypermelatonemic rats. Since luzindole has shown some noteworthy effects on glucose uptake as well as oxidation, it is clear that it has functional abilities other than its known role as melatonin receptor antagonist, and more studies are warranted to understand these functional abilities.

SUMMARY:

Evaluation of the long term influence of neonatal hypermelatonemia on pubertal strategy has revealed higher serum insulin level and potentiated glycogen and protein anabolic influences even more than in the weaning stage (Chapter 4). As a follow up of the above observations on persistent glycogenic effect and the hyperinsulinemic and hyperglycemic status in the pubertal period compared to the weaning period, the present studies on *in vitro* uptake of glucose by liver and muscle slices in presence of various uptake promoting agents singly or in combinations have been conducted further C^{14} glucose oxidation by liver and muscle slices has also been undertaken. 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 experimental rat liver slices showed significantly increased glucose uptake with Insulin (I) and Melatonin (M) and significantly reduced uptake with Acetylcholine (Ac). The combinations of M+I showed significantly increased glucose uptake as compared to other combinations by the experimental rat liver slices. Whereas the combination of L+I significantly increased the uptake promoted by liver

slices of hypermelatonemic rats, than by any other combination or, by any of the stimulant alone, L+Ac showed significantly decreased uptake than all of the combinations or by any of the stimulant alone in the hypermelatonemic rat liver slices. The uptake promoted by I and Ac is significantly greater than that of control slices, and uptake by has melatonin decreased significantly in the experimental slices. In the hypermelatonemic rat muscle slices M+Ac+I, promoted maximum uptake, whereas the uptake promoted by M+I and M+Ac was same and greater than that by I+Ac. In the experimental muscle slices the uptake promoted by Luzindole (L) is minimal as compared to all the combinations and stimulants alone but is greater than that of control muscle slices. The liver slices of experimental rats showed significantly decreased C^{14} glucose oxidation in basal state as well as with I, Ac and their combinations. However, both M and L with there combinations significantly increased the C^{14} glucose oxidation in the liver slices of experimental rats. In the muscle slices of experimental rats, the basal C^{14} glucose oxidation is significantly reduced whereas, that with M is significantly increased. However, I, Ac, I+Ac, M+I and M+Ac+I showed significantly increased C^{14} glucose oxidation, M+Ac showed significantly decreased C^{14} glucose oxidation in the muscle slices of experimental rats. L and its combinations showed significantly decreased C^{14} glucose oxidation in the muscle slices of experimental rats. It can be concluded from the present study that there is an age related increase in resistance to glucose uptake, though less marked in the hypermelatonemic rats. Concomitantly, there is decreased glucose

oxidation in hypermelatonemic rats. The relatively greater uptake capacity coupled with reduced glucose oxidation suggests an overall anabolic *milieu* in the neonatal hypermelatonemic rats.