CHAPTER – 5

INCREASED GLUCOSE UPTAKE BUT DECREASED GLUCOSE OXIDATION IN THE PUBERTAL PERIOD BY LIVER AND MUSCLE SLICES OF RATS SUBJECTED TO NEONATAL HYPOMELATONEMIA.

INTRODUCTION:

Melatonin is a vertebrate neurohormone synthesized and released by the pineal gland (Reiter, 1991; Arendt, 1995). The hormone is known to influence many physiological functions like seasonal reproduction, thermoregulation and energy metabolism in mammals. In seasonal species melatonin is known to affect body mass, adiposity and both energy intake and expenditure (Himms-Hagen, 1984; Wade and Bartness, 1984; Mc Elroy and Wade, 1986; Bartness, 1995). Species variations in the effects of melatonin are also recorded as opposite results are obtained with reference to body fat mass in Siberian (decreases) and Syrian (increases) hamsters (Wade and Bartness, 1984; Bartness and Wade, 1985; Mc Elroy and Wade, 1986; Bartness, 1995). Further in the garden mouse a melatonin agonist increases and an antagonist lowers seasonal obesity (Le Gouic *et al.*, 1996). Though the exact mechanism of action is not understood, a direct effect of melatonin on brown adipocytes (Prunnet *et al.*, 2001) and an indirect

effect via the sympathetic nervous system (Mc Elroy *et al.*, 1986; Youngstrom and Bartness, 1995) have been demonstrated. The ability of melatonin to influence energy and intermediary metabolism is gaining validity by the recent demonstration of melatonin receptors in liver, muscle and adipose tissue (Dubocovich *et al.* 1998; Pang *et al.*, 1993; Acuna-Castroviejo *et al.*, 1994). The documental influence of melatonin treatment or pinealectomy on blood glucose and tissue glycogen contents in various vertebrates supports its role on glycemic status and carbohydrate homeostasis (Ramachandran, 2002). Reports on the role of melatonin in lipid metabolism in general and its efficacy in lowering serum and tissue cholesterol levels have also appeared in literature (De Vlaming *et al.*, 1974; Mori *et al.*, 1984; Esquifino *et al.*, 1997; Aoyama *et al.*, 1988). During the course of this study involving neonatal functional hypomelatonemia by the administration of melatonin receptor antagonist luzindole, significant hyperinsulinemia and hyperglycemia along with tissue glycogenic effect and reduced tissue sensitivity and glucose uptake with insulin and other agents promoting glucose uptake have been recorded in the weaning period (Chapter 1 & 2). Concurrently, decreased tissue lipogenic response was also seen (Chapter 3). Assessment of the long term influence of neonatal hypomelatonemia on pubertal metabolic features has indicated higher scale of insulin level with decreased glycogenic effect but potentiated protein anabolic influence (Chapter 4). Parallel studies conducted with neonatal hypermelatonemic status has revealed hyperinsulinemia with potentiated glycogenic and protein anabolic

influences (Jani, 2004). As a follow up of these observations, present studies on *in vitro* uptake of glucose by liver and muscle slices in presence of various uptake promoting agents singly or in combinations and C^{14} glucose oxidation have been undertaken to have a better perception of the metabolic strategy in the pubertal period.

MATERIAL AND METHODS: See page numbers 18-38.

RESULTS:

Liver Slices:

- **Uptake in presence of Insulin, Acetylcholine and Melatonin:** Compared to liver slices of control animals, liver slices of luzindole treated rats showed significant (around double) increase in glucose uptake. While, the liver slices of control rats showed maximal glucose uptake with melatonin and least with insulin, those of the luzindole treated rats showed maximal uptake with insulin and minimal with acetylcholine (Figure and Table; 5.1).

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

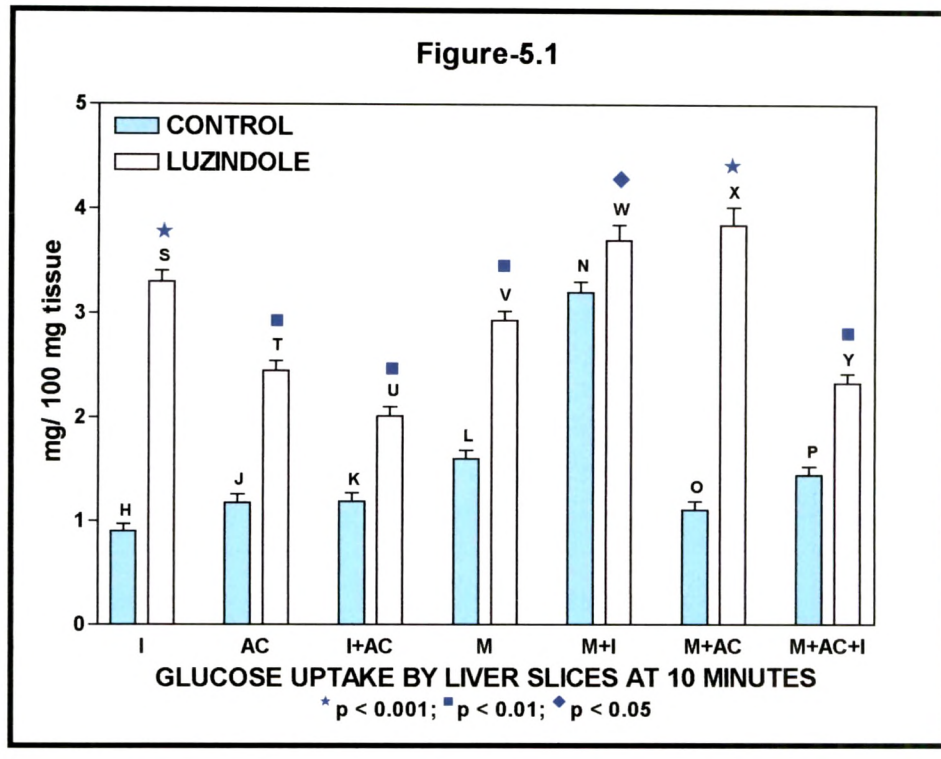

Table 5.1: Glucose uptake at 10 minutes by liver slices of pubertal rats on 45th day with combinations of insulin, acetylcholine and melatonin subjected to neonatal luzindole treatment:

	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
LUZINDOLE	*3.30$^{(S)}$ ±0.11	■2.45$^{(T)}$ ±0.092	■2.01$^{(U)}$ ±0.09	■2.93$^{(V)}$ ±0.097	◆3.7$^{(W)}$ ±0.15	*3.85$^{(X)}$ ±0.17	■2.33$^{(Y)}$ ±0.091

Values are expressed as mean ± SEM, *p < 0.001; ■ p < 0.01; ◆p < 0.05

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	*	■	■	*
	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 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 luzindole treatment:

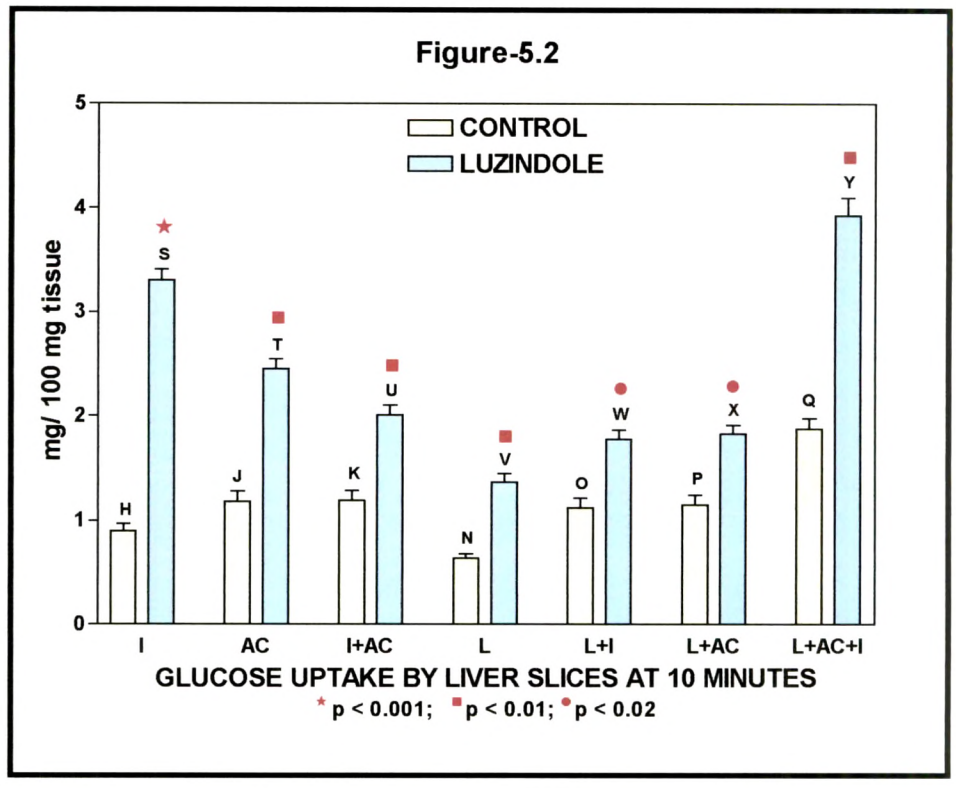

Table 5.2: Glucose uptake at 10 minutes by liver slices of pubertal rats on 45th day with combinations of insulin, acetylcholine and luzindole subjected to neonatal luzindole treatment:

	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
LUZINDOLE	*3.30$^{(S)}$ ±0.11	■2.45$^{(T)}$ ±0.092	■2.01$^{(U)}$ ±0.09	■1.37$^{(V)}$ ±0.081	●1.78$^{(W)}$ ±0.085	●1.83$^{(X)}$ ±0.086	■3.93$^{(Y)}$ ±0.17

Values are expressed as mean ± SEM, *p < 0.001; ■ p < 0.01; ●p < 0.02

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	*
	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	*	*

*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 luzindole treatment:

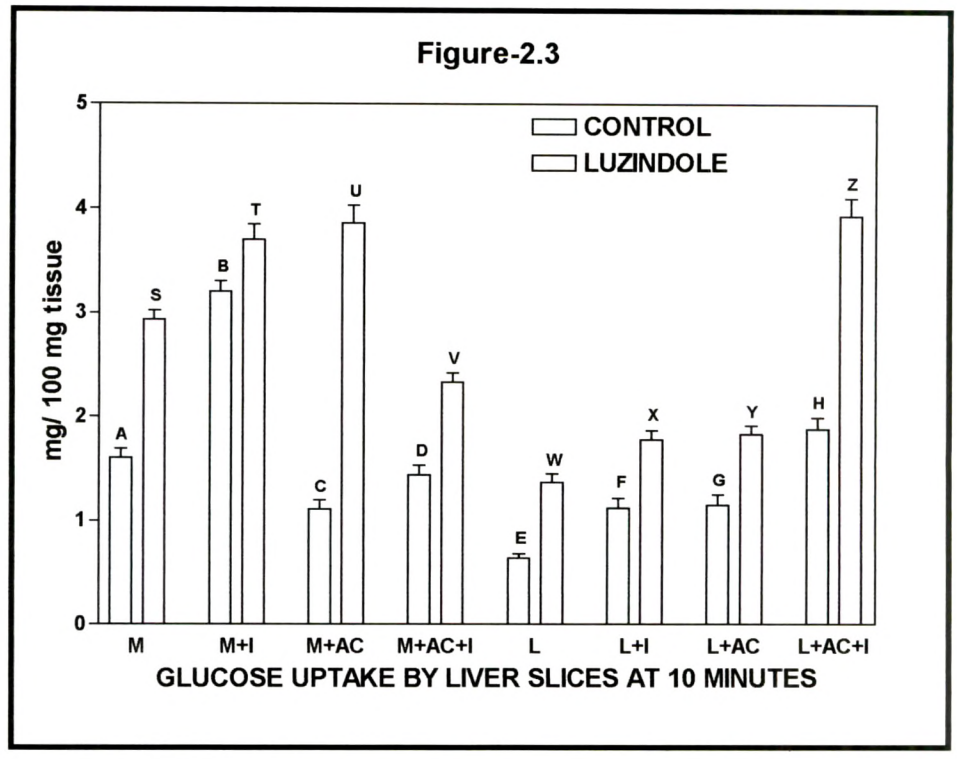

Table 5.3: Glucose uptake at 10 minutes by liver slices of pubertal rats on 45th day with combinations of insulin, acetylcholine, melatonin and luzindole subjected to neonatal luzindole treatment:

	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
LUZINDOLE	▪2.93$^{(S)}$ ±0.09	◆3.7$^{(T)}$ ±0.15	⋆3.85$^{(U)}$ ±0.18	▪2.33$^{(V)}$ ±0.091	▪1.37$^{(W)}$ ±0.081	•1.78$^{(X)}$ ±0.085	•1.83$^{(Y)}$ ±0.086	▪3.93$^{(Z)}$ ±0.17

Values are expressed as mean ± SEM, ⋆p < 0.001; ▪ p < 0.01; •p < 0.02; ◆p < 0.05

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	✶	✶	■	✶	✶	⊙	■	✶	✶	✶	✶
	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	

*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 luzindole treatment:

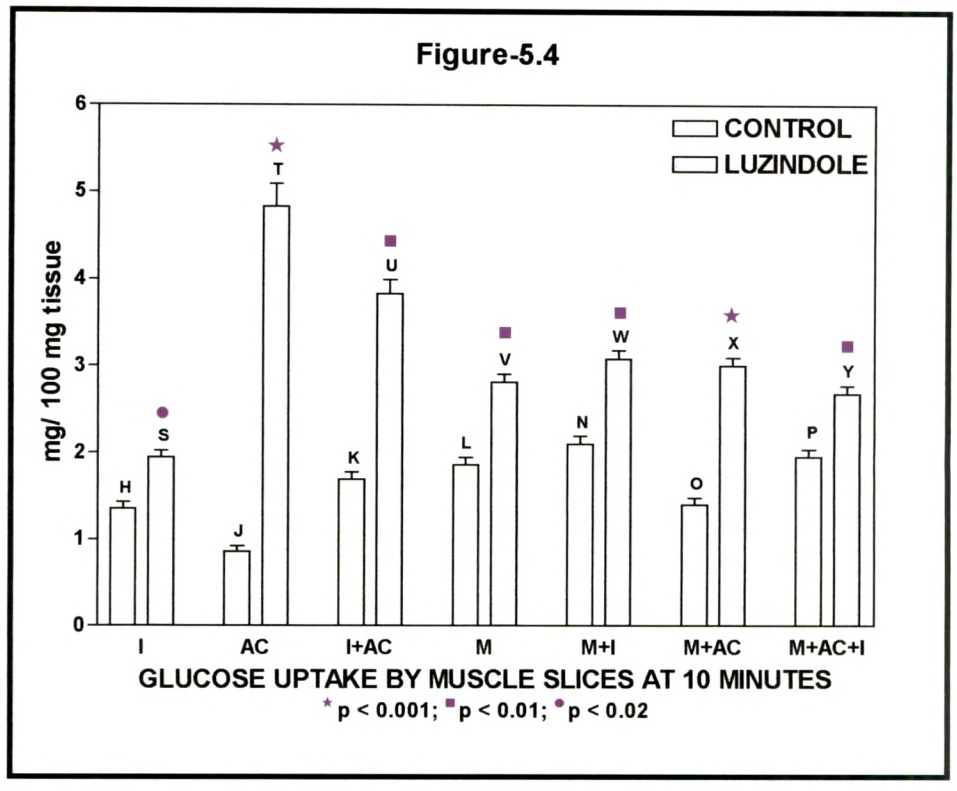

Figure 5.4: Glucose uptake at 10 minutes by muscle slices of pubertal rats on 45th day with combinations of insulin, acetylcholine and melatonin subjected to neonatal luzindole treatment:

	I	AC	I+AC	M	M+I	M+AC	M+AC+I
CONTROL	1.35(H) ±0.081	0.86(J) ±0.064	1.69(K) ±0.084	1.86(L) ±0.086	2.1(N) ±0.090	1.4(O) ±0.082	1.95(P) ±0.087
LUZINDOLE	•1.94(S) ±0.087	*4.83(T) ±0.26	■3.83(U) ±0.16	■2.81(V) ±0.097	■3.08(W) ±0.10	*3.00(X) ±0.099	■2.68(Y) ±0.094

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

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	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	

*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 luzindole treatment:

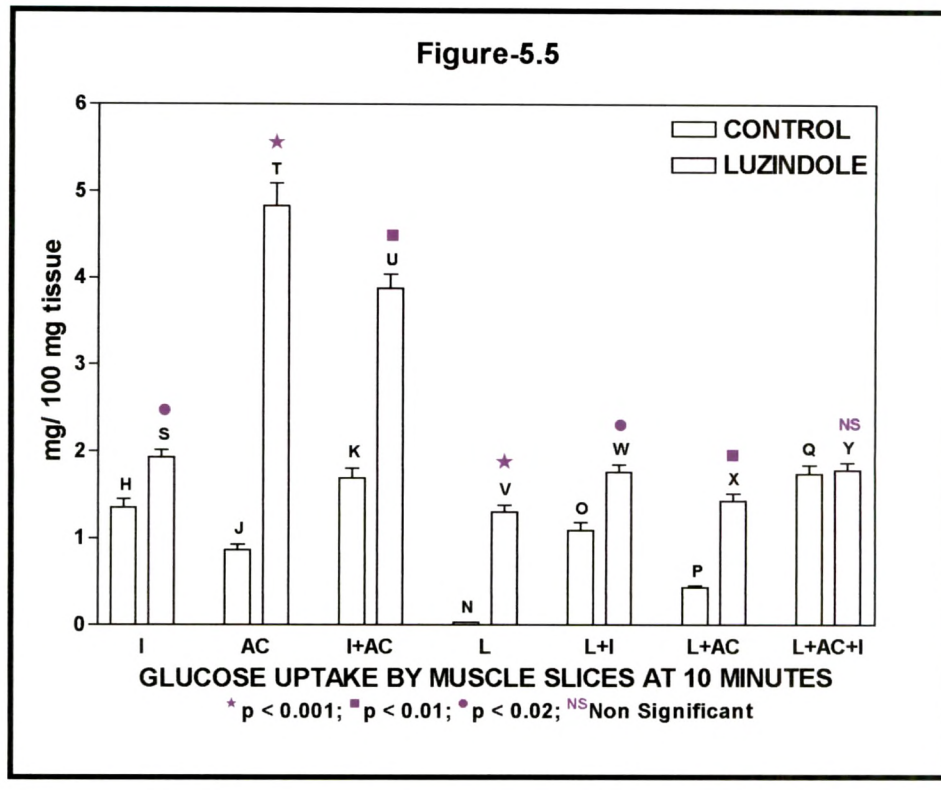

Table 5.5: Glucose uptake at 10 minutes by muscle slices of pubertal rats on 45th day with combinations of insulin, acetylcholine and luzindole subjected to neonatal luzindole treatment:

	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
LUZINDOLE	●1.93 (S) ±0.087	*4.83 (T) ±0.26	■3.88 (U) ±0.16	*1.30 (V) ±0.081	●1.76 (W) ±0.085	■1.43 (X) ±0.082	NS 1.78 (Y) ±0.085

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
	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

*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 luzindole treatment:

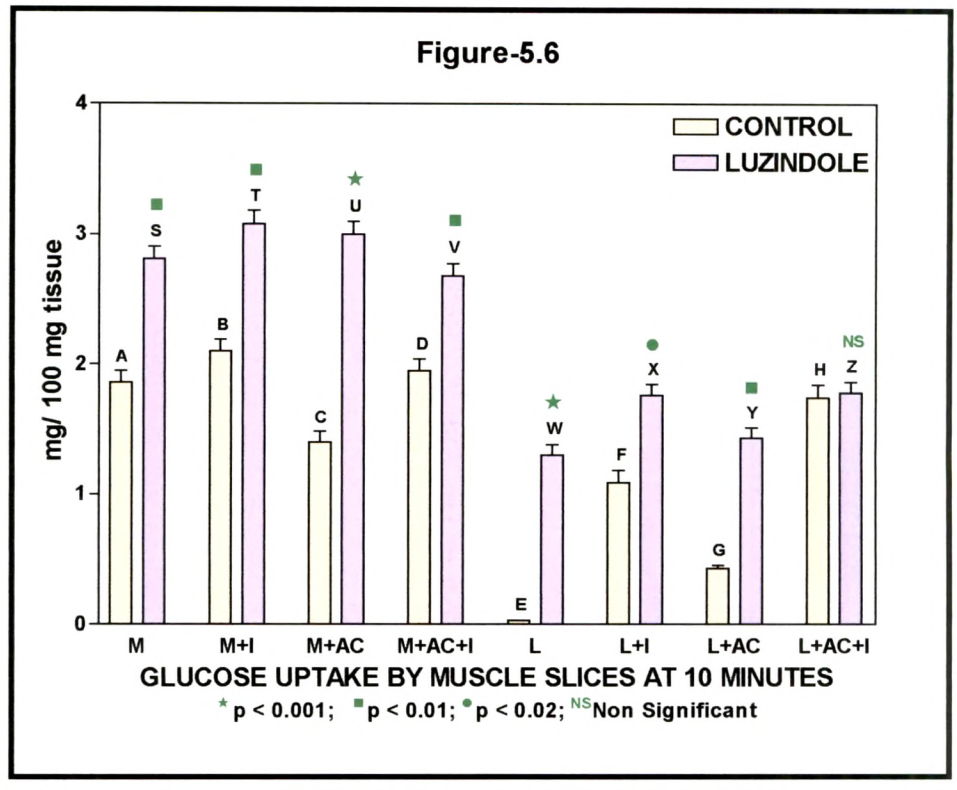

Table 5.6: Glucose uptake at 10 minutes by muscle slices of pubertal rats on 45th day with combinations of insulin, acetylcholine, melatonin and luzindole subjected to neonatal luzindole treatment:

	M	M+I	M+AC	M+AC+I	L	L+I	L+AC	L+AC+I
CONTROL	1.86 (A) ±0.086	2.10 (B) ±0.090	1.40 (C) ±0.082	1.95 (D) ±0.087	0.03 (E) ±0.0001	1.09 (F) ±0.09	0.43 (G) ±0.021	1.74 (H) ±0.098
LUZINDOLE	■2.81 (S) ±0.096	■3.08 (T) ±0.10	*3.00 (U) ±0.099	■2.68 (V) ±0.094	*1.30 (W) ±0.081	●1.76 (X) ±0.085	■1.43 (Y) ±0.082	NS 1.78 (Z) ±0.085

Values are expressed as mean ± SEM, *p < 0.001; ■ p < 0.01; ●p < 0.02; 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	✶	✶	✶	✶	✶	■	✶	✶	✶	✶	✶	✶	✶	✶
	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 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 luzindole treatment:

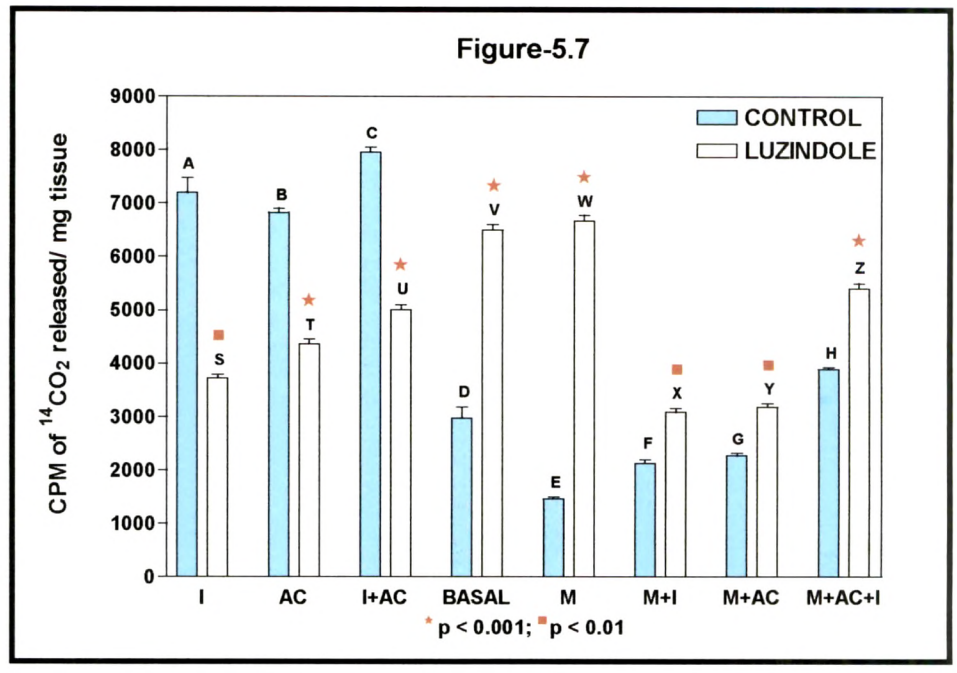

Table 5.7: Glucose oxidation by liver slices of pubertal rats on 45th day with insulin, acetylcholine, melatonin and there combinations compared to basal subjected to neonatal luzindole treatment:

	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
LUZINDOLE	■3721.03(S) ±67.33	*4366.82(T) ±85.49	*5006.62(U) ±94.75	*6498.49(V) ±103.55

	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
LUZINDOLE	*6675.47(W) ±105.55	■3086.86(X) ±90.11	■3180.27(Y) ±68.56	*5404.81(Z) ±100.76

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	*	*	*
	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 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 luzindole treatment:

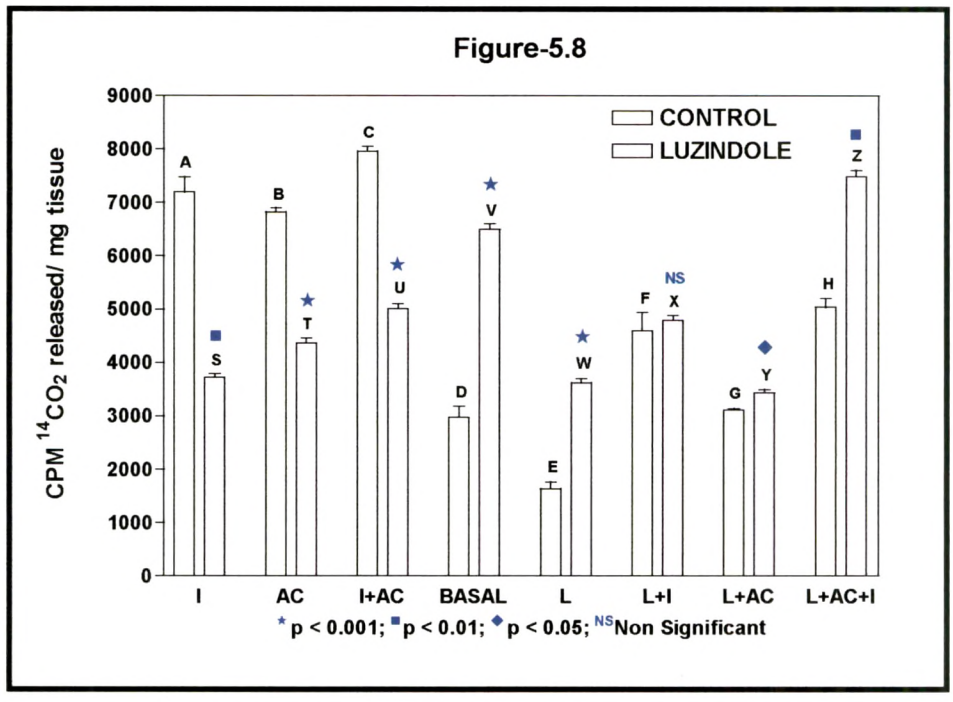

Table 5.8: Glucose oxidation by liver slices of pubertal rats on 45th day with combinations of insulin, acetylcholine and luzindole compared to basal subjected to neonatal luzindole treatment:

	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
LUZINDOLE	■3721.03(S) ±67.33	*4366.82(T) ±85.49	*5006.62(U) ±94.75	*6498.49(V) ±103.55

	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
LUZINDOLE	*3625.81(W) ±71.54	NS4789.93(X) ±89.76	◆3438.20(Y) ±69.74	■7489.38(Z) ±115.36

Values are expressed as mean ± SEM, * p < 0.001; ■ p < 0.01; ◆ p < 0.05; NS Non Significant

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	*	*	*	■

*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 luzindole treatment:

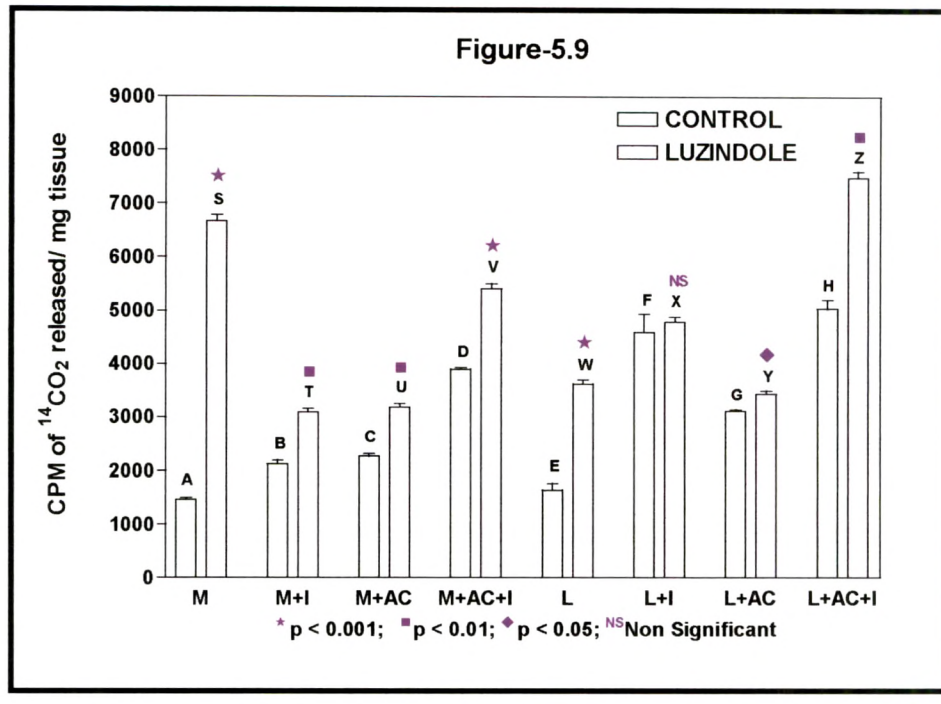

Table 5.9: Glucose oxidation by liver slices of pubertal rats on 45th day with combinations of insulin, acetylcholine melatonin and luzindole subjected to neonatal luzindole treatment:

	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
LUZINDOLE	*6675.47(S) ±105.55	■3086.86(T) ±90.11	■3180.27(U) ±68.56	*5404.81(V) ±100.76

	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
LUZINDOLE	*3625.81(W) ±71.54	NS4789.93(X) ±89.76	◆3438.20(Y) ±69.74	■7489.38(Z) ±115.36

Values are expressed as mean ± SEM, * p < 0.001; ■ p < 0.01; ◆ p < 0.05; NS Non Significant

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	*	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	⊙	*	*	*	*	*	⊙	*	*	*	*	*	*	*

*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 luzindole treatment:

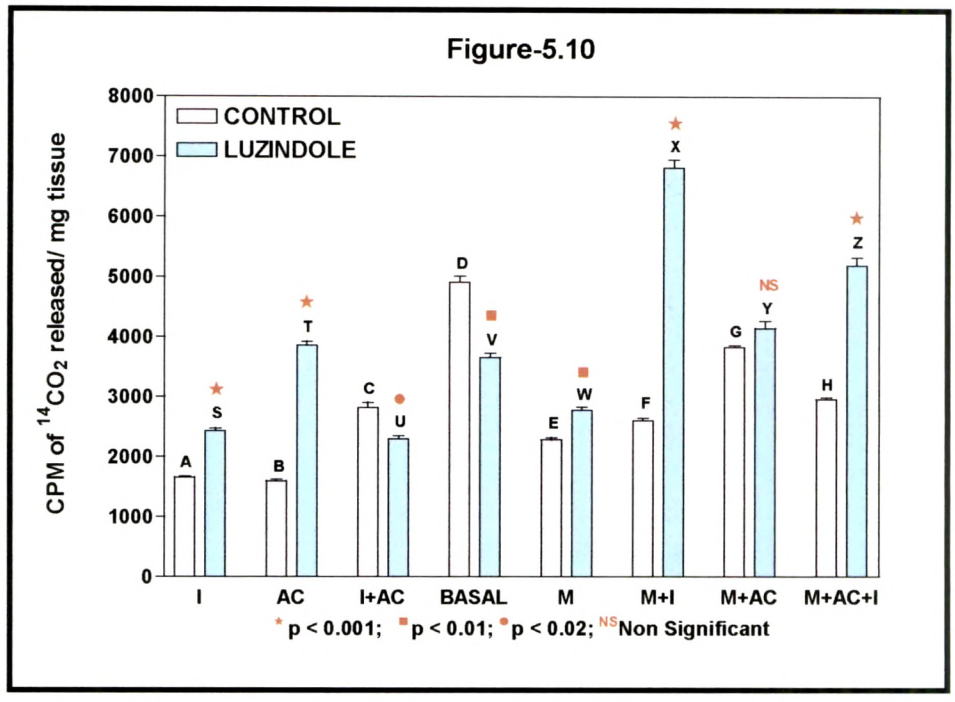

Table 5.10: Glucose oxidation by muscle slices of pubertal rats on 45th day with insulin, acetylcholine melatonin and there combinations compared to basal subjected to neonatal luzindole treatment:

	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
LUZINDOLE	*2426.94(S) ±50.86	*3861.51(T) ±63.44	•2300.94(U) ±50.31	■3652.28(V) ±75.76

	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
LUZINDOLE	■2776.43(W) ±49.13	*6817.84(X) ±130.59	NS4149.61(Y) ±116.26	*5195.93(Z) ±135.33

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

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

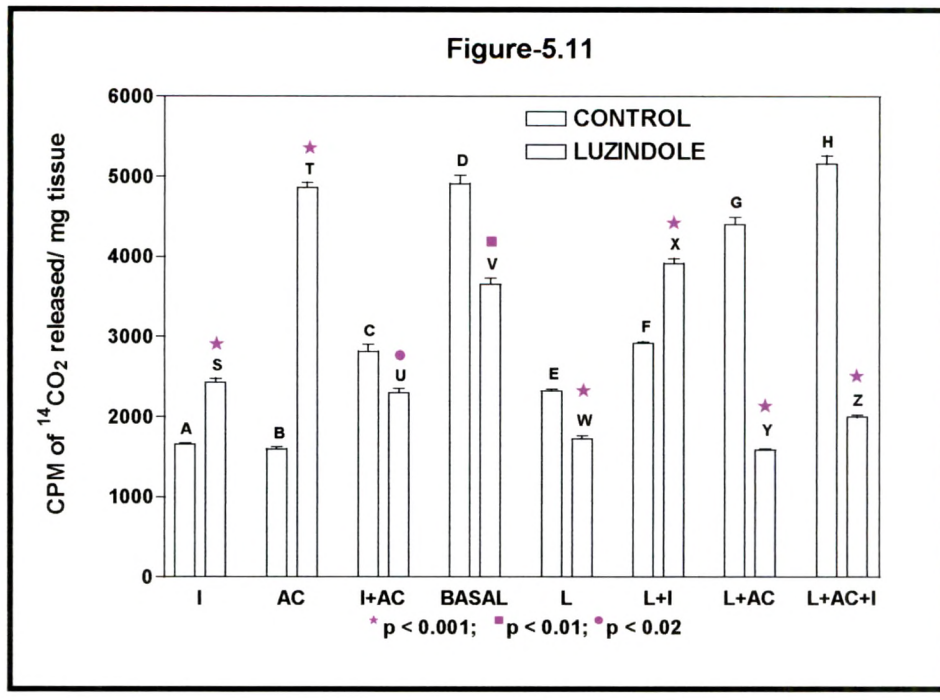

Table 5.11: Glucose oxidation by muscle slices of pubertal rats on 45th day with combinations of insulin, acetylcholine and luzindole compared to basal subjected to neonatal luzindole treatment:

	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
LUZINDOLE	*2426.94(S) ±50.86	*3861.51(T) ±63.44	•2300.94(U) ±50.31	■3652.28(V) ±75.76

	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
LUZINDOLE	*1725.49(W) ±37.76	*3916.59(X) ±60.11	*1590.21(Y) ±11.13	*1997.17(Z) ±26.75

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

Table 5.12

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	⊙	*	*	*
	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	*	*	*	*	*	*	*	*

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	*	*	*	*	*	*	*	NS	NS	*	*	NS

*p<0.001; ⊙P<0.05; NSNon Significant

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 luzindole treatment:

Figure-5.12

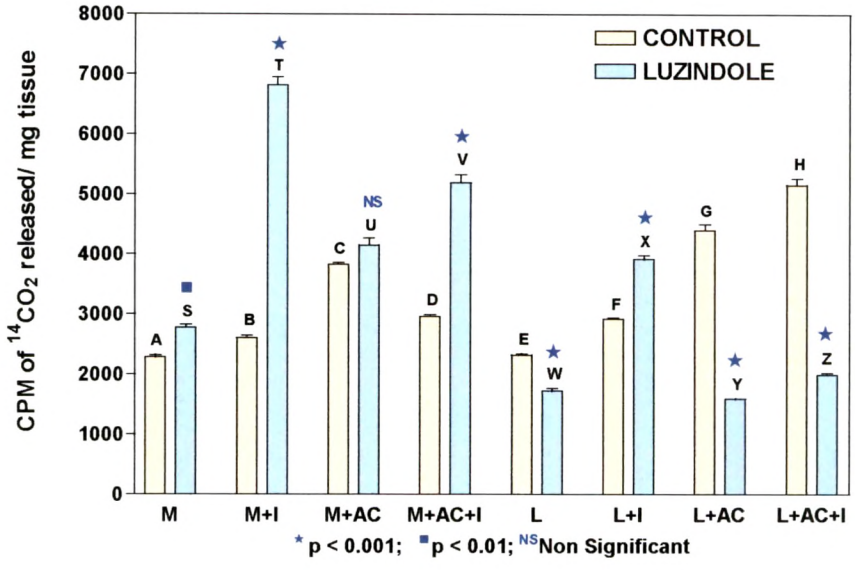

Table 5.12: Glucose oxidation by muscle slices of pubertal rats on 45th day with combinations of insulin, acetylcholine melatonin and luzindole subjected to neonatal luzindole treatment:

	M	M+I	M+AC	M+AC+I
CONTROL	■2283.77(A) ±41.05	*2605.52(B) ±43.28	NS3831.69(C) ±29.34	2959.20(D) ±27.98
LUZINDOLE	2776.43(S) ±49.13	6817.84(T) ±130.59	4149.61(U) ±116.26	*5195.93(V) ±135.33

	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
LUZINDOLE	*1725.49(W) ±37.76	*3916.59(X) ±60.11	*1590.21(Y) ±11.13	*1997.17(Z) ±26.75

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

- **Uptake by combinations of Insulin, Acetylcholine and Melatonin:** The liver slices of control rats showed significantly higher glucose uptake (almost double) with M+I than by any other combination or by any agent individually. Whereas the uptake promoted by I+Ac and M+Ac were similar to each other and to that by acetylcholine alone, that by M+Ac+I was higher but still less than M+I in the control liver slices. In the experimental rat liver slices all the combinations except for M+I showed significantly higher (almost double) glucose uptake as compared to control rat liver slices. Whereas M+I and M+Ac induced glucose uptake was similar to each other and insulin alone, I+Ac and M+Ac+I promoted glucose uptake decreased as compared to the former combinations in the experimental rat liver slices (Figure and Table; 5.1, 5.3).
- **Uptake by combinations of Insulin, Acetylcholine and Luzindole:** In the control liver slices the glucose uptake promoted by the combinations of L+I and L+Ac were similar to each other and to that of acetylcholine alone as well as its combination with insulin. Whereas, the combination of all the three promoted maximum glucose uptake in the control liver slices, that with luzindole alone was least as compared to all other agents individually or any of the combinations. The glucose uptake promoted by L+I and L+Ac in the liver slices of experimental rats was almost similar to that of each other but was significantly decreased as compared to the uptake

promoted by I, Ac and combination of I+Ac. Whereas L+Ac+I induced uptake was maximum, that by luzindole alone minimal in the liver slices of the experimental rats. The uptake promoted by luzindole individually or in any of the combination was significantly greater in the experimental rat liver slices as compared to control rat liver slices (Figure and Table; 5.2, 5.3).

Muscle Slices:

- **Uptake in presence of Insulin, Acetylcholine and Melatonin:** The muscle slices of control rats showed significantly decreased glucose uptake in presence of acetylcholine and significantly increased glucose uptake with melatonin. However the glucose uptake promoted by all the three agents in the muscle slices of luzindole treated rats was greater than that by the control rat muscle slices. Whereas, the uptake promoted by acetylcholine was maximum as compared to any other agent that, by insulin reduced significantly in the muscle slices of luzindole treated rats though greater than that by control rat muscle slices (Figure and Table; 5.4).
- **Uptake by combinations of Insulin, Acetylcholine and Melatonin:** In the muscle slices of control rats the uptake promoted by the combination of M+I increased significantly while, that by M+Ac decreased significantly. The uptake promoted by I+Ac was slightly greater than that by M+Ac whereas, the combination of all the three agents increased glucose uptake than that by I+Ac and M+Ac but was less than

that by M+I. In the muscle slices of experimental rats the uptake was almost double as compared to the control slices. Whereas, the uptake induced by I+Ac increased significantly that, by M+Ac+I decreased significantly still higher than that of control rat muscle slices. There was no significant change in the uptake promoted by M+I and M+Ac (Figure and Table; 5.4, 5.6).

- **Uptake by combinations of Insulin, Acetylcholine and Luzindole:** In the muscle slices of control rats luzindole alone did not promote any significant glucose uptake. Whereas, the uptake promoted by L+I was greater than that by acetylcholine alone that, by the combination of all the three agents was almost equal to that by insulin alone. The combination of L+Ac showed marginal glucose uptake which was less than that by any other combination. The uptake promoted by the muscle slices of luzindole treated rats was higher than that of control rats. Whereas luzindole alone and in combinations induced glucose uptake in the muscle slices of experimental rats was significantly less than that of insulin or acetylcholine or combination of I+Ac still higher than the control rat muscle slices. The uptake promoted by L+I and L+Ac+I were similar and greater than that by L+Ac and luzindole alone in the muscle slices of experimental rats (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 luzindole treated rats. Whereas the liver slices of experimental rats showed increased C^{14} glucose oxidation with L along with its combinations 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 I+Ac but, the C^{14} glucose oxidation increased significantly with I, Ac, M, M+I and M+Ac+I. However luzindole and L+I, could induce significantly decreased glucose oxidation in the muscle slices of the melatonin treated rats the glucose oxidation induced by L+Ac and L+Ac+I increased significantly in the experimental rat muscle slices. The C^{14} glucose oxidation was maximum with M+I and minimum with L+Ac 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 oxidation by control and hypomelatonemic 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 have a better perspective of the observations glucose uptake and glucose oxidation are being dealt with separately and final conclusions drawn at the end.

Glucose uptake: There is an age related decrease in glucose uptake by control liver and muscle slices as the degree of uptake presently is significantly low than that seen in the pre-weaning period (Chapter 2). However the neonatal hypomelatonemic rats seem to be resistant to this decrease in sensitivity as the degree of glucose uptake recorded by the liver in the present study is very much comparable with the values obtained in the weaning period (Chapter 2). This is in contrast to neonatal hypermelatonemia wherein the age related decrease in sensitivity was manifested though to a significantly lesser degree as compared to controls (Jani, 2004). Though the relatively higher glucose uptake seen in the hypermelatonemic rats was correlated with the higher glycogenic effect, in the present study the higher liver sensitivity towards agents promoting glucose uptake persisting in the pubertal period as well is not co relatable with an overall glycogenic effect as tissue glycogen contents have been shown to be decreased in the pubertal period (Chapter 4). Unlike the liver the muscle of hypomelatonemic rats shown the age dependent decrease in glucose uptake but still manifesting significantly higher uptake compared to the control muscle. The ability of melatonin to promote glucose uptake is a noteworthy observation which was seen even in the weaning period

(Chapter 2). Whereas in the weaning period, melatonin induced significantly higher glucose uptake in control animals which was greater than by insulin or acetylcholine in muscle and slightly lesser than by insulin in the liver (Fig. and Tab.; 5.1, 5.4). In hypermelatonemic liver and muscle, the uptake was significantly greater than by insulin or acetylcholine (Chapter 2, Jani, 2004). In contrast to the weaning period, in the pubertal stage, the hypermelatonemic liver and muscle show greater sensitivity for melatonin induced uptake which was equivalent to that promoted by insulin in the liver and greater than that by insulin in the muscle. Apparently the increasing resistance to glucose uptake occurring from the weaning to pubertal period is not only an insulin resistance but a generalized resistance against all agents promoting glucose uptake. The potency of melatonin to induce glucose uptake is clearly confirmed by the higher glucose uptakes obtained with combinations of melatonin with, insulin or acetylcholine or both. Another notable observation is the inability of luzindole to promote glucose uptake to any significant degree by either liver or muscle of both control and hypomelatonemic rats (Fig. and Tab.; 5.2, 5.5). This is again in contrast to the significant glucose uptake promoted by luzindole in both liver and muscle of control weaning rats (almost equivalent to insulin) with no apparent effect on tissues of hypomelatonemic weanings. Though there are many studies in literature on *in vitro* glucose uptake under different experimental schedules (Goodman *et al.*, 1983; Patel and Ramachandran, 1992; Seraphin et al., 1997; Lima et al., 1994; Lima et

al., 1998)) they are however not relevant to the present experimental paradigms and objectives and hence cannot be drawn to make meaningful discussions in the present context.

Glucose Oxidation: Under basal conditions a comparison between the glucose oxidation potential of control and hypomelatonemic tissues reveals a slightly higher potential in the muscle of control animals and a significantly higher potential in the liver of hypomelatonemic rats (Fig. and Tab.; 5.7, 5.10). Whereas insulin and acetylcholine significantly increases glucose oxidation by the liver slices of control animals, both had significant depressive effect in the muscle as against this both insulin and acetylcholine show depressive effect on glucose oxidative potential by hypomelatonemic liver slices and no apparent effect in the hypomelatonemic muscle slices (Fig. and Tab.; 5.7, 5.10). Whereas both melatonin and luzindole showed depressive effect on both liver and muscle of control animals, melatonin had no influence and luzindole a depressive influence on glucose oxidation by liver and muscle of hypomelatonemic rats (Fig. and Tab.; 5.8, 5.11). The depressive effect of melatonin and luzindole on control liver is strengthened by the observed depressive effect as seen with the combinations of melatonin and luzindole with insulin and acetylcholine induced uptake. However both melatonin and luzindole had a better oxidative potential in the muscle of control animals which increased in combination with insulin and acetylcholine but nevertheless than the basal potential (Fig. and Tab.; 5.7, 5.8, 5.9). Interestingly insulin and acetylcholine had depressive influence on the ability of melatonin

induced glucose oxidation in the hypomelatonemic liver slices as seen with the combinations of insulin, acetylcholine with melatonin in contrast insulin and acetylcholine can reverse the depressive effect of luzindole and a combination of both insulin and acetylcholine with luzindole increases oxidation above the basal levels. The hypomelatonemic muscle slices seem to depict significantly increased oxidation potential with combinations of melatonin, insulin and acetylcholine (Fig. and Tab.; 5.10). However the combination of insulin and acetylcholine with luzindole could not increase the oxidative potential (Fig. and Tab.; 5.11). Taken these observations together the hypomelatonemic tissues seem to have a significantly lesser oxidative potential compared to control tissues. However the hypomelatonemic tissues show increased potential for glucose uptake these two observations when taken together suggest channelisation of glucose moieties towards anabolic pathways rather than catabolic pathways. Since a reduced glycogenic effect was reported in hypomelatonemic tissues (Chapter 4), and an increased tissue lipid and protein loads has been recorded in the hypomelatonemic rats in the pubertal period (Chapter 4 & 6), it is inferable that glucose moieties are being channelised towards lipid and protein biogenesis. Obviously the pubertal period in hypomelatonemic rats is characterized by a anabolic *milieu* as against the catabolic *milieu* in the control animals. Such channelisation of glucose moieties towards lipid synthesis has been shown by Goodridge, (1968) based on his study on *in vitro* glucose oxidation by adipose tissue from embryonic and growing chicks. It is

interesting to note that even hypermelatonemic rat tissues have shown decreased potential in the pubertal period which was correlated with increased glycogen deposition rather than lipid deposition (Jani, 2004). Apparently, under altered metabolic homeostasis due to neonatal hyper or hypomelatonemia there is an altered temporal metabolic strategy from the neonatal to pubertal period which is in turn related with the differential weaning status in the two treatment groups.

Overall the present observations tend to suggest increased glucose uptake coupled with decreased oxidation in the pubertal tissues of hypomelatonemic rats and this provides validity to the reported increase in carbohydrate, lipid and protein reserves of hypomelatonemic tissues (Chapter 4 & 6).

SUMMARY:

Assessment of the long term influence of neonatal hypomelatonemia on pubertal metabolic features has indicated higher scale of insulin level with decreased glycogenic effect but potentiated protein anabolic influence. As a follow up of these observations, present studies on *in vitro* uptake of glucose by liver and muscle slices in presence of various uptake promoting agents singly, or in combinations and, C^{14} glucose oxidation, have been undertaken to have a better perception of the metabolic strategy in the pubertal period. 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 45^{th} day. The liver slices of luzindole treated rats showed significantly increased glucose uptake with insulin(I), acetylcholine(Ac),

I+Ac, melatonin(M) and their combinations. While the uptake promoted by M+Ac was maximum, that by I+Ac was minimum in the liver slices of the experimental rats. The liver slices of control rats showed maximum glucose uptake with M+I and minimum with I. Also, luzindole(L) and its combinations induced significantly increased glucose uptake by the liver slices of experimental rats. Whereas the maximum uptake was induced by L+Ac+I, least was with L alone by the liver slices of the experimental rats. The muscle slices of luzindole treated rats showed significantly increased glucose uptake with all the stimulants singly or in combinations as compared to controls. Ac induced uptake was maximum while, I induced uptake was minimum in the muscle slices of experimental rats. In the control muscle slices, the uptake induced by M+I was maximum and that by Ac was minimum. The uptake induced by L, L+I and L+Ac increased significantly in the muscle slies of experimental rats., while that by L+Ac+I showed no significant alteratins as compared to control slices. Though C^{14} glucose oxidation by liver slices of luzindole treated rats decreased significantly with I, Ac and I+Ac that by M and its combinations along with that of basal oxidation increased significantly in the liver slices of experimental rats. Also, the C^{14} glucose oxidation increased with L and L+Ac+I while the combination of L+I and L+Ac showed no significant alteration in the liver slices of experimental rats. In the muscle slices of experimental rats, the C^{14} glucose oxidation increased significantly with Ac, M+I and M+Ac+I while it decreased significantly with I+Ac and the basal state. Except for L+I which showed significantly increased glucose oxidation,

the oxidation induced by L as well as its combinations decreased significantly. Overall, the present observations tend to suggest increased glucose uptake coupled with decreased oxidation in the pubertal tissues of luzindole treated rats and this provides validity to the reported increase in carbohydrate, lipid and protein reserves of luzindole treated rat tissues.