GENERAL CONCLUSIONS

The present study was undertaken essentially to understand the impact of melatonin antagonism in the pre-weaning period on weaning, pubertal and adult carbohydrate and lipid metabolism, pancreatic function and alloxan induced diabetes. The influence of pre-weaning luzindole status on *in* vitro glucose uptake in response to different secretogogues was also studied. Experiments were essentially carried out under a photoperiod of LD 8:16 and a constant temperature regimen of 21±2° C. Individual effects of melatonin insufficiency and alloxan induced diabetes along with melatonin insufficiency have all been assessed within the framework of the above cited objectives.

Altered melatonin status has been reported to increase, decrease or have no effect on blood glucose level and is known to influence plasma insulin level, insulin secretion and even insulin action. The modulatory influence of melatonin on intermediary metabolism is known. The present study clearly indicates that pre-weaning luzindole treatment influences weaning, pubertal and adult carbohydrate and lipid homeostasis as well as the weaning alloxanisation status on

pubertal and adult carbohydrate and lipid homeostasis with or without luzindole treatment.

The realized alterations in weaning stage as immediate post-treatment effects are:

1. The body weight is decreased.
2. Relative weight of liver is increased.
3. The serum insulin level is increased while the, serum glucose level is decreased.
4. The hepatic and muscle glycogen contents are increased.
5. The Muscle glycogen synthetase and glycogen phosphorylase activities are decreased.
6. Glycogen phosphorylase and glucose-6-phosphotase activities are decreased in liver.
7. The muscle protein content is decreased.
8. The sections of pancreas show distinct hypertrophy of acinar and islet cells. The number of B cells is increased as compared to A cells.
9. The liver slices showed decreased glucose uptake with insulin, melatonin, acetylcholine as well as their combinations.
10. The muscle slices showed decreased glucose uptake with insulin, acetylcholine, melatonin as well as their combinations.
11. Luzindole, promoted glucose uptake in both liver and muscle slices although to a negligible amount in muscle.
12. The hepatic and muscle total lipid contents are decreased.

13. The hepatic cholesterol content is decreased while the, muscle cholesterol content is increased.
14. The adipose tissue total lipid content is decreased.
15. Serum phospholipid and free fatty acid levels are decreased while the, serum triglyceride level is increased.

The possible explanations for the above recorded observations are:

1. The hepatic and muscle glycogen contents have shown a tremendous increase of more than 1000% and 600% respectively.
2. The unaltered glucose-6-phosphatase activity in luzindole treated rats compared to controls is also supportive of the hyperinsulinemic glycogenic state.
3. The presently noted significantly reduced tissue glucose uptake in luzindole treated neonates does not agree with the hyperinsulinemic glycogenic effect recorded previously. Though the present results clearly show reduced tissue sensitivity towards promoters of glucose uptake, the glycogenic effect seen could be more of a consequence of the quantitative effect of hyperinsulinemia rather than a qualitative effect.
4. Since the muscle tissue of luzindole neonates has shown increased uptake in presence of acetylcholine, this could account for the glycogen loading of the muscle tissue. The significantly increased response to acetylcholine in luzindole treated neonates as against response to melatonin or I+Ac in

control animals suggests increased sensitivity to parasympathetic modulation in the absence of melatonin action.

5. It is clear that chronic luzindole treatment affects both melatonin and insulin receptors or downstream mechanisms as well as acetylcholine action. In this context, the possible role of nitric oxide in increasing glucose uptake by the liver and muscle of luzindole treated neonates may have to be speculated as, such a role for nitric oxide in increasing glucose transport independent of insulin action has been reported. In this context, it is tempting to speculate that some such mechanism might be up-regulated by chronic luzindole treatment as, it obviously down regulates both insulin and melatonin mediated glucose uptake.
6. Since the tissue lipid contents of the luzindole treated weanings are significantly lower than the control weanings and, at the same time the tissue glycogen contents are significantly higher, melatonin antagonism seems to imbalance the metabolic machinery of neonates by excessive glycogenesis at the cost of lipogenesis. Apparently, reduced melatonin action in the pre-weaning period may up-regulate glycogenic machinery and down-regulate lipogenic machinery.
7. The unchanged tissue cholesterol contents are very much in keeping with the cholesterol lowering effect of melatonin.
8. Hypomelatonemia seen in the present study contrasted with hypoinsulinemia in hypermelatonemic neonates is indicaive that

chronic melatonin treatment has the ability to suppress insulin secretion and conversely, absence of melatonin action leads to increased insulin release.

The alterations in the pubertal period due to pre-weaning luzindole treatment are:

1. There is no significant alteration in the body weight.
2. Relative weight of pancreas and adrenals are decreased while that of liver, spleen, testes and kidneys is increased.
3. The serum insulin level is increased significantly while, the serum glucose level is decreased.
4. The hepatic and muscle glycogen contents show no significant alteration.
5. The muscle glycogen synthetase activity is increased while the, liver glycogen synthetase activity is decreased.
6. The hepatic glucose-6-phosphatase activity is increased.
7. The glycogen phosphorylase activity is increased in muscle and liver.
8. The islets appear to be larger in size with higher number of B and A cells and a higher B:A cell ratio. Transdifferentiation of acinar cells into islet cells seen prominently at 22 days of luzindole treated rats is found to be still persistent though to a lesser degree.
9. The liver slices showed increased uptake with insulin, acetylcholine, melatonin as well as their combination

10. Luzindole along with its combinations could also induce increased uptake by liver slices.
11. The muscle slices showed increased uptake with all the agents singly or in combinations..
12. The C^{14} glucose oxidation in the liver slices was increased in the basal state as well as with melatonin and its combinations.
13. Melatonin as well as luzindole and their combinations increased the C^{14} glucose oxidation by the liver slices.
14. In the muscle slices, the C^{14} glucose oxidation increased with Ac, M+I and M+Ac+I while, decreased with I+Ac and in the basal state.
15. The C^{14} glucose oxidation by the muscle slices decreased with L and its combination, except for L+I, which showed increased oxidation
16. The hepatic total lipid content increased while, the hepatic cholesterol content decreased.,
17. The muscle total lipid and cholesterol contents showed no significant alterations.
18. The adipose tissue total lipid content is decreased.
19. The serum cholesterol and free fatty acid levels are increased.
20. The serum triglyceride level is decreased.

The possible explanations for the above recorded observations are:

1. Though the serum glucose level is decreased, the tissue glycogen contents (liver and muscle) are also decreased despite

increased GS:GP ratio in the former or unaltered in the latter, suggesting decreased insulin sensitivity

2. Increased glucose-6-phosphatase activity correlates well with the decreased glycogenic effect
3. Luzindole treatment in the neonatal period seems to have a protein anabolic influence from weaning to puberty.
4. Since the adrenal glands are increased in size, it is likely that there is increased corticosterone titre in these animals which could again contribute to insulin resistance/insensitivity.
5. The relatively greater glucose uptake capacity coupled with reduced glucose oxidation suggests an overall anabolic *milieu* in the neonatal hypomelatonemic rats.
6. The decreased serum triglyceride level along with increased tissue lipid content suggests a lipogenic status in the hypomelatonemic rats. Apparently, induction of lipogenic enzymes during postnatal development requires not only an optimal insulin level but also an optimal background of melatonin action. Obviously, a synergistic influence of melatonin and insulin can be presumed to be of significance in the induction of lipogenic enzymes and tissue lipid deposition in the postnatal period.
7. The increased serum cholesterol level recorded herein is a clear persisting manifestation of the hypercholesterolemic effect of absence of melatonin. The higher serum free fatty acid level

may not only serve to meet the energy needs but also serve as precursors for hepatic and muscle lipid synthesis.

The alterations in the adult period due to pre-weaning luzindole treatment are:

1. The body weight is increased.
2. Relative weight of liver, kidney and adrenals is decreased.
3. The serum insulin level is decreased while, the serum glucose level is increased.
4. The hepatic glycogen content is decreased while, the muscle glycogen content is increased
5. The hepatic and muscle glycogen synthetase activities are increased.
6. The glycogen phosphorylase activity is decreased in liver and muscle.
7. The hepatic glucose-6-phosphatase activity is decreased.
8. Both hepatic and muscle protein content are increased.
9. The pancreatic islets seem to be larger in size with greater B:A cell ratio.
10. The glucose uptake promoted by I, Ac, M as well as their combinations decreased in the liver and muscle slices at 10 and 90 minutes.
11. The uptake promoted by L and its combinations did not show any significant alteration.
12. The hepatic cholesterol content is decreased.

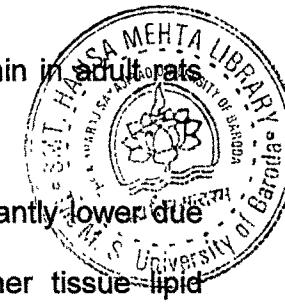
13. The muscle and adipose tissue total lipid contents are decreased while the, hepatic, muscle and adipose tissue cholesterol contents are increased.
14. The serum triglyceride, cholesterol, total lipids and free fatty acid levels are decreased.

The possible explanations for the above recorded observations are:

1. An age related decrease in hepatic glycogen content and increase in muscle glycogen content are seen in both control and experimental animals from pubertal to adult stage but, the degree of decrease in the liver and the degree of increase in the muscle are significantly greater in the experimental animals.
2. The decrease in the hepatic glycogen content of control rats is explicable in terms of the increased phosphorylase activity and decreased synthetase:phosphorylase activity ratio and, the significantly higher glycogen depletion seen in the experimental rats need an alternative explanation as, there is neither an increase in phosphorylase activity nor a change in the synthetase:phosphorylase activity ratio from the pubertal period.
3. Since the experimental rats show hypoinsulinemia and hyperglycemia, it is likely that there is decreased insulin sensitivity and reduced glucose uptake by the liver and hence observed decrease in the glycogen content.
4. A reverse situation seems to be operative for muscle glycogen content. As, the significantly increased muscle glycogen content

in the experimental animals relative to the pubertal period is explicable in terms of the decreased phosphorylase activity and increased synthetase activity with a significantly high synthetase:phosphorylase activity ratio.

5. The significantly increased glucose-6-phosphatase activity in the control rats from the pubertal period and the observed decrease in blood glucose level suggest increased carbohydrate utilization. In contrast, the unchanged glucose-6-phosphatase activity and hyperglycemia seen in the experimental rats relative to the pubertal period suggest decreased carbohydrate utilization and energy generation.
6. Apparently, the glucose uptake potential of liver of neonatal hypomelatonemic rats is consistently down regulated from weaning through pubertal to adult stage. Relatively lesser hepatic glycogen recorded in the hypomelatonemic liver is further confirmed by the observed no change in the degree of glucose uptake from 10 to 90 minutes while, the control liver slices show a significant further increment in uptake from 10 to 90 minutes.
7. The hypomelatonemic liver slices show significantly increased acetylcholine sensitivity and obviously, the possibility of an age related increase in cholinergic sensitivity which can be potentiated by melatonin need to be ascertained.
8. Though the ability of luzindole to promote glucose uptake is understandable in the context of it being an analog of melatonin,



its ability to promote higher uptake than melatonin in adult rats needs further evaluations.

9. Since the tissue glycogen contents were significantly lower due to greater glycogenolysis and unchanged higher tissue lipid stores from pubertal to young adult stage rats subjected to neonatal melatonin antagonism, it is presumable that there is increased glucose/carbohydrate oxidation and sparing effects on lipids. A steady state of tissue lipids suggests balanced turn over with no net synthesis.
10. As against the tissue lipid contents, the tissue cholesterol contents are significantly increased with concomitant decrease in serum cholesterol level suggesting increasing conversion of serum cholesterol into tissue cholesterol esters in the experimental rats. Possibility of decreased cholesterol utilization as a long term impact of neonatal melatonin antagonism is another metabolic feature worth investigating.
11. The decreased tissue lipids and serum lipid fractions are suggestive of the long term protective influence of neonatal blockade of melatonin action. Whether this long term consequential effect is due to potentiated melatonin action by increased receptor sensitivity or, due to an increase in the number of melatonin receptors, is a topic of relevance for future investigations.

12. Apparently there could be an age dependent difference in the partitioning of carbohydrates among tissues and intro cellular pathways.

The observed alterations in the pubertal period due to neonatal luzindole treatment and weaning alloxanisation on the 22nd day are:

1. The body weight of LA(100) and LA(75) rats is decreased as compared to CA(100) and age matched controls.
2. The relative weight of liver of LA(100) rats is decreased significantly while, the relative weight of spleen of LA(75) rats is increased.
3. The hepatic glycogen content is decreased while; the muscle glycogen content is increased in all the alloxanised rats.
4. The serum glucose level is decreased while, the serum insulin level of LA(100) and LA(75) rats is increased as compared to all other groups.
5. The hepatic glycogen synthetase and glycogen phosphorylase activities are decreased in all the alloxanised rats.
6. The muscle glycogen synthetase and glycogen phosphorylase activities of all the alloxanised rats are decreased as compared to nLT rats.
7. The muscle protein content is increased in all the alloxanised rats.
8. The hepatic total lipid content of LA(75) rats is increased as compared to all other groups.

9. The muscle total lipid content of LA(100) rats is decreased as compared to control rats while, the muscle cholesterol content of LA(100) rats is increased as compared to all other groups.
10. The adipose tissue total lipid content of LA(100) and LA(75) rats is decreased as compared to CA(100) and control rats while, the adipose tissue cholesterol content of all the alloxanised rats is decreased as compared to the controls.
11. The serum triglyceride, cholesterol and phospholipid levels are decreased in all the alloxanised rats.
12. The serum total lipids and free fatty acid levels of LA(100) and LA(75) rats are increased significantly as compared to CA(100) and control rats.
13. Both the control and nLT rat islets show B cell neogenesis from acinar cells with the result that the areas between the islet and acinar components has got befudged.

The possible explanations for the above recorded observations are:

1. The increase in the relative weight of all the organs and decrease in body weight suggest altered growth kinetics in nLT rats whose underlying cause is inexplicable at this juncture.
2. The significant hyperglycemia seen in the alloxanised control animals suggest poor recovery of B cells from alloxan challenge. This is reflected in the depleted hepatic glycogen content and hypoinsulinemia seen in these rats.
3. The nLT rats however show significant hypoglycemia with relatively higher hepatic glycogen content which would suggest

a faster recovery of the B cells after alloxan induced damage. Apparently, reduced melatonin action in the neonatal period has a favorable influence on B cell recovery/regeneration

4. Histological observations reveal enlarged islets/islet cell mass with increased number of B cells generated probably by both proliferation of surviving B cells as well as neogenesis by transdifferentiation from acinar cell in nLT rats after alloxan induced damage, more pronouncedly, in the nL(75) rats.
5. Based on the present study it is inferable that B cell growth, recovery and regeneration are all hightened under a low neonatal melatonin background.
6. A greater insulin action is seen in nL(75) rats as indicated by not only increased hepatic and muscle glycogen contents but also the significantly higher lipid deposition in these organs.
7. Obviously, alloxan induced diabetogenic effects are effectively countered by prior neonatal functional deficiency of melatonin. This antidiabetogenic effects are also substantiated by the relatively lesser protein anabolism seen in these rats as marked by the hepatic and muscle protein contents.

The observed alterations in the adult period due to neonatal melatonin treatment and weaning alloxanisation on the 22nd day are:

1. The body weight is decreased in all the alloxanised groups.
2. Relative weight of pancreas of LA(100) and LA(75) rats are decreased as compared to CA(100) and control rats while, the relative weight of spleen is increased in the LA(75) rats as

compared to all other groups. Also the relative weight of kidney of all the alloxanised animals is decreased as compared to nLT and control rats.

3. The serum insulin level of LA(100) and LA(75) rats is decreased as compared to CA(100) and control rats while, the serum glucose level of LA(100) and LA(75) rats is increased as compared to CA(100) and control rats.
4. The hepatic glycogen content is decreased while, the muscle glycogen content is increased
5. The hepatic and muscle glycogen contents of LA(100) rats are increased as compared to all other groups.
6. The hepatic and muscle glycogen synthetase activity of all the alloxanised rats is decreased as compared to nLT rats.
7. The hepatic glycogen phosphorylase activity of LA(100) rats is increased significantly as compared to all other groups while, the muscle glycogen phosphorylase activity of LA(75) rats is decreased as compared to all other groups.
8. The hepatic glucose-6-phosphatase activity of the alloxanised rats is decreased.
9. The muscle protein content of all the alloxanised rats is increased.
10. The hepatic total lipid content of LA(75) rats is increased as compared to all other groups while, the hepatic cholesterol content of all the alloxanised rats is decreased as compared to nLT rats.

11. The muscle total lipid content of LA(100) and LA(75) rats is decreased as compared to CA(100) rats while, the muscle cholesterol content of all the alloxanised rats is decreased as compared to nLT rats.
12. The adipose tissue total lipid content of all the alloxanised rats is decreased as compared to control rats while, the adipose tissue cholesterol content of all the alloxanised rats is decreased as compared to the nLT rats.
13. The serum triglyceride, cholesterol, total lipids and free fatty acid levels are decreased in all the alloxanised rats while, the serum phospholipid level of LA(100) and LA(75) is decreased as compared to CA(100) and control rats
14. The serum cholesterol level of MA(100) rats is increased.
15. The pancreatic islets of CA(100) rats show pronounced recovery from alloxan induced damage. Islets of alloxan treated nLT rats showed prominent B cell loss.

The possible explanations for the above recorded observations are:

1. It is obvious that there is a favorable influence for organ growth due to neonatal melatonin functional deficiency respectively a retardatory influence on body growth. The underlined cause for the paradoxical effect does not find any valid explanation at this juncture. However, the significantly increased adrenal weights might suggest activation of hypothalamo hypophysial adrenal axis with probably increased corticosterone and catecholamine out put.

2. The increased serum glucose level and decreased indulin livel can be understood in the context of histologically observable increased B cell loss in nLT rats and increasing recovery in the control rats
3. The changes in hepatic and serum lipid levels as well as the observed glycemic and insulinemic status are clearly relatable with the decreased B cell mass with increased degenerative loss reminiscent of a developing of type 2 diabetes mellitus.
4. The increased B cell degeneration observable could be a long term consequence of reduced neonatal melatonin action resulting in permanent alteration of B cell dynamics and increased generation of oxidative stress. This might suggest the need for an optimal melatonin action during the neonatal period for long term protection of pancreatic B cell and decreased generation of reactive oxygen species.
5. The herein observed changes in the lipid contents and serum glucose level suggest a link between neonatal low melatonin status and potentiated diabetogenic inclination. Further studies are needed on these lines to take the observations to there logical conclusions.
6. It can be concluded that neonatal hypomelatonemia could render animals vulnerable to diabetogenic agents, conditions and result in insulin resistance and even type 1 diabetes by increased B cell loss