“We dance in a ring and suppose, but the secret sits in the middle and knows”

- Robert Frost
In this study we simulated complications associated with untreated DM patients namely, glycemic and intestinal motility alterations using suitable physiological conditions and pharmacological agents to find any relationship existed between each other (in healthy animals). Further, we evaluated effect of insulin administration on SIT in vivo and in vitro models and investigated pathways involved in insulin action.

6.1. Physiological and drug-induced hypoglycemia - Intestinal transit - Insulin level

Hypoglycemia can be induced by prolonged fasting, insulin administration or its secretogogues or hepatectomy. We find food deprivation for different periods as a physiological means of achieving hypoglycemia. Hypoglycemia with insulin or its secretogogues may affect other organs and interfere with investigations. Hepatectomized animals exhibit absolute hypoglycemia but surgical manipulation may add another variable on findings. In view of these consequences, we opted for food deprivation for more than 24 h to induce hypoglycemic condition.

Fed state: Fed state refers to the period of active nutrient digestion and absorption from gastrointestinal tract. Duration of the fed state normally lasts for 4-6 h and also depends upon amount and composition of food ingested and animal species used. After the ingestion of meals, the glucose is released from dietary carbohydrates as the enzymatic digestion process initiated, glucose gets absorbed from duodenum and ileum and elevates blood glucose level in 30-45 min. After 4-5 h of food deprivation the plasma glucose level falls rapidly due to faster glucose consumption coupled with reduction in glucose availability from dietary source. This is followed by optimization of blood glucose level to maintain supply to the brain. The concentration of glucose in the blood is regulated by a complex interplay of multiple pathways and modulated by a number of hormones.
**Post absorptive state:** In humans, post absorptive state is attained 10-15 h after meal consumption. When the animal is deprived from food for more than 10 h blood glucose level falls to basal level, then liver becomes the sole organ of maintaining the glycemic state to support for brain. Insulin level is reduced to allow hepatic glucose production, at the same time glucagon, adrenaline, cortisol and growth hormone are elevated to maintain glycemic state. Glucagon plays primary counter regulatory role during fasting. Our results shows a generalized fall in blood glucose levels from 6-48 h. In case of 12 h food deprivation we observed minimal fall in BG when compared with 6 h period, the reason may be that reflex release of glucose can occur from liver during acute stress induced by short period of fasting. However, the hypoglycemic levels were observed after 24 h fasting (<56 mg/dL).

**Blood glucose - Small intestinal transit**

Dog is the most suitable animal model for the study of GI motility. However, in view of ethical reasons we selected mice, being small and easily manageable experimental animal.

**Fed state:** Feeding disrupts pattern of MMC and initiates a continuous spiking activity. Forty four percent of the contractions in the fed state are segmental, the rest are propulsive. Thus, the intestine of the fed animals exhibits both segmental and peristaltic contractions indicating absorption of nutrients and propulsion of undigested food material. The net transport of contents in the fed state is just as rapid as that occurring during phase III of MMC. In our experimental animals, fed state SIT varied in between 52-73%.
Fasting state: In humans, about 4 to 6 h after the meal is taken, the fed state pattern changes quite abruptly and fasting pattern persist until the next meal is taken\(^{205}\). The fasting pattern occurs as Phase I, II, III & IV\(^{206}\). Phase III occurs about 5% of the cycle, begins abruptly at an interval in which ring contractions occurs at the maximum possible frequency and amplitude, ends abruptly returning the intestine to the quietude of phase I. This cycle recurs over and over again with a total period of about 90-100 min until the next meal was taken.

Many reports are available studying the effect of hypoglycemia on gastric motility but very few reports are available studying the hypoglycemic effect on small intestine transit.

Hypoglycemia was reported to accelerate motor activity of stomach\(^{163}\) and inherent hypoglycemia reported to accelerate gastric motility using isolated head technique in dogs\(^{207}\) and in rabbits\(^{166}\). But it was not clear whether they had compared with free fed or overnight fasted animals. Some workers assumed that vagal pathways mediate the excitatory effect of hypoglycemia on GI motility\(^{165, 166}\) and dorsal nucleus and diencephalons are involved at central level\(^{173}\). Schvarcz et al (1995)\(^{9}\) also suggested from their finding that in humans, vagal activity seems to be an important determinant in gastric emptying during hypoglycemia. To study the effect of hypoglycemia on small intestine, we have to fast the animals for more than 24 h as physiological hypoglycemia prevails from this period of time and a blood glucose level of 50 mg/dL is generally accepted as a requirement for adequate stimulation of GI motility\(^{173}\).

When animals were fasted up to 48 h at six hour intervals, we observed a mild deceleration in the 30 h group of SIT whereas other groups did not significantly differ from the free fed group. Earlier literature indicated hypoglycemia cause acceleratory effect on gastric emptying and intestinal transit in some parts\(^{163, 204}\). Our results do not indicate similar effect on small intestinal transit when compared with free fed group. We suggest that gradual hypoglycemic effect do not alter the SIT.
Food deprivation - Serum insulin levels - Small intestinal transit

Insulin is an anabolic hormone which stimulates the transportation of glucose into adipose tissue and muscle, promotes the conversion of glucose to glycogen, inhibits glucose production by the liver and proteins breakdown\(^\text{30}\). Insulin secretion is stimulated by glucose, amino acids, pancreatic and GI hormones (glucagon, gastrin, secretin, pancreozymin and GI polypeptide) and some drugs (sulphonylureas, \(\beta\)-agonists). Insulin secretion is inhibited by hypoglycemia, somatostatin and some drugs (\(\alpha\)-agonists, \(\beta\)-blockers, diazoxide, phenytoin, phenothiazines and nicotinic acid)\(^\text{47}\).

In all the complementary groups of food deprivation from 6 – 48 h for the measurement of serum insulin level we observed the serum insulin levels were either equal to free fed group or slightly lower than free fed group. It is surprising to observe in this animal species that when fasting exceeded more than 24 h, insulin levels were not significantly decreased as seen in human beings, which deserves further investigation in this model. We verified with C-peptide of insulin levels in similar experimental protocol. In that experiment, we observed C-peptide levels during short periods of fasting were even significantly higher than free fed animals (Free fed: 0.325 ±0.02 ng/ml; 6 h 0.0.646±0.14***; 12 h 0.541±0.046***; 18 h 0.556±0.06***; 24 h 0.595±0.05*** ng/ml).

These higher insulin levels are associated with hypoglycemic condition achieved with prolonged fasting, for reasons unknown or in this animal species fasting state may not lower the insulin level. These results may support the view that optimum insulin levels are required for adequate maintenance of SIT. Lowering of insulin levels may lead to mild attenuation of SIT. In all the groups of fasting, all the parameters except blood glucose level, showed no significant differences from free fed groups. We used similarly treated animals for the measurement of serum insulin levels omitting the SIT measurement as the results obtained are globally accepted.
Glibenclamide - Hypoglycemia - SIT

Glibenclamide is one of the widely used oral hypoglycemic drugs. Its binding to SUR or \(K^+_\text{ATP} \) channels results in release of insulin from pancreas and disposal of glucose. Our results indicate that glibenclamide at the dose of 10 mg/kg produced significant reduction in blood glucose level \((P<0.001)\) and acceleration of marker along the length of small intestine. Acceleration of SIT in this protocol might be due to sudden fall in BG level or elevated endogenous insulin or glibenclamide itself. As we had seen the fall in BG level here, may not produce typical hypoglycemic condition. However, the typical hypoglycemia can be induced by increasing the dose of glibenclamide, may produce the response of drug-induced hypoglycemia. We speculate that a sudden fall in BG level might have produced acceleration of SIT rather than gradual fall in BG level taken place during fasting state. Involvement of endogenous insulin in accelerating SIT cannot be ruled out.

Glibenclamide –Serum Insulin – SIT

Glibenclamide (10 mg/kg) \textit{per se} elevated serum insulin levels significantly \((P<0.05)\) and in the corresponding group acceleration of SIT was observed. Our finding was in agreement with Simonson (1990) in accelerating SIT as glibenclamide improves insulin sensitivity. Such results provide a possibility that insulin might be involved in acceleration of SIT in this study.

In Fig 1, a fall in blood glucose is associated with deceleration of SIT in four groups out of eight groups of fasting periods. An opposite association existed in four groups and with glibenclamide treated group. These findings does not indicate existence of any relationship between blood glucose and SIT, in different groups of hypoglycemia induced states.

In Fig 4, a fall in serum insulin level is associated with deceleration of SIT in four groups, whereas an elevation of insulin is associated with acceleration of SIT in glibenclamide treated group. This may indicate association of insulin in acceleration of SIT.
6.2. Effect of hyperglycemia on intestinal transit and insulin level

**Acute hyperglycemia - Small Intestinal transit**

Variable reports are available about effects of hyperglycemia on GI motility but many reports support the depression of motor function of stomach\textsuperscript{183,187}, weak reversible effect on motor function\textsuperscript{210}. Hyperglycemia inhibits parasympathetic nervous system\textsuperscript{211} or acts as cholinergic antagonist\textsuperscript{212-213}. Hyperglycemia attenuates motor activity of stomach\textsuperscript{214}. Fasting MMC prevails even during intravenously-induced hyperglycemia.

Our experiments with acute effect of parenterally-induced hyperglycemia on SIT showed that mild to moderate blood glucose levels did not affect SIT, only very high blood glucose level attenuated the SIT significantly ($P<0.01$). Our finding was supported by Bjornsson et al (1994)\textsuperscript{215} that hyperglycemia not only reduced the motility in the stomach but also inhibited motility of small intestine in healthy volunteers. Very high dose of glucose might have utilized osmoceptive effect or inhibition of PNS or exerted anti cholinergic effect or some non-specific mechanisms might have produced attenuation of GI motility.

**Acute hyperglycemia - Serum insulin level - Small intestinal transit**

Insulin secretion is a tightly regulated process designed to provide stable concentrations of glucose in blood during both fasting and feeding. Insulin is secreted in a pulsatile fashion. Intravenously administered glucose stimulates release of insulin in two phases. First phase begins in 1-2 min after elevation of blood glucose surrounding fluids of β-cells, and ends within 10 min. The second phase begins at the point where the first phase ends and lasts until the normoglycemia has been restored (usually in 60-120 min).
In all the acutely treated groups from mild – moderate – severe hyperglycemia, we observed significant elevation of serum insulin levels after 30 min of dextrose administration. When we compared SIT in complementary groups, SIT was significantly attenuated ($P<0.01$) only with very high dose of glucose (4 g/kg). Normally when glucose is administered intravenously, insulin is released reflexly from pancreas to maintain normoglycemic state. So any effect that has resulted, in addition to elevated BG effect, insulin effect could also be associated. We therefore, postulate that insulin might have counteracted the deceleration by mild to moderate hyperglycemia on SIT and failed to counteract attenuation induced by severe hyperglycemic effect.

**Euglycemia – Small Intestinal transit**

The objective of this experiment was to find out the effect of euglycemic state on intestinal transit. We observed that the euglycemic state did not affect the SIT. This finding clearly indicates glycemic alterations do not affect the SIT except the severe hyperglycemia.

**Clonidine - Blood glucose**

Clonidine, an established antihypertensive drug, has a side effect to produce hyperglycemia. In this study clonidine was used to produce hyperglycemia in experimental animals to study this effect on SIT and serum insulin. The suggested actions were about release of hormones involved in the elevation of blood glucose (somatotropin, growth hormone) or suppression of hormone that regulate the glucose metabolism or transient stimulation of peripheral $\alpha$-receptors or direct inhibitory effect on pancreas or inhibition of islet monoamine oxidase activity. In dogs, clonidine produced prompt elevation of blood glucose in 2 h. We also observed in our study that clonidine (0.1 mg/kg) elevated blood glucose levels significantly, this might have happened by utilizing all or any one of mechanisms stated above.
Clonidine - Blood glucose - SIT

Our results indicate that SIT was attenuated significantly ($P<0.001$) in clonidine treated animals. Therapeutic dose of clonidine administration would also affect gut motor function\textsuperscript{215}. Antidiarrhoel effect of clonidine is in part due to prolonging orocecal transit in healthy volunteers\textsuperscript{217} or small bowel transit time\textsuperscript{218}.

Clonidine - Serum insulin - SIT

In our study clonidine (0.1 mg/kg) produced attenuation of serum insulin level significantly ($P<0.001$) 30 min after its administration. This finding confirms that clonidine elevates BG level. Clonidine has been reported to produce elevation of BG by inhibiting insulin secretion\textsuperscript{91,96,97} through stimulation of postsynaptic $\alpha_2$ receptors in pancreas\textsuperscript{98}. This effect was antagonized by yohimbine\textsuperscript{99}. Clonidine also suppresses insulin secretion through direct inhibition of Ca$^{2+}$ current through voltage dependent Ca$^{2+}$ channels\textsuperscript{219}. In the corresponding group, attenuation of SIT was observed. We suspect a fall in insulin level might have favoured attenuation of SIT. These reports indicate that suppression of insulin secretion would contribute to hyperglycemic attenuation of SIT.

Fig 2 shows that, only the highest dose of dextrose and clonidine-induced hyperglycemia attenuated the SIT. This indicates in healthy animals only the severe hyperglycemia attenuates SIT and not the normal elevation of blood glucose.

In Fig 5, a fall in insulin level may favour deceleration of SIT in clonidine treated group. However, the similar relationship can not be drawn in dextrose treated group.

Thus, the hypoglycemia induced by food deprivation has not affected SIT, however the glibenclamide induced hypoglycemia accelerated SIT. Euglycemic or mild to moderate hyperglycemic states do not affect SIT. In addition, the elevated serum insulin achieved with glibenclamide has some effect on SIT. Reduction in insulin levels may contribute for attenuation of SIT atleast after clonidine administration.
6.3. Effect of intestinal transit alterations on blood glucose and insulin levels

**Metoclopramide - Blood glucose – SIT**

*Glycemic state:* The objective of this experiment was to find out whether any alteration induced on SIT can reflect in blood glucose level. We observed that metoclopramide induced alteration of SIT elevated blood glucose level ($P<0.05$). Our finding is in line with Morricone *et al.* (1990) that acute i.v., administration of metoclopramide (2.5 mg) has elevated BG level in healthy volunteers.

Metoclopramide is an established prokinetic drug. It was found useful in the treatment of diabetic gastroparesis. It produces prokinetic action by enhancing ACh release from post ganglionic cholinergic nerve endings and to antagonize the dopamine action. It was shown to stimulate gastric antral motility but not duodenal, ileal or colonic motility. We also observed, metoclopramide significantly accelerating the SIT ($P<0.05$).

**Metoclopramide - Serum insulin – SIT**

We observed that metoclopramide (5 mg/kg) could slightly elevate serum insulin levels in this species. This observation is in contrast to findings of Morricone *et al.* (1990). In that study, 2.5 mg of metoclopramide significantly decreased basal serum insulin level in healthy subjects. In the corresponding group treated with metoclopramide showed a significant acceleration of SIT. These findings of our study strengthens an idea that insulin might be also involved in acceleration of SIT.

**Atropine - Blood glucose – SIT**

The parasympathetic nerves dominate the overall tone and motility of GIT. Intestine has a complex system of intramural nerve plexuses that are mainly responsible for motility and impulses from the CNS only modify the effects of the intrinsic reflexes. The terminal neurons of the intramural plexuses consist of cholinergic and ganglionic nicotinic receptors and the effects of their activity can be blocked by atropine. Atropine abolishes or prevents the excess motor activity of the GIT induced by
parasympathomimetic drugs and anti-acetylcholinesterase agents, has led to use atropine as an antispasmodic agent for GIT disorders[1]. The local hormones and non-cholinergic neurons in enteric nervous system also modulate GI function. Even a complete muscarinic block cannot totally abolish minimal activity of GIT. This might be the reason for maintenance of minimal motility of intestine reported in our study. Our observation with atropine was in contrast to Ruwart et al (1979)[22] where they reported cholinergic system do not play a role in acceleration of SIT in rats. In mice, we observed participation of cholinergic system in accelerating SIT.

The objective of this experiment was to evaluate whether deceleration of GI motility can reflect any change in blood glucose level. We used atropine for its quality of antispasmodic activity or attenuation of GI motility[148,155]. In our study, the regular dose of atropine produced significant inhibition of SIT ($P<0.01$) without affecting blood glucose and insulin levels. Atropine 1 mg/kg, produced 25% inhibition of SIT when compared with vehicle treated group.

Atropine (1 mg/kg) administration did not alter normal blood glucose level. This finding indicate that our results were in line with Lautt and Wong (1978)[222] and Lima et al (1998)[223]. Because, a central action cannot be ruled out even when it was administered peripherally, it seems atropine can only prevent central cholinergically stimulated blood glucose level[224]. These findings indicate atropine per se has no effect on glycemic state. Similarly, atropine did not affect insulin levels. Cholinergic stimulation lead to insulin secretion in $\beta$-cells[225]. It seems atropine dose selected in our study did not affect physiological cholinergically stimulated insulin level. We observed only a slight elevation of insulin levels. This slight elevation of insulin level could not accelerate the SIT due to strong decelerating effect of atropine.

In Fig 3 and 6, we tried to draw any relationship between the drugs altering the SIT, on BG and serum insulin levels. We observed alterations in SIT produced similar effect on blood glucose and insulin level with both the drugs. This finding indicates alterations of SIT with pharmacological manoeuvres do not significantly alter the serum insulin levels.
Thus, only the drug-induced acceleration of SIT was observed with alteration in glycemic state. A moderate elevation of serum insulin achieved with prokinetic drug may contribute for potentiating inherent effect on SIT.

We used above stated pharmacological agents to alter blood glucose or insulin levels or intestinal transit, as they possess definite role in interventions adopted in this study. Moreover, some drugs were frequently prescribed for diabetic patients.

6.4. Effect of exogenously administered insulin on Intestinal transit

Our study with insulin administration comes out with a novel finding that insulin at lower doses significantly accelerated small intestinal transit without altering glucose level. Our finding was in line with Takeshita and Yamaguchi (1997)\(^6\) where they reported insulin at lower doses produced antinociceptive action without attenuating BG level.

The objective of this experiment was to find out whether any dose relationship of insulin with SIT and its effect on blood glucose under the influence of exogenously administered insulin existed. We observed that all the doses employed in the protocol produced consistent acceleration of SIT. Surprisingly, we observed the lowest dose of insulin (2 \(\mu\)U) especially accelerated SIT significantly \((P<0.05)\) without affecting blood glucose level. The first report is available in using insulin to produce hypoglycemic effect in dogs.\(^{163}\) In that model, insulin produced an increase in the height and frequency of gastric contractions and gastric tonus. These effects depend on blood glucose level\(^{167}\) and acceleration of gastric emptying was proposed to be due to hypoglycemic effect\(^6\).

In our experiment, blood glucose level was not affected by the lowest dose of insulin but produced significant acceleration of SIT. We suggest that blood glucose level does not play a role at least in lower doses of insulin. Insulin acts indirectly on stomach through vagus nerve.\(^{168,146}\) In fact it was emphasized that insulin injection could be used as a challenge to test the functional integrity of vagus fibers to the stomach. At some time, insulin effect on GI motility was recommended as a model to compare prokinetic drugs on GI motility, which can be comparable to metoclopramide.\(^{146,146}\) In addition, it was also reported that insulin may have a permissive role in basal pattern motility of
Intestines in sheep\textsuperscript{169}. Some contrasting reports are also available about depressant effect on GI motility by releasing norepinephrine in rats\textsuperscript{170,171} and some attributed this inhibitory effect due to hyperpolarization of smooth muscle\textsuperscript{44} or phenol content of commercial insulin\textsuperscript{174}. Moreover, these observations were recorded at higher concentration of insulin\textsuperscript{163,168,169}. Our finding strongly refutes the statements of Ozturk \textit{et al} (1996)\textsuperscript{44} in their review article that insulin produced inhibitory effect on smooth muscle contractility.

To rule out the influence of endogenous insulin on SIT, we felt it is necessary to evaluate serum insulin level. We observed that serum insulin levels were significantly elevated in all treated groups at 50 min after insulin administration. Further to know the role of endogenous insulin on SIT effect, we evaluated C-peptide of insulin levels. C-peptide of insulin is released in equimolar ratio with insulin from islet cells and not extracted by liver. Hence, the C-peptide measurement reflects absolute status of endogenous insulin production. Normally the C-peptide level is expected to comedown by supplementation of insulin. Surprisingly, we noticed that C-peptide levels in serum were elevated. This confirms that exogenous insulin might have a reflex action on secretion of insulin from pancreas in healthy animals. In these animals there might be a role played by elevated endogenous insulin in acceleration of SIT, but the effect on SIT was not proportionate to total insulin level. This indicates insulin has rate limiting effect on acceleration of SIT. To find the effect of exogenous insulin \textit{per se} on SIT, pancreatectomized animals need to be utilized. But this model is associated with surgery induced stress and stress factor is likely to affect the parameters to be measured. Characterization of insulin effect further may be possible with insulin antagonists, however at present we do not have a specific antagonist of insulin at its receptor site. Hence, it is difficult to characterize the effect of insulin. We selected s.c route for insulin administration which is based on general observation that insulin is frequently administered by s.c route. Therefore, our findings are more reasonable.
6.5. Effect of repeated administration of insulin on intestinal transit

Tolerance is a state of reduced responsiveness of the biological system to any repetitive stimuli. Six hourly repeated administration of insulin with a dose that can produce maximum acceleration did not show reduction in response. This finding indicates repeated administration of insulin did not produce tolerance. In other words no extra dose of insulin is required to produce adequate effect in regular administration of insulin. Hence, insulin repeated administration is free from development of tolerance.

6.6. In vitro effect of insulin on intestinal ileal segment

This experiment reveals about direct effect of insulin on GI smooth muscle. The functional components of the isolated intestine are terminal sympathetic and parasympathetic synapses as well as parasympathetic ganglionic synapse. A sustained submaximal contractions or inherent tone might be due to intramural prostaglandins. Available literature indicates insulin produces inhibitory effect on isolated GI smooth muscle. It was attributed to a preservative associated with commercial formulation (metacresol). Later it was observed that a transitory inhibition was followed by marked increase in tonus. Our results also indicate similar phenomenon but without transitory inhibition with all the range of doses of insulin, when compared with standard myogenic drugs. This observation indicates insulin possess a direct effect on smooth muscle producing tonus. It may produce this effect significantly through peripheral nervous system. Pavel and Mlicou (1932) report also indicated that with prolonged insulin therapy in human subjects produced direct stimulatory effect on intestinal muscle tone in vivo. We suppose that insulin acts as a neurotransmitter to elicit myogenic effect on GI motility. In light of our observation, we find the reports of Altan et al (1989) and Ozturk et al (1996) statements were controversial as we could not observe the stated phenomenon of attenuation in in vitro experiments.
6.7. Evaluation of different mechanisms in insulin-induced hypermotility of small intestine

The major pathways involved in GI motility are cholinergic, adrenergic, opioidergic and calcium channels. Pretreatment with suitable antagonist reveals about evidence of involvement of pathway. Acceleration of GI motility can be achieved by direct stimulation of GI muscle, by activation of excitatory neural pathways or by inhibition of inhibitory pathways.

**Atropine –Exogenous insulin – SIT**

Atropine is frequently used as a tool for identifying mechanism involving cholinergic pathways. When atropine group (1 mg/kg) was post treated with insulin, SIT failed to be reversed significantly. This observation indicates atropine was successful in counteracting accelerating effect of insulin atleast by 50%. This finding shows insulin acts through cholinergic pathways to accelerate SIT and some associated pathways as atropine could not completely prevent the acceleratory effect of insulin. Further, cholinergic mechanism can be evaluated by increasing the dose of atropine.

**Clonidine –Exogenous insulin – SIT**

Clonidine has presynaptic \( \alpha_2 \)- agonistic activity. Stimulation of \( \alpha_2 \)-receptors present on excitatory cholinergic intramural neurons in the intestine\(^\text{28}\) which attenuates release of ACh presynaptically thereby producing depression of intestinal motility. It is well known that clonidine acts as agonist of presynaptic \( \alpha_2 \)-receptors which produce attenuation of noradrenaline secretion, but herein enteric nervous system \( \alpha_2 \)-stimulation leads to attenuation of ACh secretion, this finding indicates clonidine interacts with cholinergic system also.

When clonidine treated mice (0.1 mg/kg), post-treated with insulin (2 \( \mu \) U/kg), the SIT was inhibited by 63%. Even though the insulin could reverse the SIT the inhibitory effect of clonidine was still significant. This finding indicates \( \alpha_2 \)-adrenergic pathways are more powerful in producing acceleratory effect of insulin. Since clonidine could not completely
inhibit the SIT, we can suggest that insulin action is associated with α-adrenergic pathways and other pathways playing a minor role. It can be consolidated at this stage that insulin cause facilitation of ACh release which is known for its myogenic effect on intestinal smooth muscle.

**Naloxone – Exogenous insulin – SIT**

The results with naloxone treated group indicates a surprising finding that naloxone per se produced significant inhibition of SIT ($P<0.01$). It is known that endogenous opioids reduce the SIT by binding to respective receptors. Naloxone prevents the action of opioids by competitively binding to its receptors. Therefore, naloxone is expected to produce normal SIT. In contrast we observed that naloxone produced inhibition of SIT. This finding indicate that naloxone acted similar to opioids. We traced out some similar contrasting reports. Champion et al (1982) reported that naloxone (2 mg) delays gastric emptying of radio-opaque material in healthy volunteers. They suggested that naloxone inhibits an endogenous opiate system which normally stimulates gastric emptying and they had used the dose of naloxone two to five times large than those usually given to reverse narcotic induced respiratory depression and in large doses naloxone itself may inhibit gastric emptying. Naloxone may act as an opiate agonist or have pharmacological action unrelated to the opiate receptor system. Shea-Donohue et al (1981) have reported in their abstract that 2 micro g/kg/min infusions of naloxone in rhesus monkeys inhibited gastric emptying of water while larger doses have no effect. They suggest that this apparent paradoxical effect on gastric emptying represents an opiate agonist action of naloxone. Asai and Power (1999) reported naloxone (0.01-10 mg/kg; i.p) per se had significantly inhibited gastric emptying in rats. Some contrasting reports available about stimulatory effect of opioids on SIT. Bitar and Makhlouf (1985) study showed that opioids act directly to cause contraction of circular muscle. Giuliani et al (1996) reported that opiates contract intestinal smooth muscle.

The objective of this experiment is to evaluate the involvement of opioid pathways in acceleration of SIT. In our study also we used higher dose of naloxone to block all the receptors to mask the effects of endogenous opioids. When naloxone treated group
(5 mg/kg) was post treated with insulin, the SIT was reversed significantly in comparison with naloxone per se treated group. Naloxone could only produce about 28% inhibition. However, the naloxone produced inhibition of SIT was still significant from insulin per se treated group. This tends to suggest that both naloxone and insulin act by independent pathways.

**Verapamil – Exogenous insulin – SIT**

Verapamil is a Ca^{2+} channel blocker, act by blocking calcium channels on the surface of smooth muscle, it relaxes smooth muscle. The objective of this experiment was to evaluate the involvement of Ca^{2+} channels in insulin-induced acceleration of SIT. Verapamil (8 mg/kg) per se inhibited SIT significantly, indicating the involvement of Ca^{2+} channels in normal physiology of small intestinal motility. In verapamil pretreated group insulin administration could partly reverse the inhibition produced by verapamil. This indicates calcium channels are partly involved in insulin action.

**Glibenclamide – Exogenous insulin – SIT**

We speculated that glibenclamide-induced acceleration of SIT might be associated with elevated endogenous insulin levels (Table 11). The objective of this study was to find whether endogenous insulin can potentiate the exogenously administered insulin. We observed that insulin group pretreated with glibenclamide, SIT was further accelerated significantly ($P < 0.001$). This observation indicate that both insulins from different sources might have acted at the same site of action producing acceleration of SIT.

Thus, the externally administered insulin (2 µU/kg) inherently accelerates SIT through cholinergic, adrenergic and Ca^{2+} channels, without the development of tolerance or altering glycemic state.
6.8. Centrally mediated effects of insulin on intestinal transit

In the past, it was considered that peptides do not cross blood brain barrier. However, most of them have a higher concentration in CSF than in plasma. This might have resulted from a de novo brain synthesis. About 25 fold greater concentration of insulin was found in the brain. It's action is still unclear but some workers suggested that insulin central action may be associated with food intake and body weight. Insulin enters the brain by transcytosis through microvessels, endothelial cells and governed by saturable mechanisms. High concentration of specific insulin receptors have been described on the brain endothelial cells, and found not to undergo much up and down regulation. These receptors mediate the transcytosis of circulating insulin from the blood to brain interstitial fluid. This transport process explains the origin of insulin in brain, since the de novo synthesis of insulin does not occur in brain in vivo. In addition to its role in growth, insulin provides glucose for glial cells in CNS.

Recently, an inherent antinociceptive property for centrally administered insulin was reported which was found to be independent of hypoglycemic effect. Like antinociceptive effect of insulin (i.c.v), it was thought worthwhile to study physiological effect of centrally administered insulin on SIT in mice.

Our earlier part of the study indicated that all the doses (2 μ, 2 m or 2 U/kg) of subcutaneously administered insulin accelerated the SIT, but in centrally administered insulin groups, lowest dose attenuated SIT and highest dose accelerated significantly. It seems lowest dose acts differently from the highest dose of insulin. Centrally administered insulin has less effect on % SIT, only the highest dose (2 U/kg) accelerated the SIT. No hypoglycemic symptoms were observed even at the higher doses of insulin.

We used the commercial plain bovine insulin as injectable form (40 IU/ml), containing metacresol as preservative (0.25% w/v). Takeshita and Yamaguchi had used bovine insulin, shown antinociceptive effect in normal mice. However, some previous reports did not reveal the animal source of insulin.