EFFECTS OF INSULIN ON SLEEP
IN PATIENTS WITH INSULIN-DEPENDENT DIABETES MELLITUS

A Thesis

Submitted to the College Of Graduate Studies and Research
in Partial Fulfillment of the Requirements
for the Degree of Doctor of Philosophy
in the Department of Pharmacology
University of Saskatchewan
Saskatoon

By

Agus Abdurahim Dahlan
Fall, 1996

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College of Graduate Studies and Research

SUMMARY OF DISSERTATION
Submitted in partial fulfillment
of the requirements for the
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by
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University of Saskatchewan
Fall 1996

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EFFECTS OF INSULIN ON SLEEP
IN PATIENTS WITH INSULIN-DEPENDENT DIABETES MELLITUS

Insulin receptors have been identified in the central nervous system, particularly in the brain stem and limbic regions. Binding to the insulin receptor is regulated by the distribution of insulin in the brain, and this by the level of insulin in the peripheral circulation. Studies in animals suggest that insulin modulates a number of different neuronal activities including sleep-wake cycle patterns and satiety.

Based on these observations, we hypothesized insulin also had effects on sleep and daytime cognitive performance in humans and that there may be differences between the effect of different insulin species or modes of insulin administration due to their differing time-effect profiles. Thus, we performed a crossover comparison of natural sleep measured by overnight polysomnography (PSG), day-time sleepiness measured by the Multiple Sleep Latency Test (MSLT), and daytime cognitive performance measured by four psychological tests; namely, the Digit Symbol Substitution Test, the Symbol Digit Modalities Test, the Grooved Pegboard Test and the Manual Finger Tapping Test, in subjects with insulin-dependent diabetes mellitus (IDDM) randomized to one-monthly treatments with human R/N insulin, beef/pork R/NPH insulin, beef/pork Iletin R/N insulin, lispro/human ultralente insulin, and continuous subcutaneous (beef/pork Iletin R or lispro) insulin using an insulin pump. They were also compared to eight age-, gender-, and weight-matched non-diabetic healthy control subjects. Our result showed that diabetic patients had a very disrupted sleep, characterized by a 30% shorter REM onset latency, 30% less time in SWS and REMS and a high frequency of stage shifts, REMS periods and awakenings after sleep onset. They were also significantly more sleepy during the day on the MSLT. Cognitive performance on psychometric testing was similar to that of non-diabetic controls, but showed significantly greater fluctuation throughout the day. Human R/N insulin-treated diabetics fell asleep faster on both the PSG and MSLT, and had 25% more REMS time and a better sleep distribution than beef/pork R/NPH insulin-treated diabetics. Conversely, lispro/human ultralente treated diabetics spent more time in stage 1
sleep, and less in REMS. Insulin pumping, particularly with beef/pork insulin increased the duration of REMS and REMS distribution, increased the time to fall asleep on the MSLT, and generally improved overall daytime cognitive function, although the pattern of sleep disruption persisted. Thus, we conclude that insulin has important effects on sleep and daytime performance in humans, and that different insulin have different effects, depending upon their pharmacokinetic profiles.

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PUBLICATIONS


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ABSTRACT

Insulin receptors have been identified in the central nervous system, particularly in the brain stem and limbic regions. Binding to the insulin receptor is regulated by the distribution of insulin in the brain, and this by the level of insulin in the peripheral circulation. Studies in animals suggest that insulin modulates a number of different neuronal activities including sleep-awake cycle patterns and satiety.

Based on these observations, we hypothesized insulin also had effects on sleep and daytime cognitive performance in humans and that there may be differences between the effect of different insulin species or modes of insulin administration due to their differing time-effect profiles. Thus, we performed a crossover comparison of natural sleep measured by overnight polysomnography (PSG), daytime sleepiness measured by the Multiple Sleep Latency Test (MSLT), daytime cognitive performance measured by four psychological tests; namely, the Digit Symbol Substitution Test, the Symbol Digit Modalities Test, the Grooved Pegboard Test and the Manual Finger Tapping Test, in subjects with insulin-dependent diabetes mellitus (IDDM) randomized to one-monthly treatments with human R/N insulin, beef/pork R/NPH insulin, beef/pork Iletin R/N insulin, lispro/human ultralente insulin, and continuous subcutaneous (beef/pork Iletin R or lispro) insulin using an insulin pump. They were also compared to eight age-, gender-, and weight-matched non-diabetic healthy control subjects. Our result showed that diabetic patients had a very disrupted sleep, characterized by a 30% shorter REM onset latency, 30% less time in SWS and REMS and a high frequency of stage shifts, REMS periods and awakenings after sleep onset. They were also significantly more sleepy during the day on the
MSLT. Cognitive performance on psychometric testing was similar to that of non-diabetic controls, but showed significantly greater fluctuation throughout the day. Human R/N insulin-treated diabetics fell asleep faster on both the PSG and MSLT, and had 25% more REMS time and a better sleep distribution than beef/pork R/NPH insulin-treated diabetics. Conversely, lispro/human ultralente treated diabetics spent more time in stage 1 sleep, and less in REMS. Insulin pumping, particularly with beef/pork insulin increased the duration of REMS and REMS distribution, increased the time to fall asleep on the MSLT, and generally improved overall daytime cognitive function, although the pattern of sleep disruption persisted. Thus, we conclude that insulin has important effects on sleep and daytime performance in humans, and that different insulin have different effects, depending upon their pharmacokinetic profiles.
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Dr. C. M. Shapiro introduced me to the domain of sleep medicine and its research possibilities. He showed me the fascinating realm of sleep medicine. My special appreciation is extended to him. I would also like to acknowledge Dr. M.J. Flanigan who devoted her time to train me in sleep recording and scoring techniques and who assisted me in the research.

The participation of the research subjects is sincerely appreciated. Without them this research would not have been possible. Also, I would like to express my appreciation to the Government of Indonesia, especially the Six University Development and Rehabilitation (SUDR) Project, for the scholarship I received.

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their warm friendship which allowed me to feel at home even being so far from my home country. Special thanks also to Fran McCauley, Elsie Habbick, Noella Johnson, and Bob Wilcox, who made everything possible for me during my study.

My deepest and most sincere gratitude goes to my parents, Dumyanah and Ali A. Dahlan, for their great love and support that always keep me going. My thanks also to my brothers, sisters and to my parents-in-law for their encouragement and supports. Finally, I would like to thank my wife, Titis Lubis, who is always beside me going through the hardships and joys during my study at the Department of Pharmacology, University of Saskatchewan, as well as in our journey of life. Being with her, all kinds of trouble are endured. May Allah subhanahu wa ta’ala always bless them all.
DEDICATION

This thesis is dedicated to my parents, Dumyanah and Ali A. Dahlan, to Mrs. Nuraini Dahlan, my late grandmother who suffered from diabetes mellitus, and all persons with diabetes mellitus and/or sleep disorders.

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<tr>
<td>5HT</td>
<td>Serotonin</td>
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<tr>
<td>ATP</td>
<td>adenosine triphosphate</td>
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<tr>
<td>BBB</td>
<td>Blood-Brain Barrier</td>
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<tr>
<td>cAMP</td>
<td>cyclic 3',5'-adenosine monophosphate</td>
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<tr>
<td>cGMP</td>
<td>cyclic 3',5'-guanosine monophosphate</td>
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<tr>
<td>C&lt;sub&gt;max&lt;/sub&gt;</td>
<td>maximum concentration</td>
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<tr>
<td>CNS</td>
<td>Central Nervous System</td>
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<tr>
<td>CSF-1</td>
<td>Colony-Stimulating Factor-1</td>
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<td>CSII</td>
<td>Continuous Subcutaneous Insulin Infusion</td>
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<td>DCCT</td>
<td>Diabetes Control and Complications Trial</td>
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<td>df</td>
<td>degrees of freedom</td>
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<td>DSST</td>
<td>Digit Symbol Substitution Test</td>
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<td>EC</td>
<td>Enterochromaffin</td>
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<td>EEG</td>
<td>Electroencephalography</td>
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<td>Electromyography</td>
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<td>Electrooculography</td>
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<td>FGF</td>
<td>Fibroblast Growth Factor</td>
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<td>GABA</td>
<td>γ-aminobutyric acid</td>
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<td>GH</td>
<td>Growth Hormone</td>
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<td>GLUT</td>
<td>Glucose Transporter</td>
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<td>GPBT</td>
<td>Grooved Pegboard Test</td>
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<td>Hu.</td>
<td>Human Insulin</td>
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<tr>
<td>IDDM</td>
<td>Insulin-Dependent Diabetes Mellitus</td>
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<td>IFN</td>
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<td>Neutral Protamine Hagedorn Insulin</td>
</tr>
<tr>
<td>NREM</td>
<td>Non Rapid Eye Movement</td>
</tr>
<tr>
<td>NREMS</td>
<td>Non Rapid Eye Movement Sleep</td>
</tr>
<tr>
<td>PDGF</td>
<td>Platelet-Derived Growth Factor</td>
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<tr>
<td>PZI</td>
<td>Protamine Zinc Insulin</td>
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<tr>
<td>R</td>
<td>Regular Insulin</td>
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<tr>
<td>REM</td>
<td>Rapid Eye Movement</td>
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<tr>
<td>REMS</td>
<td>Rapid Eye Movement Sleep</td>
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<tr>
<td>S.E.</td>
<td>Standard Error</td>
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<td>SDMT</td>
<td>Symbol Digit Modalities Test</td>
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<tr>
<td>SPT</td>
<td>Sleep Period Time</td>
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<td>SWS</td>
<td>Slow Wave Sleep</td>
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<tr>
<td>t&lt;sub&gt;h&lt;/sub&gt;</td>
<td>half-life</td>
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<tr>
<td>TBT</td>
<td>Total Bed Time</td>
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<tr>
<td>T&lt;sub&gt;max&lt;/sub&gt;</td>
<td>Time to peak concentration</td>
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</table>
TNF = Tumor Necrosis Factor
TST = Total Sleep Time
UL = Ultralente Insulin
WASO = Wake After Sleep Onset
1. INTRODUCTION

1.1 INSULIN

1.1.1 The History of Insulin

Frequent and voluminous urination accompanied by unquenchable thirst, excessive hunger, fatigue and rapid weight loss led to the recognition of the disease now known to be diabetes mellitus thousands of years ago by the Greeks and Egyptians. It was thought that in diabetics food was not fully transformed or metabolized into the form of energy. Instead, nutrients derived from food merely passed through the metabolic system. This belief was the reason why the Greeks called the disease diabetes which means “siphon” or “pipe-like”.

Until the early 19th century, when doctors finally developed a chemical test to indicate and measure the presence of sugar in the urine, diabetes was diagnosed by means of tasting the urine. Even after the diagnosis of diabetes was made easy by this discovery, the treatment was still a mystery. In 1866, Bouchardat, a French physician, proposed the theory of the pancreatic origin of diabetes. Bouchardat's theory was confirmed in 1889 by Oskar Minkowski and Joseph von Mering when it was noticed that their pancreatectomized dog was suffering from polyuria and glycosuria indistinguishable from that of diabetes mellitus in humans. It was not only the first successful total pancreatectomy, but also the one that generated the new hypothesis that diabetes was caused by the absence of a functioning pancreas.

Thirty years latter, Moses Barron, an American pathologist, reported in the November 1920 issue of “The Journal of Surgery, Gynecology and Obstetrics”, that
with total obstruction in the main pancreatic duct, all acinar cells disappeared through atrophy, but that most of the islet cells remained intact. Inspired by Barron's report, Frederick G. Banting, a young Canadian surgeon and a demonstrator in surgery and anatomy at the University of Western Ontario, convinced J. J. R. Macleod at the University of Toronto, to allow him access Macleod's laboratory to search for the antidiabetic principle in the internal secretions of the pancreas. Being unaware of much previous work in diabetes research and insulin, Banting, assisted by a medical student, Charles H. Best, developed a method of extracting the active principle of the endocrine pancreas, namely insulin. The method was similar to that developed by Scott and Dewitt 10 years earlier, except that the dog's pancreas was ligated by an experienced surgeon and remained in the abdominal cavity for a longer period (i.e. 6-8 weeks) to allow for the total degeneration of the pancreatic acinar cells. The remaining islet tissue was then extracted with ethanol and acid. The extract, called isletin, showed an antidiabetic effect which normalized the blood sugar level in pancreatectomized dogs. Hampered by the difficulties in obtaining a reproducible extract, Banting sought help from Macleod, and J.B. Collip, a professor at the University of Alberta. In 1922, the quartet finally produced a pure extract (insulin), and successfully normalized the blood sugar in the first insulin-treated human diabetic patient, Leonard Thomson. One year later, The Nobel Prize in Medicine and Physiology was awarded to Banting and Macleod which they then shared with Best and Collip.

History shows that insulin was the first substitution therapy to be introduced in the area of human medicine. Also, insulin was the first protein to have its amino acid structure elucidated. Furthermore, it was the first product of recombinant DNA technology, the first hormone for which a radioimmunoassay was developed, and the second protein to be crystallized. New approaches in treating diabetes mellitus, in
controlling its symptoms and complications, and recent innovations in the
development of new human insulin analogues and methods of administering insulin,
mark the exciting history of insulin and diabetes mellitus today (Bliss, M., 1982;

1.1.2 Pharmacology of Insulin

1.1.2.1 Chemistry of Insulin

1.1.2.1.1 Insulin Biosynthesis

The β cells of the pancreatic islets synthesize insulin from a single chain
polypeptide precursor, termed preproinsulin, which consists of 110 amino acids.
Preproinsulin can be found in the intact islets of Langerhans in low concentration, and
is rapidly cleaved by microsomal peptidases into proinsulin which has 86 amino
acids. Proinsulin is converted into insulin by the enzymatic cleavage and ultimate
removal of an intervening peptide, called C-peptide. having two basic amino acid
residues, Lys-Arg 64-65 and Arg-Arg 31-32, at each end of the cleavage sites. The
result is that insulin consists of two separate polypeptide chains, A and B, which are
joined by disulfide bridges. The A chain consists of 21 amino acids and the B chain
consists of 30 amino acids. Thus, human insulin contains 51 amino acids and has a
1.1.2.1.2 Amino Acid Structure of Insulin

The amino acid sequence of insulin has been highly conserved in biological evolution. Species differences in the structure of insulin consist mainly of interchain substitutions at positions A-8, -9, and -10, and at the carboxy terminus of the B chain. Beef insulin has alanine-serine-valine at positions A-8, -9, -10 and alanine at B-30. Pork and human insulin have the same amino acid sequence at A-8, -9, -10, i.e. threonine-serine-isoleucine. However, at position B-30, pork insulin has an alanine residue, whereas human insulin has threonine (Brown, H., et al., 1955; Sanger, F., 1959; Nicol, D.S.H.W., and Smith, L. F., 1960). The structure-activity relationships of a wide variety of insulin species suggests that some of the amino acid regions are related to the interaction between insulin and its receptor, while others are related to insulin immunogenicity. For example, amino acids A-1, -4, -5, -19, -21 and B-25 are contact points for the binding of insulin to the human insulin receptor, while B-21, -22, -23 and B-24 are important in supporting the insulin structure so as to facilitate that interaction. Positions A-8 to A-11, B-3 and B-30 are related to insulin immunogenicity (De Meyts, P., et al., 1978; Galloway, J.A. and deShazo, R.D., 1990; Kahn, C.R. and Rosenthal, A.S., 1979; Pullen, R.A., et al., 1976).

Insulin and Insulin-like growth factor (IGF) possess very similar tertiary structures and amino acid homologies that range from 40-50%. However, their physiological, biochemical and pharmacological activities are very different. Twenty four amino acids are strictly conserved between insulin and IGF-I. These conserved residues are crucial for maintaining the IGF and insulin structure. The remaining structure determines its affinity for insulin or IGF receptors. The inversion of B-28 proline and B-29 lysine of human insulin to B-28 lysine and B-29 proline as in IGF-I changes the affinity of the insulin for IGF-I receptors to two-fold greater, but that is
still only 0.2% of the affinity of IGF-I for the IGF receptor (DiMarchi, R.D., et al., 1994).

1.1.2.1.3 Crystalline Structure of Insulin

Studies of the crystalline structure of insulin in solution shows that insulin can exist as a monomer, a dimer or as a trimer of dimer, that is, a hexamer. Insulin is stored in the β cells of the pancreas in the hexameric form each containing two molecules of Zn$^{2+}$. Zn$^{2+}$ has a functional role in the formation and stability of the insulin crystals. Furthermore, crystallization facilitates the conversion of proinsulin into insulin (Davis, S.N. and Granner, D.K., 1996). The ability of insulin to self-associate, is dependent upon its amino acid structure (DiMarchi, R.D., et al., 1994).

1.1.2.2 Secretion, Distribution, Degradation and Excretion of Insulin
1.1.2.2.1 Histology of the Islets of Langerhans

The islets of Langerhans are composed of seven different types of cells, each of which synthesizes and secretes a distinct polypeptide hormone: glucagon by the α cell, insulin by the β cell, somatostatin by the δ cell, vasoactive intestinal peptide by the δ₁ cell, substance P and serotonin by the enterochromaffin (EC) cell, gastrin by the gastrin G₁ cell and pancreatic polypeptide by the pancreatic polypeptide-producing PP cell. The β cells make up the central core of the islets and are wrapped by a discontinuous mantle of cells about three cell layers thick of the other cell types (Bonner-Weir, S. and Smith, F.E., 1994). The arterioles enter the islets and ramify into a glomerular-like capillary mass in the β cell core, and then flow to the α cells and δ
cells. This anatomical arrangement suggests the β cells are the primary glucose sensors for the islet, and that the other cell types react to the high concentration or to the effects of insulin (Davis, S. N. and Granner D. K., 1996).

1.1.2.2.2 Secretion of Insulin

The interaction between insulin and other hormones in the islets is designed to provide a stable concentration of glucose in the blood during both fasting and feeding. Several hormones, the β2 adrenergic, α2 adrenergic and cholinergic nervous systems, neurotransmitters, glucose and other nutrient substrates (fatty acids, amino acids, and ketone bodies), and gastrointestinal enzymes are also involved in the intrinsic control system which regulates insulin secretion (Henquin, J.C., 1994).

Insulin is stored in the mature secretory granule until it is released by exocytosis. Granules move along microtubules from the golgi complex to the plasma membrane (Shoelson, S.E. and Halban, P.A., 1994). Exocytosis arises by the fusion of the granule membrane and the plasma membrane, with subsequent discharge of the granule contents into the extracellular space. There is a good evidence that secretion of insulin is regulated by transport within the cytoskeletal network of microtubules and microfilaments.

Insulin secretion evoked by a rapid increase in plasma glucose level is biphasic. The first phase reaches a peak very quickly, that is, within 1 to 2 minutes, and is short in duration. This phase is followed by a nadir and a slowly rising second phase which has a longer duration. It is proposed that insulin is stored in two compartments based on the size of the granules. The smaller granules are rapidly transported along the microtubules and are responsible for the first phase of insulin secretion. The larger granules are transported more slowly and it is believed that the second phase results
in a slower response to plasma glucose due to changes in the magnitude of the triggering signal (Henquin J.C., 1994).

Glucose is the principal stimulus for insulin secretion in humans. Glucose evokes insulin secretion more efficiently if it is given by mouth. This is because oral administration of glucose induces the secretion of insulin not only through a rising plasma glucose level, but also by stimulating the secretion of a number of gastrointestinal hormones and increasing vagal tone (Davis, S.N. and Granner, D.K., 1996). Hormonal and neurologic control of normal insulin secretion by the β cells of the pancreatic islets involves a very complex regulation. Indeed, any activity which influences the autonomic nervous system, such as stress, exercise, heat, etc., affects insulin secretion in one way or another. Counterregulatory hormones, on the other hand, oppose the actions of insulin (Nolte, M.S., 1992).

There is another insulin secretory system that is not affected by hormonal or neurologic control. This insulin secretion is pulsatile in fashion and is controlled by a pacemaker which originates within the pancreatic islets. Pulsatile secretion is differentiated into two kinds according to the frequency of the pulse. The first kind is a high-frequency pulse, wherein insulin is released every 10-15 minutes (Marchetti, P., et al., 1994). The second is a low-frequency pulse, in which insulin is released in longer cycles recurring every 90 to 120 minutes (Nolte, M.S., 1992). Insulin secretion also has a diurnal rhythm with an elevated early morning surge of insulin secretion and higher post-breakfast insulin levels relative to meal-stimulated increases later in the day (O’Rahilly, S., et al., 1988). Interestingly, insulin resistance has been found to be associated with a loss of the regular periodicity in insulin secretion (Peiris, A.N., et al., 1992).
1.1.2.2.3 **Distribution of Insulin**

Following secretion into the blood stream, insulin circulates as a free monomer and is distributed into a volume equal to the volume of the extracellular fluid space. Under basal conditions, the pancreas secretes insulin at a rate of one unit of insulin per hour and achieves a basal plasma insulin concentration in the portal vein of about 50 to 100 \( \mu \text{U/ml} \), and a concentration in the peripheral circulation of about 12 \( \mu \text{U/ml} \). Ingestion of the meal will heighten the insulin level in the portal vein. However, elevation of insulin in the portal vein is followed by only a slight increase in insulin concentration in the peripheral circulation. Thus, the ingestion of a meal augments the concentration ratio of insulin between the two circulations (Polonsky, K.S. and Rubenstein, A.H., 1986; Waldhausl, W., *et al.*, 1979).

1.1.2.2.4 **Degradation and Excretion of Insulin**

Insulin is metabolized primarily in the liver. About 50% of the insulin that reaches the liver via the portal vein is destroyed and never reaches the general circulation. The rest is degraded in the kidney and in muscle. The degradation of insulin is brought about mainly by intracellular receptor-mediated degradation and by proteolytic enzymes on the surface of the cell. The binding of insulin to its receptor is irreversible. Thus, following binding and initiation of the insulin response, insulin is captured on the cell surface and is transported across the plasma membrane, ending in intracellular proteolytic degradation. Receptor-mediated internalization and degradation of insulin is an efficient mechanism for rapidly terminating the insulin response because it rapidly removes both the hormone and the receptor target (Gliemann, J., *et al.*, 1975). Thus, the binding of insulin to its receptor leads not only
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these regions impairs receptor signal transmission. The juxtamembrane region, contains a fundamental tyrosine autophosphorylation site which blocks further binding to the insulin receptor. The ATP-binding domain is a lysyl-rich glycine residue which is very crucial in the receptor signal transmission. Any amino acid substitution in the ATP-binding domain blocks the autophosphorylation sequence and biological response. The regulatory domain consists of three major insulin-stimulated autophosphorylation sites. These autophosphorylation sites are activated by insulin binding to the α-subunit of one αβ-dimer and stimulate the phosphorylation of the adjacent covalently linked β-subunit. The autophosphorylation of these three tyrosine residues inactivates the insulin receptor tyrosine kinase (White, M.F. and Kahn, C.R., 1994).

1.1.2.3.2 Post Receptor Signaling System

The binding of insulin with the α subunit at the cell membrane surface activates tyrosine kinase on the β-subunit. This binding also leads to the autophosphorylation of several tyrosine residues which subsequently autocatalyze the activity of the enzyme toward other substrates within the cell. Many details of insulin receptor function are well understood. However, the molecular mechanisms that link the receptor to insulin action are not well studied. Two mechanisms have emerged as the foundation of insulin-receptor signal transduction. First, insulin-receptor signal transduction involves tyrosine phosphorylation of cellular proteins by the activated insulin-receptor kinase. Second, the autophosphorylated β-subunit mediates the noncovalent stable interaction between the receptor and other cellular proteins. These two mechanisms require intact ATP-binding sites, receptor autophosphorylation and
cellular substrates. However, thus far, these mechanisms are still not able to explain the signaling process caused by insulin (White, M.F. and Kahn, C.R., 1994).

Three compounds have been proposed or found as cellular substrates for insulin receptors. First, IRS-1, insulin-receptor substrate-1, which is purified from insulin-stimulated rat liver, is a unique protein with a molecular mass of 131 kd. It contains ATP and GTP binding sites and phosphorylation sites that may serve as a target for protein kinase C, and cAMP- and cGMP-dependent protein kinases. The insulin-receptor complex phosphorylates IRS-1, and phosphorylated IRS-1 activates PI 3'-kinase and then increases the cellular level of phosphatidylinositol-3,4,5-triphosphate. Second, P21ras has been suggested as an integral component of the insulin signaling pathway. The biochemical properties of p21ras are closely related to G-protein action in the modulation of signal transduction. P21ras is involved in signaling for cell proliferation and differentiation. The last, phosphatidylinositol-glycan, is stimulated by insulin action through the activation of phospholipase C by an unknown mechanism. Kinase activity of insulin receptors is required to activate phospholipase C (White, M.F. and Kahn, C.R., 1994).

1.1.2.3.3 Insulin Receptor Kinetics

Insulin-receptor binding kinetics show negative cooperativity between receptors. At low concentrations, insulin binds to receptors with high affinity. This condition is shifted to a low-affinity binding state as insulin levels rise. Thus, insulin sensitivity is maintained at low insulin levels and the cellular response is attenuated as the concentration of insulin increases. This characteristic of insulin receptor binding plays an important physiological role as a buffer against fluctuations in insulin secretion, especially in the liver which is exposed to large oscillations of insulin.
secretion from the pancreas. Negative cooperativity may not be physiologically important in tissues where insulin concentrations are less variable, such as in adipose tissue (Gammeltoft, S., 1984).

Receptor-mediated insulin degradation is involved in the ligand-induced downregulation of receptors and in cellular desensitization to insulin (Krupp, M. and Lane, M.D., 1981). In clinical settings the concentration of insulin receptors is reduced in association with elevated blood levels of circulating insulin. This downregulation of insulin receptors provides an intrinsic mechanism in which target cells limit their responsiveness to excessive hormone concentration. Desensitization of insulin receptors has been shown to occur with a time course of four to 24 hours (Karam, J.H., 1995).

Experimental evidence indicates that insulin receptors differ in various tissues and that these differences relate to functional characteristics of the receptors as well as differences in structural composition. The existence of different insulin receptor subtypes has been linked to different physiological actions of insulin and to the level of insulin in the organ. Differences in receptor functional characteristics relate to the phenomena of spare receptors. Maximum effects of insulin in the adipose tissue at steady-state are obtained by receptor occupancies of 5-10% compared to the liver cells which require about 90% occupancy for full effect. Thus, spare receptors are absent in the liver tissue, however, it is an obvious phenomenon in adipose tissue. High levels of insulin in the portal vein obviates the need for spare receptors in this location. Conversely, the high sensitivity of fat cells correlates well with the low physiological insulin levels in this tissue (Gammeltoft, S., 1984).
1.1.2.4 Insulin Action

The main actions of insulin take place in the liver, adipose tissue, and muscle. In these organs, insulin has important effects on cell growth and nutrient metabolism, particularly in regulating the availability of energy sources such as glucose and fat. Insulin stimulates the conversion of glucose into glycogen in the liver and inhibits glycogenolysis, gluconeogenesis, and the formation of keto acids from fatty acids and amino acids. In the muscle, insulin increases glucose and amino acid transport and induces glycogen and protein synthesis. Insulin's action on adipose tissue is mainly to stimulate glucose uptake and to increase the storage of triglycerides (Karam, J., 1995).

One major effect of insulin is stimulating the glucose uptake into the cells. The movement of glucose into cells occurs through a series of membrane spanning glycoproteins called glucose transporters (GLUT). There are six such transporters. First, GLUT1 functions in basal glucose uptake in all kinds of tissue. GLUT1 facilitates glucose transport into the central nervous system across the blood-brain barrier (Pardridge, W.M., et al., 1990). Second, GLUT2, which is located in the β-cells of the pancreas, regulates the insulin release, and while in kidney, liver and gut, it manages the glucose homeostasis. Third, GLUT3 controls glucose uptake into the neurons in the brain. GLUT3 is also involved in modulating glucose uptake into tissues such as kidney and placenta. Fourth, GLUT4 exists in the muscle and adipose tissues. Fifth, GLUT5 particularly facilitates the intestinal absorption of fructose. Lastly, GLUT7 is found in the endoplasmic reticulum. Its function as a glucose transporter is not yet understood (Karam, J., 1995). Sodium-independent facilitated diffusion of glucose into the cells involves GLUT1 to GLUT5.

Glucose transport involving GLUT1 and GLUT4 is insulin dependent. Insulin promotes the energy-dependent translocation of intracellular vesicle glucose
transporter to the cell membranes by an exocytic mechanism. The fusion of vesicles to
the plasma membrane will expose the glucose transporter to the extracellular milieu.
The exposure, furthermore, increases the transport of glucose into the cells. Upon
degradation or removal of insulin from its receptor, the glucose transporters are
retranslocated back to the intracellular pool by an endocytic process (Simpson, I.A.,
and Cushman, S.W., 1986). Even though GLUT1 and GLUT4 are insulin-dependent
-glucose transporters, only the synthesis of GLUT4 is regulated by insulin (Davis,
S.N. and Granner, D.K., 1995). Furthermore, some studies in diabetic rats show that
the synthesis of insulin-dependent glucose transporters is suppressed when there is

1.1.2.5 The Issues in Bovine, Human and Porcine Insulin

Human insulin has been claimed to cause an increasing incidence of
hypoglycemia unawareness, and even sudden death during sleep among young people
with Type-I diabetes mellitus (Egger, M., et al., 1991; Teuscher, A. and Berger, W.G.,
1987), although others dispute these findings (Colagiuri, S., et al., 1992; Maran, A., et
al., 1993; Tattersall, R.B. and Gill, G.V., 1991). Another study on hormonal counter
regulation during hypoglycemia shows that mean plasma noradrenaline concentrations
and concentrations of pancreatic polypeptide during porcine insulin therapy are
significantly higher than during human insulin therapy. Glucagon, growth hormone,
cortisol, and adrenaline levels in the plasma were identical for both insulin species
hypoglycemia, found that patients on porcine insulin experienced and reported that
their hypoglycemic symptoms were more obvious than when they were on human
insulin. The awareness of the symptoms is believed to be independent from hormonal
counter regulation and does not involve the afferent nerve transmission of auditory and somatosensory systems. There are two other points that should be made in regard to this study. First, while patients were on treatment with human insulin they were more insulin resistant than while on treatment with porcine insulin. Secondly, while patients were on human insulin, and during hypoglycemia-hyperinsulinemia, there was a decrease in insulin effects on adrenergic and noradrenergic neurons as shown by lower catecholamine levels in the peripheral circulation. (Lingenfelser, Th., et al., 1992). Finally, a study of auditory evoked potentials during insulin-induced hypoglycemia showed that human and porcine insulins exert different influences on sensory function. These differences contribute to the distinction between human and porcine insulins on hypoglycemia warning symptoms in patients with diabetes (Kern, W., et al., 1990). The difference between human and porcine insulins on sensory function during hypoglycemia is also supported by a study on visual evoked potential (Kern W., et al., 1994). Thus, there is reasonable evidence to suggest that there are changes in sensory processing during hypoglycemia related to differences between insulin species.

Human insulin, insulin analogues and a variety of different species of animal insulins, have been used in studies of the structural-activity relationships of insulin. Most natural and synthetic insulin analogues exhibit identical metabolic activity relative to porcine insulin. Other insulin species show a range of potencies. Interestingly, there is no partial agonist or competitive antagonist for insulin. However, for some insulin species, their precise roles and their interaction with receptors and effects on other tissues require further study (Gammeltoft, S., 1984).

The binding of human and porcine insulins to the human insulin receptor are generally believed to be identical. A study on the difference in the affinity of human insulin receptors for human, porcine and bovine insulins concludes that: 1) there are
no differences in the relative binding of the human, porcine and bovine insulins to the human insulin receptors in the brain, muscle, and adipocytes. 2) there are no differences in the degradation rates for human, porcine and bovine insulins in homogenates of human autopsy brain (Kotzke, G., et al., 1995). However, in the presence of radioionated monoclonal antibodies to the insulin receptors, human and porcine insulin have been shown to differentially bind to the human insulin receptor. The biological significance of these findings remains unknown (Sesti, G., et al, 1991).

The suspicion that human insulin has different effects on human insulin receptors than bovine or porcine insulins is based on the differences in amino acid sequence among the three insulins. The suggestion is that small changes in the primary amino acid sequence of insulin could introduce changes in insulin structure and function. Certainly, such an effect has been shown for substitutions of B-28 proline and B-29 lysine for the IGF-1 receptor (DiMarchi, R.D., et al., 1994). However, the structural differences between human and porcine insulins causes other differences in chemical characteristics between them. Human insulin B-29 lysine and B-30 threonine have less affinity to Zn$^{2+}$ than porcine insulin B-29 lysine and B-30 alanine (Kitabchi, A.E., et al., 1990). Because of this characteristic, the ability of human insulin to self-associate is less than that of porcine insulin. Thus, the dissociation rate of human insulin from its hexameric form is higher than that of porcine insulin (Brange, J., et al., 1990). Amino acid structural differences also change the hydrophobic nature of insulin. Porcine insulin is more lipophilic than human insulin (Brogden, R.N. and Heel, R.C., 1987).

The higher dissociation rate and less ability to self-associate make human insulin more likely to be in a monomeric form than porcine insulin. Furthermore, its monomeric and hydrophilic natures allow human insulin to be more readily absorbed from subcutaneous depots. Finally, immunogenicity is one of the significant variables
influencing insulin kinetics. Human insulin is less antigenic than porcine and bovine insulins and, thus, more likely to exist in a free, unbound, state. These differences in characteristics are the cause of differences in pharmacokinetic profiles including the distribution and the overall bioavailability of the three insulin species (Davis, S.N. and Granner, D.K., 1996).
1.2 Insulin-Dependent Diabetes Mellitus

1.2.1 Classification and Epidemiology of IDDM

Diabetes mellitus is a syndrome characterized by chronic hyperglycemia and disturbances of carbohydrate, fat, and protein metabolism associated with an absolute or relative deficiency in insulin secretion and/or insulin action. According to the National Diabetes Data Group (1979) and the World Health Organization (1980 and 1985), this syndrome is classified into four distinct categories namely 1) Insulin-dependent diabetes mellitus (IDDM), 2) Non insulin-dependent diabetes mellitus (NIDDM), 3) malnutrition-related diabetes mellitus, and 4) other types of diabetes associated with certain conditions and syndromes. NIDDM is the most common type of diabetes. The prevalence of diabetes in the USA was estimated to be about 3.1% or about 7.8 million in 1993. However, only 300,000-500,000 of these individuals have been diagnosed as having IDDM and very small proportion of other types (La Porte, R.A., et al., 1995). The remainder, about a half of all patients with diabetes, are undiagnosed.

Insulin-dependent diabetes mellitus is defined as an absolute deficiency of insulin which causes the necessity for insulin treatment to control hyperglycemia, classical symptoms of diabetes such as thirst, polydipsia, polyphagia, weight loss, polyuria, and proneness to ketoacidosis (Bennett, P.H., 1994). IDDM is a chronic disease caused by the destruction of the pancreatic β-cells. IDDM is the most common form of diabetes in children and young adults, particularly those of northern European origin. The incidence of IDDM in Canada is estimated to be around 9.0 per 100,000 per year (Tull, E.S and La Porte, R.E., 1992). The highest incidence of IDDM occurs among people in Finland which is 35.3 per 100,000 population per
year, while the lowest is among the Japanese which is about 0.8 per 100,000 population per year. The incidence of IDDM also varies by age. In the year 1990, it was noted that the incidence of IDDM in the United States was 18.2 per 100,000 in the population under the age of 20, but only 9.2/100,000 in the population age 20 and older. The incidence of IDDM in USA also differs among races. Among white Americans the incidence is 12-21 per 100,000 per year, in Hispanics it is 3-15 per 100,000 per year, and in Blacks it is 2-11 per 100,000 population per year. The incidence also varies between males and females (La Porte, R.A., et al., 1995). Other evidence suggests that the risk of Type-I diabetes correlates with the average yearly temperature and the distance from the equator.

1.2.2 Pathogenesis of IDDM

Epidemiological data and evidence from a number of other studies show that the development of IDDM is related to both genetic and environment factors. The genetic susceptibility to IDDM is mediated, in part, by genes in the human leukocyte antigen region that either predispose or protect a person from developing the disease. The presence of human leukocyte antigen DR3 and/or DR4 are believed to be related to the risk of developing IDDM. Conversely, the presence of HLA DR2 is protective. However, the majority of individuals with these disease-associated genes do not develop IDDM (Eisenbarth, G.S., et al., 1994), so that other genes, or, non genetic factors, appear to be important.

The genetic factors in IDDM are well connected to the genes that control the immune system. Evidence shows that IDDM is an autoimmune disease. Antibodies to the pancreatic β-cells can be detected in about 80% patients with IDDM early in the development of the disease and these cause permanent and complete destruction of
insulin secreting cells. There is also evidence that viruses may play a role in initiating the autoimmune process. This is supported by three observations. First, viruses can cause destruction of pancreatic β-cells through direct cytotoxicity to these cells. Secondly, viral infection triggers autoimmunity to the pancreatic β-cells. Thirdly, pharmacological intervention with immunomodulators early in the pathogenesis can halt the progression of the disease, at least in animal models (Atkinson, M.A. and MacLaren, N.K., 1994).

There are relatively few well defined mechanisms that are known to activate autoimmunity in genetically susceptible individuals. Epstein-Barr virus infection, rheumatic fever, and paraneoplastic syndromes have been associated with the development of IDDM. The activation of autoimmune processes stimulates many factors, such as macrophages and lymphocytes, to produce cytokines. This activation, furthermore, distracts β-cells. Immune cells play a role in the process leading to the disappearance of β-cells, although this has not been determined clearly. It is possible that the destruction of β-cells is due to the existence of cytotoxic T lymphocytes specific to one or more β-cell antigens. Another possibility is that β-cells are surrounded by macrophages or natural killer cells (NK cells) which then cause the destruction of β-cells by secreting high local levels of interleukin-1 (IL-1), tumor necrosis factor (TNF) and interferon which are toxic to β-cells. Finally, it is possible that islet cell antibodies initiate complement-dependent or antibody-dependent cellular toxicity. It has been shown that IgG is deposited within the islets shortly after the clinical onset of IDDM (Palmer, J.P. and Lernmark, A., 1990).
1.2.3 Insulin Therapy in IDDM

1.2.3.1 The Objectives of Insulin Therapy

There are three main objectives to the management of diabetes mellitus. The first objective is to preserve the life of the diabetic patient by relieving the symptoms of acute insulin deficiency and hyperglycemia, such as ketoacidosis and hyperosmolar coma. The fact that patients with Type-I diabetes mellitus are insulin-deficient and have practically no β-cell activity, requires that the number one objective of treatment must be the correction of the primary defect through insulin replacement therapy. The second objective of treatment is to avoid the long-term complications of diabetes by establishing and maintaining good metabolic control (Rosenzweig, J.L., 1994). The goal, as supported by the finding of the DCCT (the DCCT Research Group, 1993), is maintenance of euglycemia. However, insulin replacement, even with the most careful adjustment, is empirical and other factors are important in regulating blood sugar control. Thus, insulin therapy should be accompanied by rigorous attention to diet, by reduction and avoidance of obesity and by adequate physical activity. Finally, in fulfilling objectives one and two, one should see that these treatments improve the overall quality of life of diabetic patients, and allow them to have as normal a life as possible. This later objective is most critical in ensuring the success of the former two objectives.

Ideally, insulin treatment should be able to replicate the physiological secretion and actions of insulin in the normal body. This goal may be achieved by one of two basic strategies, namely, conventional insulin therapy and intensive insulin therapy. Conventional therapy consists of once or twice daily injection of intermediate- or long-acting insulin mixed with regular insulin. The intermediate- or long-acting insulin is intended to control basal, non-meal, glucose production, whereas the short-acting
regular insulin is directed at controlling post-prandial glucose excursions. Intensive insulin therapy refers to three or more subcutaneous injections or continuous subcutaneous insulin infusion using an insulin pump (Rozensweig, J.L., 1994). Intensive therapy was recommended by the 1993 report of The Diabetes Control and Complications Trial (DCCT Research Group) for patients with IDDM. Intensive insulin therapy may be the best strategy to control the blood sugar level because it offers the best blood sugar control and the greatest freedom to diabetics to adjust dosing time and dosage in coordination with meals, exercise and other life activities (Rozensweig, J.L., 1994).

1.2.3.2 Short-Acting Insulins

Short-acting insulin has a rapid onset and a short duration of action. It may be given subcutaneously, intravenously or intramuscularly, although from a practical standpoint, the subcutaneous administration is most often used for long-term diabetic control. There are three kinds of short-acting insulin preparations; namely, regular insulin, semilente insulin, and monomeric insulin analogs.

1.2.3.2.1 Regular Insulin

Regular insulin is a crystalline zinc insulin which is widely used to supplement intermediate- and long-acting insulin preparations. It is also the insulin used in infusion pumps because it is freely soluble in water and less likely to crystallize in the tubing during the slow infusion. In Canada, regular insulin is commonly referred to as Toronto insulin and in the United Kingdom it is referred to as soluble insulin. Regular insulin is available in four different preparations from three different species: human,
porcine, bovine or a mixture of bovine and porcine insulin. Porcine insulin and human insulin are believed to have similar potency in controlling blood glucose level. However, human insulin is less antigenic than porcine or bovine insulin. It appears that immunogenicity is one of the significant variables influencing insulin kinetics; it delays the onset of action and also reduces the bioavailability of insulin (Francis, A.J.I., et al., 1985). When antibody reactions do not occur, overall bioavailability of these two types of insulin are the same.

All regular insulin given subcutaneously, regardless the species origin, has an onset of action within 0.3 and 1 hour, peaks within 2-4 hours, and has a duration of action of 5-8 hours. Regular insulin should be injected 30-45 minutes before meals. A tendency for a more rapid initial absorption in human regular insulin than porcine regular insulin has been noted in most studies. Peak concentrations of regular human insulin are 50% higher than porcine insulin (Galloway, J.A., et al., 1982). This difference may relate to the more hydrophilic nature of human insulin than porcine insulin (Brogden, R.N. and Heel, R.C., 1987). The differences in pharmacokinetics may also relate to variations in the interaction between human or porcine insulin, and zinc crystals (Davis, S.N. and Granner, D.K., 1996).

1.2.3.2.2 Semilente Insulin

Semilente insulin has almost the same kinetic profile as regular insulin. However, it has a much longer duration of action. It was originally used in place of regular insulin when the latter was available only in a phosphate buffer which could not be mixed with lente or ultralente insulin. Semilente insulin has become obsolete and is rarely used in the treatment of diabetes today (Skyler, J.S., 1988).
1.2.3.2.3 Lispro Insulin

Lispro insulin is an insulin analogue in which the natural amino acid sequence of the B-chain at positions 28-29 is inverted. Thus, B-28 proline and B-29 lysine are switched to B-28 lysine and B-29 proline. Positions B-28 and B-29 are association sites for insulin aggregation. The changes reduce the capacity of lispro insulin to self-association (Howey, D.C., et al., 1995; Trautmann, M.E., 1994; Galloway, J.A. and Chance, R.E., 1994).

Conventional regular insulin exists in solution as hexamers. Following subcutaneous injections, hexamers break down into dimers and monomers and only the dimers and monomers can leave the subcutaneous tissue to enter the blood stream. The speed of dissociation of hexameric insulin varies among the different insulin preparations. Thus, lispro insulin is more likely to remain in the monomeric state and is less likely to bind zinc in solution. These conditions favor the absorption and distribution of lispro insulin so that its onset of action is faster. This also diminishes the depot-effect in the subcutaneous tissue so that the duration of action is shortened. The addition of zinc into the solution increases the long-term stability of Lispro insulin (Howey, D.C., et al., 1994). Lispro insulin is manufactured by Eli Lilly Company, Indianapolis, Indiana. Insulin lispro has been approved by the United States Adopted Names as an official nonproprietary name for this compound with CAS registration number 133107-64-9 (USAN Council, 1995).

Following the subcutaneous injection of lispro insulin, the time to peak concentration \( (T_{max}) \) is achieved in about 45 minutes, compared to human regular insulin which is about 2 hours. Lispro insulin achieves much higher maximum concentration \( (C_{max}) \) than human regular insulin, 698 ± 227 pM compared to 308 ±
132 pM. The half life ($t_{1/2}$) of subcutaneous lispro insulin (46 minutes) is also much faster than subcutaneous human regular insulin (82.5 minutes), but mimics the $t_{1/2}$ of intravenous human regular insulin (46.3 minutes) more closely. However, the overall area under curve (AUC) of the two insulins is equal. Mixing lispro with zinc will significantly change the kinetics of lispro but it still has a significantly shorter onset $T_{\text{max}}$, higher $C_{\text{max}}$ and faster $t_{1/2}$ than human regular insulin (Torlone, E., et al., 1994).

The pharmacokinetic properties of lispro suggest that this insulin may be injected immediately prior to the meal. Also, the short duration action of lispro insulin reduces the possibility of iatrogenic hypoglycemia during exercise later after the meal. The potential benefits of lispro may improve the compliance of diabetics pursuing intensive insulin therapy because of the greater flexibility of lispro insulin to be fitted into the diabetic lifestyle (Tuominen, J.A., et al., 1995).

**1.2.3.3 Intermediate-Acting Insulins**

The need for longer-acting insulin to control fasting glucose production has been recognized since the introduction of insulin therapy. The pharmacological basis for prolonging the biological action of insulin was to decrease the rate of insulin absorption. This was done by complexing insulin with various proteins or by changing the physical form of insulin by altering the zinc content in the buffer. Hagedorn and co-workers in 1936 introduced the first insulin preparation to have a prolonged action. This prolonged-acting insulin was called protamine insulin. They used protamine, obtained from fish sperm, complexed with insulin. Unfortunately, protamine insulin was not a stable preparation, so that it was never used widely (Skyler, J.S., 1988).
Presently, there are two intermediate-acting insulin preparations that are frequently used, neutral protamine Hagedorn insulin (NPH) and lente insulin. Neutral protamine Hagedorn (isophane insulin suspension) was introduced by Krayerbuhl and Rosenberg in 1946, by complexing insulin and protamine at a neutral pH in a stochiometric ratio and in the presence of a small quantity of zinc. Lente insulin is a mixture of 30% semilente with 70% ultralente insulin. These insulins will dissolve slowly when administered subcutaneously, so that they have a longer duration of action than regular insulin alone. Intermediate-acting insulins are normally given once or twice a day. They have an onset of action of about 1-2 hours, a time to peak effect of 6-12 hours, and a duration of action of approximately 18-26 hours (Skyler, J.S., 1994).

Bovine, porcine and biosynthetic and semisynthetic human insulin have been marketed as intermediate-acting insulin formulations. As with shorter acting insulins, the pharmacokinetic properties of intermediate-acting insulins are slightly different between different species. For example, human insulin has a faster onset of action and a shorter duration of action than porcine insulin. This difference may relate to the more hydrophilic nature of human insulin than porcine insulin, or to the delaying effects of insulin antibodies. Differences in pharmacokinetics may also relate to the variation of interaction between human or porcine insulin, with protamine and zinc (Davis, S.N. and Granner, D.K., 1996). Because human insulin has a shorter time course of action, if it is given immediately before dinner, the treated diabetic patients may experience early next morning insulin deficiency and morning hyperglycemia. When lente insulin is mixed with regular insulin, after several hours some of the regular insulin will form a complex with protamine or Zn\(^{2+}\). This slows the absorption of the shorter acting insulin component. Such an interaction does not occur when short-
acting insulin is mixed with NPH insulin. This advantage has made NPH the most widely used intermediate-acting insulin in the world (Davis, S.N., et al., 1991).

1.2.3.4 Long-Acting Insulins

Protamine zinc insulin was the first stable insulin with prolonged action. It was developed by Scott and Fisher in 1936. The other long-acting insulin is ultralente insulin. Both insulins have a very long delay in onset of action, a prolonged duration and a relatively peakless activity profile. These insulins are administered subcutaneously to provide a low basal concentration of insulin around the clock. Long-acting insulin lowers blood glucose over a period of 28-36 hours. Onset of action occurs within 4-6 hours with a peak action in 8-24 hours. Since the half-lives of these two insulins are very long, it is difficult to determine the optimal dosage. Several days of treatment is required to achieve a steady-state concentration of circulating insulin. It may be given once or twice daily and adjusted according to the blood glucose concentration (Skyler, J.S., 1988).

When long-acting insulins are given with rapid-acting insulins, regular insulin should be given by separate injection. If regular insulin is mixed with ultralente or PZI, the activity of the rapid-acting insulin may be diminished by the formation of a complex between regular insulin and Zn$^{2+}$ and/or protamine (Bennet, D.R., et al., 1992). This condition is minimized when the mixture of insulin is injected immediately after the mixing (Rosenzweig, J.L., 1994).

Ultralente insulin shows a species-related feature. Ultralente human insulin clearly produces an insulin peak within 15 hours after injection compared to beef/pork ultralente which is virtually peakless. Moreover, the peak concentration (120-180 µ
U/ml) is almost three times higher than that of other insulin species (60 μU/ml) or 12-36 times higher than that of non-diabetic basal insulin levels (Seigler, D.D., et al., 1991). This high human insulin level persists for almost 30 hours. The explanation for these effects is the same as that mentioned for other human insulin formulations (Davis, S.N. and Granner, D.K., 1996).

1.2.3.5 Insulin Delivery Systems

There are several ways to deliver insulin into the body, including subcutaneous injection, subcutaneous infusion, intravenous, nasal, and intramuscular injection. Insulin is administered intravenously in short-term emergency situations such as during treatment of ketoacidosis and intraoperatorically. The other purpose of intravenous administration is to mimic the normal meal-stimulated secretion of insulin. However, this approach is too invasive to have long-term benefit. When intravenous access is limited, insulin may be given intramuscularly. Nasal insulin administration may appear to offer promise as a non-invasive method to control metabolism around meal time. However, the absorption of insulin through the nasal mucosa is incomplete and unpredictable. Besides, the long-term safety of nasal administration is not established (Galloway, J.A. and Chance, R.E., 1990).

1.2.3.5.1 Subcutaneous Insulin Injection

Subcutaneous injection is the most popular way of administering insulin. With this method, there is always a depot of insulin, particularly long-acting insulin to guard against insulin deficiency. The disadvantage of this method is that there may be continuing high levels of insulin and so there is a risk of low blood sugar when
carbohydrate intake is curtailed. This method is also characterized by variability of insulin absorption into the circulation. There are several factors that may modify the rate of insulin absorption from its subcutaneous depot. These include the site of injection, subcutaneous blood flow, regional muscular activity at the site of injection, the volume and concentration of the injected insulin, the insulin formulation and species, and the depth of injection. Massage, hot water, change in body position and exercise, all increase subcutaneous blood flow and increase the rate of insulin absorption. These phenomena are the cause of daily problems for diabetics in adjusting time to time insulin and glucose requirements (Koivisto, V.A. and Felig, P., 1978; Galloway, J.A., et al., 1981).

Sites on the abdominal wall, buttock, anterior thigh and dorsal arm are usually chosen as the location for subcutaneous insulin administration. The insulin absorption rate among these different areas varies greatly. The abdominal wall is the preferred site of insulin injection, since insulin absorption from this site is about 20-30% faster than that of other sites. It is also less subject to changes in temperature and muscular activity. Rotation of injection in the same area may also reduce variability in the insulin absorption rate (The American Diabetes Association, 1991).

Prolonged-acting insulins are generally more affected by factors that alter the rate of absorption than shorter-acting insulins. Because short-acting insulin minimizes the amount of insulin depot and has a shorter and more predictable time course following injection, three daily injections of regular insulin at meal time and one injection of intermediate-acting insulin before bedtime, have been shown to produce a better glucose-insulin control in diabetic patients. Still, this method of insulin therapy is unpredictable and causes an increase in the incidence of hypoglycemia diabetic patients (The DCCT Research Group, 1993).
Continuous Subcutaneous Insulin Infusion

Continuous subcutaneous insulin infusion (CSII) using an insulin pump has been introduced to overcome the burden of multiple injections of insulin. This method uses only regular insulin or lispro insulin in a special buffer. The dosage of the intermittent insulin boluses can be more closely related to the meal and the level of the ambient blood sugar. Also, a peakless low level of basal insulin can be easily set. In this way, insulin pump therapy mimics the normal physiologic pattern of insulin secretion. However, this method of insulin delivery requires frequent blood sugar monitoring and is suitable only for patients who are interested in intensive therapy (Davis, S.N. and Granner, D.K., 1996).

The method can reduce the incidence of iatrogenic hypoglycemia significantly, because the insulin depot is minimal. Indeed, lack of the subcutaneous insulin depot means that insulin deficiency develops rapidly when the pump is accidentally interrupted due to mechanical or electrical failure, and ketoacidosis is a risk. Infection from the infusion apparatus is a problem of pump therapy (Mecklenburg, R.S., et al., 1984), but is easily managed by regular tubing changes and attention to aseptic technique (Mecklenburg, R.S., 1989). The insulin pump is the only strategy that has the potential to produce a constant low level of insulin throughout the non-meal periods. However, the use of a pump is not necessarily free from hyperinsulinemia. During insulin pump therapy, hyperinsulinemia has been detected between 1:00 am and 7:00 am. In this instance, hyperinsulinemia is the result of the cumulative effects of the preceding dinner bolus infusions (Galloway, J.A. and Chance, R.E., 1990).

At an equal insulin daily dosage, insulin pump therapy has been shown to control daily blood glucose as good as conventional therapy or multiple injection intensive therapy. Insulin pump therapy has not been shown to be superior to
intermittent multiple injection therapy (Reeves, M.L., *et al.*, 1982); long-term, glycemic control is no better than with either conventional therapy or multiple injection intensive therapy. Interestingly, even though human insulin has a shorter duration of action and a faster onset of action, differences in insulin/glycemic control between human regular insulin and porcine regular insulin during insulin pump therapy have not been detected (Sonnenberg, G.E., *et al.*, 1982).

1.2.3.6 Daily Insulin Dosage

A population study of patients with IDDM shows that, on average, diabetic patients inject 0.6 to 0.7 U of insulin/kg of body weight per day with a range of dosages between 0.2 to 2 U/kg/day. Insulin secretion, on the other hand, in healthy non-diabetic individuals averages 18 to 40 Units per day or 0.2 to 0.5 Unit/kg of body weight. This means that diabetic patients require a much higher dosage of insulin, compared to non-diabetic individuals. This occurs because insulin is absorbed from subcutaneous tissues directly into the general circulation, thereby bypassing the liver. This has two important consequences. First of all, systemic administration obviates the 50% first-pass metabolism of insulin by the liver. Secondly, it relieves that organ of the suppressive effects of high insulin concentrations on hepatic glucose output. Daily insulin requirements may also be used to predict the endogenous production of insulin in Type-I diabetes mellitus patients. Patients who require insulin more than 0.5 U of insulin/kg body weight per day are likely to have a complete destruction of pancreatic β-cells (Davis, S. N., and Granner, D. K., 1996).

The daily insulin dosage can be divided into basal and postprandial doses. The basal insulin requirement is usually met by the intermediate- or long-acting insulin injection. The postprandial insulin requirements are usually met by shorter-acting
insulin. The basal insulin dosage is about 40-60% of the total daily insulin requirement. The basal dosage is adjusted according to the need to suppress hepatic output of glucose which is usually reflected by the morning fasting blood glucose levels. Postprandial insulin requirement is measured by pre-meal blood-glucose levels. The basal and postprandial insulin requirements are adjusted up and down by trial and error (Davis, S.N and Granner, D.K., 1996).

Subcutaneous insulin injection may be given two, three or four times a day. The twice a day regimen or split-mixed regimen is the most frequent method adopted by diabetics. With this method, diabetics are given mixed short- and intermediate-acting insulin before breakfast and before dinner. A third insulin injection may be given as a pre-noon injection of short-acting insulin. Insulin requirements increase in the early morning hours. This is the so called, “dawn phenomenon” (Nolte, M.S., 1992). When the morning fasting blood glucose is not well controlled by the evening intermediate acting insulin, a fourth insulin injection is sometimes given at bedtime.

1.2.3.7 Advantage And Disadvantage of Insulin Therapy

The natural history of IDDM has changed dramatically since the introduction of insulin into clinical medicine in 1921. Before insulin, 50% of patients died within 20 months of being diagnosed with IDDM. Only 10% of them survived more than 5 years and these usually passed away from ketoacidosis. Only a few juvenile-onset diabetics survived to the age of 20 years. The introduction of insulin prolonged the life expectancy of patients with IDDM. However, half of them still die before the age of 55 years. Thus, while insulin treatment has dramatically improved the survival of patients with IDDM, they still have a median life expectancy that is 20 years less than that of the general population (Krolewski, A.S., and Warram, J.H., 1994).
The Diabetes Control and Complications Trial published in the New England Journal of Medicine, September 1993, established the conclusion that effective glycemic control will delay the development and progression of diabetic retinopathy, nephropathy, and neuropathy and reduce the incidence of microvascular disease. Moreover, intensive insulin therapy was shown to provide significantly better insulin/glycemic control in Type-I diabetic patients than conventional therapy. During the nine year observation period, mean HbA1c values of the intensive insulin therapy group were 7.23 ± 0.96 % versus 9.11 ± 1.31% in the conventional insulin therapy group (Crofford, O.B., 1995). Continuous subcutaneous insulin infusion showed almost equal level of insulin/glycemic control as intensive insulin therapy (Bischof, F., et al., 1994). A lower HbA1c value was related to a 50% reduction in the incidence of retinopathy, a 69% reduction in the incidence of diabetic neuropathy and a 34 to 43% reduction of the risk of microalbuminuria (The DCCT Research Group, 1993).

However, intensive insulin therapy has inherent limitations. Besides the greater risk of hypoglycemia and the associated costs of multiple injections and close blood sugar monitoring (Galloway, J.A. and Chance, R.E., 1994), current insulin therapy is incapable of replicating the natural pattern of insulin action. In a non-diabetic individual, insulin secretion is characterized by a basal and a meal-stimulated insulin phase. In the meal-stimulated phase, insulin concentrations increase from 5 to 15 μU/ml up to 60 to 80 μU/ml within a few minutes after a meal and last 30 minutes in duration. This results in the immediate disposal of glucose and other nutrients from the general circulation into peripheral tissues. Within 2-4 hours, insulin secretion returns to its basal levels. Thus, the dynamics of normal insulin secretion are absent with conventional insulin replacement therapy. Specifically, insulin therapy fails to replicate the peak insulin effects that occur following meals. Secondly, insulin therapy fails to replicate a normal basal insulin secretion pattern between meals and through
the night. Thirdly, insulin therapy always produces systemic hyperinsulinemia. And finally, there are commonly large intraindividual variations in a patient’s response to the same dose of insulin injected at the same site (Rosenzweig, J.L., 1994).

The above problems pose certain risks to patients with IDDM. On average, iatrogenic hypoglycemia occurs at a rate of one episode per week in patients practicing conventional therapy. The number of hypoglycemic episodes increases sharply by about three fold in IDDM patients on intensive insulin therapy (The DCCT Research Group, 1993). Moreover, 25% of patients with IDDM suffer at least one episode of severe, temporarily disabling, hypoglycemia, and in 4% of patients, death may be attributed to hypoglycemia. Thus iatrogenic hypoglycemia causes mortality, and recurrent physical and psychosocial morbidity. This factor limits the management of insulin therapy for IDDM (Widom, B. and Simonson, D.C., 1994).

Other factors may not be as obvious, but may pose even greater risks. Regular human insulin when injected subcutaneously causes postprandial iatrogenic hyperinsulinemia. The action of regular insulin formulations lasts for about 6-8 hours postprandially, compared to 2-4 hours for endogenously secreted insulin in non-diabetic individuals. This phenomenon is aggravated even more so by the administration of longer-acting insulins that are needed during non-meal conditions. Intermediate- and long-acting insulins have been shown to cause hyperinsulinemia. Thus, normal basal insulin levels cannot be achieved by administering subcutaneous insulin since the administration of intermediate- and long-acting insulin results in elevated serum insulin and prolonged peaks or plateaus of hyperinsulinemia that will persist while prolonged-acting insulin is administered. Chronic hyperinsulinemia is related to down regulation of insulin receptors causing insulin resistance in the periphery (Galloway, J.A. and Chance, R.E., 1994). Hyperinsulinemia is also believed
1.3 Sleep and Wakefulness

Sleep and dreams together with love and conflict have been the subject of art and science since the beginning of human history. Indeed, sleep behavior has attracted the attention of some of the world's greatest thinkers from Aristotle and Hippocrates to Freud, Pavlov and others. The definition of sleep has changed from time to time, simultaneously with the development of behavioral science. However, one definition proposed in 1834 by Robert MacNish, a member of the Faculty of Physicians and Surgeons of Glasgow, pre-dated the modern era of sleep understanding. As he explains in his book, The Philosophy of Sleep, "sleep is the intermediate between wakefulness, the active state of the animal and intellectual functions, and death, total suspension of animal and intellectual functions". This definition points out the duality of sleep; sleep as an active state as well as sleep as a passive state. Today, sleep is defined as a reversible behavioral state of perceptual disengagement from, and unresponsiveness to, the environment (Dement, W.C., 1994). The differences between sleep and wakefulness are characterized by a set of three cardinal physiological parameters; namely, brain wave activity (electroencephalogram, EEG), eye movement, and muscle tone. According to the configuration of these three cardinal physiologic parameters, sleep can be further differentiated into two states, non-rapid eye movement (NREM) sleep and rapid eye movement (REM) sleep. The sleep dichotomy is characterized by the variability in brain and skeletal muscle activities. In NREM sleep it is the inactive brain in a moveable body, whereas in REM sleep it is the activated brain in a paralyzed body. (Carskadon, M.A. and Dement, W.C., 1994).
1.3.1 Normal Sleep

Normally, a human enters sleep through NREM sleep. NREM sleep is divided into four stages according to the appearance of the brain waves on the electroencephalogram: stage-1 sleep, stage-2 sleep, stage-3 sleep and stage-4 sleep. These stages are parallel to the depth of the sleep. Stage-1 and stage-2 sleep together are called light sleep, which usually has a low threshold for arousal. Stage-3 and stage-4 sleep are called deep sleep or slow-wave or delta sleep, in which the arousal threshold is usually quite high. In healthy, young adults, 75 - 80% of the total sleep is spent in NREM sleep. Typically, this is comprised of 2-5% stage-1 sleep, 45-55% stage-2 sleep, 3-8% stage-3 sleep, and 10-15 % stage-4 sleep. REM sleep is characterized by three parameters; namely, an active EEG, muscle atonia, and episodic bursts of rapid eye movement. Generally, REM sleep does not occur until 70 minutes or longer after the onset of sleep. REM sleep usually comprises about 20-25% of sleep and occurs in four to six discrete cycles. REM sleep can be further differentiated into tonic and phasic REM sleep, based on the period of eye movement. REM sleep is tonic when the eyes are quiescence and phasic when the eyes are restless (Carskadon, M.A. and Dement, W.C., 1994).

Normally, NREM is the state where sleep starts. As a subject enters sleep, the EEG pattern in the occipital region changes from a dominant alpha activity (8-13 cycle per second) to a low voltage, mixed-frequency pattern which is characteristic of stage-1 sleep. Slow and asynchronous eye movements usually precede and accompany these changes, but disappear as sleep consolidates. Stage-1 sleep, generally lasts 1-7 minutes and then re-appears as a transitional stage later in the night (Rechtschaffen, A. and Kales, A., 1968).
The onset of stage-2 sleep is signaled by the appearance of sleep spindles and K-complexes on the EEG. In the first cycle of sleep, stage-2 continues for about 10-25 minutes. As this progresses, there is an increasing occurrence of high-voltage slow wave activity on the EEG. This activity is a sign of progression of the depth of the sleep. Eventually, when the high-voltage slow wave activity occupies more than 20% of the electroencephalogram activity, stage-2 is replaced by stage-3 sleep (Rechtschaffen, A. and Kales, A., 1968).

Stage-3 sleep is defined by the EEG as delta wave activity dominating between 20-50% of sleep activity with the frequency of 2 cycles per second and an amplitude greater than 75 µV. In the first cycle of sleep, stage-3 usually occurs for only a very short period and is merely a transitional stage. As more and more slow wave sleep (SWS) activity predominates (i.e., more than 50% of sleep), the sleep continues into the deepest stage, that is, stage-4 sleep. SWS continues about 20-40 minutes or longer. When the first burst of SWS is gone, before the conclusion of the first sleep cycle by a short episode (1-5 minutes) of REM sleep, a series of body movements and stage-2 sleep occur (Rechtschaffen, A. and Kales, A., 1968).

Overall, sleep contains 3-5 cycles where NREM and REM sleep emerge alternately. On average, the first NREM-REM sleep cycle is 70-100 minutes in length. The length of the other cycles is about 90-120 minutes. SWS predominates in the first one third of the night. Thus, the SWS episodes occupy less and less time as the night progresses and then disappear altogether. Afterwards, the NREM sleep portion of the episode is mostly occupied by stage-2 sleep. Alternatively, REM sleep is usually distributed toward the last one third of the night. NREM and REM sleep are always distributed in balanced proportions across the night during sleep. REM sleep distribution is believed to be controlled by a circadian oscillator, and NREM
sleep by prior wakefulness, initiation, the length of previous wakefulness, and the
time course of sleep (Carskadon, M.A. and Dement, W.C., 1994).

Most young adults report that they sleep about 7.5-8 hours on weekday nights
and slightly longer on weekend nights (Johns, M.W., et al., 1971). The normal length
of sleep is not established yet, since the need for sleep very much depends on a large
number of factors and is very individualized. Genetics, age, sex, well being, and
medication are some of the factors that affect the need for sleep. Prior sleep history, is
also an important factor in determining the need for sleep. Sleep loss in one or more
nights will change the sleep pattern (Carskadon, M.A. and Dement W.C., 1994). On
the first recovery night, SWS will predominate the pattern, while REM sleep may
show a rebound in the second recovery night (Boberly, A.A, et al., 1981). Chronic
sleep deprivation can cause a peculiar sleep pattern and also a premature REM sleep
onset (Carskadon, M.A. and Dement W.C., 1994).

In sleep research, there is one very important factor that may affect sleep
distribution and pattern, and that is the first-night effect (Agnew, et al., 1966). The
first-night effect is a disruption of normal sleep which is not related to the previous
sleep history. It is related to the laboratory setting and a subject’s adaptation to that
setting (Browman, C.P., and Cartwright, R.D., 1980). Quietness, cleanliness and a
nice setting of the bedroom in the laboratory, the friendliness and the openness of the
researcher, are very important factors in the adaptation of subjects to the sleep
environment (Coble, P., et al., 1974). Observations over four consecutive nights of
sleep in a sleep laboratory show that first-night effects are characterized by delayed
REM onset latency and a reduced total amount of REM episodes, prolonged
wakefulness after sleep onset time and an increasing number of stage changes. NREM,
especially SWS, is usually not altered dramatically (Agnew, H.W.Jr., et al., 1966).
The sleep pattern of subjects studied in a well designed laboratory setting do not
show the above characteristics, except that they may have a delayed REM sleep onset (Coble, P., et al., 1974).

1.3.2 Neurobiology of Sleep and Wakefulness
1.3.2.1 Neurobiology of Wakefulness

Visceral, somatic and special sensory input are necessary for the initiation and maintenance of wakefulness. These sensory input systems have collaterals to the brain stem reticular formation, which extends from the medulla to the midbrain. Afterwards, the sensory inputs are transmitted to the forebrain and cortex cerebri via nonspecific thalamocortical projection systems and via the subthalamus, hypothalamus and basal forebrain areas. Studies, where the cell bodies of the posterior hypothalamus are destroyed by neurotoxic lesions, show some decrease in wakefulness. The lesions of cells in the basal forebrain, which project to the cortex, also result in a loss of cortical activation of wakefulness. Neurons in the ascending reticular formation system, thalamus, lateral hypothalamus and basal forebrain have a higher spontaneous firing rate during wakefulness, than during SWS. Thus, wakefulness is believed to be associated with activation of the ascending reticular formation (Jones, B.E., 1994).

The ascending reticular activating system overlaps extensively with the sympathetic nervous system in the midbrain and hypothalamus. The involvement of catecholamines in the sleep-awake cycle is established by the finding of catecholamine-containing neurons in the brain stem reticular formation (Jones, B.E., 1972). Norepinephrine neurons in the locus ceruleus of the brain stem are projected to the forebrain along reticular formation pathways. From the basal forebrain, the norepinephrine-containing neurons project to the entire cortical area. Norepinephrine
neurons are mainly involved in cortical activation. Locus ceruleus neurons are very active during tense and stressful conditions. Relaxed wakefulness slows down the firing rate, SWS slows down further the firing rate, and REM sleep causes complete cessation of neuronal firing in the locus ceruleus neurons (Jones, B.E., 1994).

Several lines of evidence suggest that central catecholamines are involved in arousal and wakefulness. Depletion of monoamines by reserpine produces inactivity and sleepiness. In contrast, the release of norepinephrine and dopamine by amphetamine produces an intense arousal and prolonged vigilance. This pattern of mental activity is also produced by inhibition of catecholamine reuptake by cocaine and inhibition of catecholamine metabolism by monoamine oxidase inhibitors, such as tranylcyromine. On the contrary, inhibition of catecholamine synthesis decreases wakefulness (Jones, B.E., 1994).

Dopamine-containing neurons are located in the midbrain, especially in the substantia nigra, ventral tegmental area, hypothalamus and subthalamus. Dopamine neurons in the substantia nigra project to the hypothalamus and neostriatum, and together with the ventral tegmental dopamine neurons, innervate the basal forebrain, the nucleus accumbens, the septal area, the amygdala, and the frontal cortex. These pathways mediate the activity of dopamine on motor and limbic systems whose effects are obvious on behavioral arousal and responsiveness, but not on the cortical activation of wakefulness, per se. (Jones, B.E., 1994).

Cholinergic cell bodies, such as found in the pedunculopontine tegmentum and the lateral dorsal tegmentum, form a large collection of cholinergic cells in the pontine tegmentum. From these cell bodies, cholinergic axons project along the caudal mesencephalic-oral pontine reticular formation, into the forebrain, especially into the thalamic nuclei, basal forebrain, frontal cortex and then widely disperse into the entire cortex. Discrete neurotoxic lesions of the mesopontine cholinergic neurons do not
produce any obvious reduction of cortical activity or wakefulness. Massive loss of cholinergic innervation of the cerebral cortex and the degeneration of cholinergic neurons of the basal forebrain, such as seen in Alzheimer disease, lead to some changes in sleep pattern especially loss of stage-3 sleep, stage-4 sleep, sleep spindles and REM sleep. However, cholinergic muscarinic blockers, such as atropine, produce a decrease in alertness due to deprivation of cortical activation. Conversely, cholinesterase inhibitors, such as neostigmine, enhance vigilance by prolonging the action of acetylcholine at cholinergic postsynaptic receptors. Thus, cholinergic neurons are very important in modulating wakefulness and cortical activation (Jones, B.E., 1994).

The other modulator of neural activity that may be involved in wakefulness is glutamate, an excitatory amino acid neurotransmitter. Glutamate is contained within large projection neurons scattered throughout the brain stem reticular formation, the thalamus and the cerebral cortex. Stimulation of the midbrain reticular formation evokes cortical activation which is associated with the release of glutamate in the pyramidal cells of the cerebral cortex (Jones, B.E., 1994).

Intraventricular administration of histamine causes arousal. The effects of histamine on wakefulness are related to the endogenous activity of histamine-containing neurons in the posterior hypothalamus. Therefore, inhibition of the activities of these neurons by antihistamines produces a sedative action. Other chemicals are suspected to be present in the cerebrospinal fluid as wake-promoting factors. These factors appear to be neuropeptides which are co-localized in neurons with catecholamines or acetylcholine. Other neuropeptides, such as substance P, corticotrophin-releasing factor, thyrotropin-releasing factor and vasoactive intestinal peptide, also cause behavioral arousal associated with enhanced and prolonged cortical activation (Jones, B.E., 1994).
1.3.2.2 Neurobiology of NREM Sleep

Certain types of sensory input such as warmth and satiation activate sleep-inducing neurons which reduce the cyclic activity of the reticular activating formation, modulate cortical EEG activation through the forebrain, and promote cortical slow wave activity. Serotonin-containing neurons in the raphe, are important in diminishing sensory input and inhibiting motor output to promote sleep onset. Sleep-inducing neurons are localized in the lower brain stem reticular formation, the solitary tract nucleus, the rostral hypothalamus, and the preoptic and basal forebrain. Therefore, total destruction of the raphe nuclei produces total insomnia in the cat, while subtotal lesioning of the raphe nuclei is associated with a decrease in the amount of SWS. The sleep-inducing neurons extensively overlap with the autonomic nervous system, particularly the parasympathetic regulatory system (Jones, B.E., 1994).

Serotonin plays a critical role in the initiation and maintenance of sleep, and in modulating the depth of sleep. Serotonin restores SWS and REM sleep activities in insomniacs (Jones, B.E., 1994). An L-tryptophan free diet lengthens sleep onset latency but does not affect the amount of SWS (Bhatti, T., et al., 1995). However, an L-tryptophan enriched diet, shortens the sleep onset latency, augments the brain serotonin level and increases the amount of slow-wave sleep (Hartmann, E., and Spinweber, C.L., 1979). It is generally accepted that L-tryptophan has hypnotic effects and increases delta sleep (Gillin, J.C., et al., 1995). The involvement of serotonin in generating SWS is still unclear since pharmacological stimulation of serotonin receptors shows the reverse effects or inconsistencies. Serotonin dynamics are very complex because serotonin neurotransmission involves at least 7 types of receptors (Burkhalter, A., et al., 1995). Ipsapirone, a 5HT1A agonist, does not affect the amount of SWS in humans (Ruiz, C., et al., 1995). Other studies in rats show that
the 5HT₁A agonist, buspirone, or the 5HT₁B agonist, RU 24969, reduce SWS activity. On the other hand, ritanserine and risperidone, two 5HT₂ antagonists, increase SWS in human volunteers (Dugovic, C., et al., 1991).

Gama-aminobutyric acid (GABA) is known to have a role in sleep. Benzodiazepines show sedative and hypnotic effects due to the enhancement of the postsynaptic action of GABA through binding to and alteration of GABA receptors. Other drugs that increase the affinity and the binding of GABA in the brain, such as barbiturates, also have sedative and hypnotic effects. Enhancing the GABA level in the brain can amplify the SWS activity. GABA containing neurons are distributed throughout the brain stem, diencephalon, and forebrain. From the thalamus, hypothalamus and forebrain, these neurons project to the cerebral cortex where they make inhibitory. Lesions of the reticular formations increase GABA release in the cortex and activates the sleep EEG. GABA also exists in interneurons in the cortex cerebri and these neurons provide most of the inhibitory influence during SWS (Jones, B.E., 1994).

Another neurotransmitter that increases SWS activity is adenosine. Blocking adenosine receptors produces a central stimulation, as is shown by the ingestion of coffee (Jones, B.E., 1994). Also, glutamate acts on N-methyl-D-aspartic acid (NMDA) receptors and has been shown to be involved in the generation of spontaneous burst activity of cortical neurons during stage-3 and 4 sleep. Administering an NMDA agonist increases cortical burst duration and the number of spikes per burst, while an NMDA antagonist suppresses spontaneous burst activity of layer V cortical neurons during SWS (Armstrong-James, M., and Fox, K., 1988).

Intraventricular injection of the cerebrospinal, or brain extract removed from sleep-deprived animals or from animals in the sleep-intense part of the cycle, promotes sleep, particularly during the active period of the day. The isolation and
characterization of cerebrospinal fluid-born sleep-inducing factors are incomplete. However, somatostatin and opiate peptides are known to be two of these factors. Somatostatin acts as a central nervous system depressant. The intraventricular injection of somatostatin produces analgesia and akinesia, depresses EEG activity, and also potentiates barbiturate-induced anesthesia. Somatostatin-containing neurons are located in the raphe nuclei, the solitary tract nucleus, and in certain neurons, somatostatin is co-localized with GABA. Other peptides, such as the opiates, produce analgesia, sensory anesthesia, and also influence the sleep-wake state. Opiates do not increase the amount of SWS, nonetheless, they can be very important in the onset of the sleep through the effects on sensory modulation and analgesia. Opiates are distributed widely in all parts of the brain, including the solitary tract nucleus and the preoptic area. In raphe nuclei, opiates are also co-localized with serotonin (Jones, B.E, 1994).

Insulin, cholecystokinin and bombesin are other sleep-inducing substances. Food ingestion increases the release of cholecystokinin and bombesin, which eventually induce SWS. The main actions of these two hormones are to regulate satiety and to induce sleep by direct actions on the circumventricular organs or indirectly via vagal afferents to the solitary tract nucleus (Jones, B.E, 1994).

Finally, sleep may play preventive and therapeutic role in response to certain infectious diseases. This idea was originally brought to medical attention by Hippocrates who advised his patients to follow the natural habit of sleep to cure their illnesses. Sleep deprivation in laboratory animals appears to put an animal at risk for infections. Conversely, healing may be preceded by an enhancement of sleep. Moreover, sleep-awake activity may relate to the activity of natural killer cells (NK cells) and to the concentration of certain immune parameters, especially interleukin-1 (IL-1). The IL-1β, IL-1 receptor antagonist (IL-1RA) and IL-1 receptors are found in
the normal brain (Krueger, J.M. and Toth, L.A., 1994). IL-1 increases NREM sleep and decreases REM sleep. A very high dose of IL-1 inhibits both NREM and REM sleep. Substances that inhibit the actions, the release or the production of IL-1, such as α-melanocyte-stimulating hormone, corticotrophin-releasing hormone, glucocorticoids and prostaglandines E\textsubscript{2}, also inhibit sleep (Krueger, J.M. and Obál, F.Jr., 1993). IL-1 affects sleep indirectly through its influence on the release of growth-hormone releasing-hormone (GHRH). Anti-GHRH blocks IL-1-induced NREM sleep. Growth hormone (GH) release is associated with NREM sleep, however, the GH level is not well paired with NREM sleep. Furthermore, GHRH induces excessive NREM sleep and anti-GHRH inhibits normal and rebound sleep after deprivation (Krueger, J.M. and Obál, F.Jr., 1993). IL-1 and other cytokines also induce the synthesis of nitric oxide (NO). The inhibition of the NO production by arginine analogues, greatly suppresses sleep. IL-1β directly alters the firing pattern of the hypothalamus, and activates hypothalamic adrenergic, dopaminergic and serotonergic neurons (Kapas, L., et al., 1994). In the hippocampus, IL-1β also increases synaptic inhibition. Tumor necrosis factor (TNF), another cytokine, also affects sleep similar to IL-1. TNF induced NREM sleep, enhances the delta wave amplitude during SWS, and inhibits REM sleep. Thus, anti-TNF antibodies inhibit normal sleep. Interferon (IFN), another interesting cytokine, causes excessive sleepiness and fatigue. IFN induces REM sleep in monkeys and NREM sleep in the rabbit (Krueger, J.M. and Toth, L.A., 1994). The specific mechanisms that differentiate these various cytokines are not well understood.
1.3.2.3 Neurobiology of REM sleep

Anatomical studies suggest that the midpons is necessary for REM sleep. Destruction of the caudal and/or oral pontine tegmentum, including the lateral dorsal tegmental and pedunculopontine tegmental nuclei, markedly reduces or eliminates the amount of REM sleep. Cholinergic neurons in the lateral dorsal tegmentum and pedunculopontine tegmentum activate REM sleep, while adrenergic neurons in the locus ceruleus and dorsal raphe nuclei turn off REM sleep. Adrenergic neurons are at their lowest activity preceding or during the REM episode, whereas cholinergic activity becomes uninhibited and is very active during episodes of REM sleep (Jones, B.E., 1994).

The muscle atonia that occurs during REM sleep is mediated by the peri locus ceruleus non-aminergic neurons. These neurons project through the tegmentoreticular tract to the bulbous magnocellularis, where they excite neurons within the reticular formation, and then finally, through the ventrolateral reticulospinal tract to the spinal motor neurons where they induce muscle atonia by hyperpolarizing the cells (Gillin, J.C., et al., 1995).

Ipsapirone, a 5HT₁A agonist, significantly inhibits REM sleep both in patients with affective disorders and in normal control subjects (Ruiz, C., et al., 1995). Similar effects have also been observed in rats with the partial 5HT₁A agonist, buspirone (Lerman, J.A., et al., 1986). A low dose of buspirone initially inhibits sleep in rats, but subsequently causes a rebound phenomenon (Dugovic, C., et al., 1991). However, a study reveals that buspirone has no sedative or hypnotic properties in anxiety patients (De Roeck, J., et al., 1989). RU 24969, an 5HT₁B agonist, similarly abolishes paradoxical sleep in rats. Furthermore, the 5HT₂ agonist also produces a
dose-related deficit in both SWS and REM sleep in rats. Ritanserin, a 5HT$_2$ antagonist, specifically increases SWS in human volunteers at the expense of REM sleep. Both 5HT$_2$ receptor agonists and antagonists and any drugs that regulate serotonin-mediated transmission, reduce REM sleep (Dugovic, C., et al., 1991). Thus, the involvement of serotonin in generating and maintaining REM sleep is still vague. Pharmacological effects after the stimulation of serotonin receptors shows inconsistencies on REM sleep. It is possible that the involvement of serotonin in REM sleep is not as simple as involving two receptors as reported in the studies above. It is also possible that the serotonin-mediated transmission has an indirect interaction with the adrenergic system which plays an important role in REM sleep inhibition. Other approaches attempting to alter tryptophan in the diet show that a tryptophan-free diet significantly shortens the REM onset latency and lengthens the time spent in REM sleep (Bhatti, T., et al., 1995). However, a high L-tryptophan diet does not affect the REM onset latency or the amount of REM sleep (Hartman, E., and Spinweber, C.L., 1979).

1.3.3 Daytime Sleepiness

The observation that loss of sleep impaired human psychological or physiological performance was first reported in Psychological Reviews in 1896 (Patrick, G.T.W., and Gilbert, J.A., 1896). Today, it is well known that sleep deprivation reduces concentration, memory, and reasoning abilities, and leads to complaints of lethargy, fatigue, loss of energy, weariness, lack of initiative, and lassitude. Psychometric and electroencephalographic analyses show that sleep loss increases a person’s reaction time, increases the frequency of errors on vigilance testing, and decreases EEG alpha amplitude (Williams, H.L., et al., 1959). This
suggests that changes in physiological and psychological functions due to sleep deprivation occur at the expense of daytime wakefulness.

Sleep and wakefulness are alternating and reciprocal phases in the daily life cycle. Sleepiness is the most predictable outcome of the interaction between daytime wakefulness and nighttime sleep. This state exists in the wakeful condition as a necessary consequence of an inability to achieve adequate nighttime sleep (Roth, T., et al., 1994). Optimal wakefulness produces alertness, feelings of well being, activity, vitality, and an ability to learn or to be motivated. On the other hand, sleepiness results in poor cognitive function, lack of initiative and motivation, and absence of activity. Earlier, these symptoms were attributed to laziness, malingering or lifestyle excesses. Nowadays, alertness and sleepiness are the measurements of human motoric, sensoric, and higher cognitive functions. They are also the measurements of the physiological ability to remain awake in a sleep stimulating environments (Carskadon, M.A. and Dement, W.C., 1987).

There are a number of factors that may contribute to the physiological sleep tendency including age, gender, life sustaining functions such as thirst or hunger and drugs and other exogenous influences (Roth, T., et al., 1994). Although the main point is that daytime sleepiness is attributed to deficits in nighttime sleep, there are six important circumstances that contribute to this conclusion. First, inadequate sleep may relate to the length of nighttime sleep, which is proven to correlate well with the degree of sleepiness. Extending the period of nocturnal sleep, as in sleep-deprived young adult individuals, reduces daytime sleepiness (Carskadon, M.A., and Dement, W.C., 1982), whereas reducing night sleep increases daytime sleepiness (Carskadon, M.A., and Dement, W.C., 1987). Secondly, aging and maturation are also important determining factors of sleepiness. A large population study found that the mean daytime sleep onset latency during MSLT in college students aged between 18-29
years, with eight hours of night time sleep, is about 11.10 minutes (n = 129). This compares to 12.5 minutes in older adults (Levine, B., et al., 1988). Thirdly, the continuity of sleep at night is important in determining the level of daytime functioning (Bonnet, M.H., 1985). The more disrupted the sleep, the sleepier the subject is. The continuity of sleep is related to arousal during sleep that may be due to internal or external stimuli. Furthermore, arousal during sleep is found to be an age-related phenomenon. Daytime sleepiness among the older population is less correlated to nighttime sleep disruption, than among the younger population. Meanwhile, an inability to maintain sleep is more common among the elderly. Fourthly, sleep and wakefulness are circadian phenomena. Intensification of sleepiness usually occurs in the middle of the night and at midday. Midday sleepiness is usually regarded as a postprandial response. Some observations using numerous performance tests show what is termed as the "post-lunch dip" phenomena (Broughton, R., 1975). However, midday sleepiness is probably not related to meals at all, since a similar reduction of sleep latency does not occur following breakfast or supper. A study shows that, even with an equal proportion of liquid meals given every hour, a midday reduction in sleep latency is still present. This leads us to conclude that midday sleepiness is a rhythmic phenomenon and a normal diurnal variation in sleep tendency. However, meals may augment sleep tendency in sleepy individuals (Carskadon, M.A., and Dement, W.C., 1987). Fifthly, excessive daytime sleepiness may be an indication of a sleep disorder. Sleepiness is a major symptom in narcolepsy, hypersomnia, insomnia, the sleep apnea syndrome, restless leg syndrome, periodic limb movement, and certain medical and psychiatric sleep disorders. In these patients, sleepiness may relate to reductions in overall sleep time, disturbances in sleep continuity, and/or alteration in circadian rhythm. The last factor that may affect sleepiness is the use of drugs that induce sleep
or sleepiness, such as benzodiazepines, antihistamines, antidepressants, and barbiturates, or drugs that enhance wakefulness such as amphetamines.

About 0.5 - 5% of the North American population complain about excessive daytime sleepiness (Roth, T., et al., 1994). In San Marino, an epidemiological survey indicated that the prevalence of excessive daytime sleepiness is related to the quality of sleep. Fifteen percent of poor sleepers complain of excessive daytime sleepiness, compared to 8.8% of good sleepers. Interestingly, daytime postprandial sleepiness is more common among good sleepers than bad sleepers (Lugaresi, E., et al., 1983). A five year longitudinal self-assessment study in sleepiness and sleep, in 2,929 students of the San Diego Naval School of Health Sciences, indicated that, performance of poor sleepers results in poor promotion and paygrade advancement (Johnson, L.C., and Spinweber, C.L., 1983). Sleepiness is also related to hundreds of thousands of motor vehicle accidents in the United States every year. It is found that driver inattention and carelessness plays an important role in a variety of accidents. Sleepiness is also considered as a contributor to human error in major occupational disasters, such as those at the Chernobyl and Three Mile Island nuclear power plants, the NASA space shuttle program, and the Exxon Valdez oil spill. These human errors cause loss of life, bodily damage and billions of dollars in public costs (Mitler, M.M., et al., 1988; Carskadon, M., 1993; Leger, D., 1994).

Performance on a panel of psychometric tests has been used as a surrogate measure of daytime sleepiness since the end of 19th century. However, these tests may not be sensitive enough to measure sleepiness. Moreover, sleepiness can be temporarily masked by high motivation, excitement, muscle activity and other environmental factors. To demonstrate sleepiness on performance testing, the completion time of the tasks must be long enough to be able to indicate the impairment. Tasks that require less than ten minutes to complete are rarely able to
detect sleepiness. Tasks that require ten to twenty minutes to complete can be expected to detect performance-related sleepiness (Roth, T et al., 1992). However, tasks with long completion times may also cause boredom, decreasing motivation and interest, which themselves precipitate sleepiness. Thus, these tests may be confounded by false positive sleepiness (Mavjee, V., and Horne, J.A., 1994).

Another objective measurement of sleepiness can be detected by pupilometry. This particular test is based on the fact that the stability of the peripheral autonomic nervous system is related to the cycle of sleep-wakefulness. Thus, the pupil diameter of a sleepy person is unstable when she or he is adapting to the dark (Yoss, R.E., et al., 1969). Another objective sleepiness measurement is Auditory Evoked Potentials as developed by Broughton et al. in 1981 (Guilleminault, C., 1994). It is quite sensitive to increased daytime sleepiness, but it is not widely used as a diagnostic tool.

The Stanford Sleepiness Scale (Hoddes, E., et al., 1973) and Epworth Sleepiness Scale (Johns, M.W., 1991) are self-assessment tools used to diagnose and measure the severity of sleepiness. The Stanford Sleepiness Scale is quite subjective and not a sensitive measure of sleepiness, because patients with chronic excessive daytime sleepiness have no good reference to normal wakefulness. This limitation causes an inability to judge one’s own state of wakefulness (Hartse, K.M., et al., 1982). Some patients also deny or hide the degree of their sleepiness to avoid the possible social consequences, such as suspension of a driving license. The Epworth Sleepiness Scale (ESS) is a more recent approach to measuring subjective sleepiness. This method is well correlated with the respiratory disturbance index in sleep apnea syndrome, but is not helpful in diagnosing other types of hypersomnia. ESS scores for a normal population are 5.9±2.2, for mild obstructive sleep apnea syndrome (OSAS) they are
9.5±3.3, for moderate OSAS they are 11.5±4.2, for severe OSAS they are 16.0±4.4, for narcolepsy they are 17.5±3.5, and for idiopathic hypersomnia they are 17.9±3.1.

The Multiple Sleep Latency Test (MSLT) is the only objective tool to measure excessive daytime sleepiness severity and to diagnose certain sleep disorders (Carskadon, M.A., et al., 1986). MSLT is also the basis for classifying the severity of sleep disorders. (the American Sleep Disorders Association, 1992). Later, in the Methodology section, the method will be described in detail. A sleep onset latency on the MSLT of less than 5 minutes and sleep onset REM periods in two separate naps on the MSLT, are clues for the diagnosis of narcolepsy (Roehrs, T. and Roth, T., 1992).

The International Classification of Sleep Disorders (the American Sleep Disorders Association, 1990) divides the severity of sleepiness as follows:

1. Mild sleepiness: This term describes sleep episodes that are present only during times of rest or when little attention is required. Situations in which mild sleepiness can become evident include but are not limited to lying down in quiet room, watching television or reading, and being a passenger in a moving vehicle. Mild sleepiness may not be present every day. The symptoms of mild sleepiness produce a minor impairment of social or occupational function.

   This degree of sleepiness is usually associated with a multiple sleep latency test (MSLT) mean sleep latency of 10-15 minutes.

2. Moderate sleepiness: This term describes sleep episodes that are present daily and that occur during very mild physical activities requiring, at most, a moderate degree of attention. Examples of situations in which moderate sleepiness may occur include driving and attending concerts, movies, the theater, or similar group meetings. The symptoms of moderate sleepiness produce a moderate impairment of social or occupational function.

   This degree of sleepiness is usually associated with multiple sleep latency test mean sleep latency of 5-10 minutes.

3. Severe sleepiness: This term describes sleep episodes that are present daily and at times of physical activities that require mild to moderate
attention. Examples of situations in which severe sleepiness may occur include eating, direct personal conversation, driving, walking, and physical activities. The symptoms of severe sleepiness produce a marked impairment of social or occupational function.

This degree of sleepiness is usually associated with a multiple sleep latency test mean sleep latency of less than 5 minutes.
1.4 Insulin, CNS and IDDM

1.4.1 Distribution of Insulin into the CNS

The central nervous system has traditionally been considered a tissue that is independent of the actions of insulin. However, evidence has accumulated showing that there is both insulin and its receptor in brain tissue (Havrankova, J., and Roth, J., 1978; Havrankova, J., et al., 1979; 1983; Van Houten, M., et al., 1979). While it is generally believed that most of the brain insulin is taken up from plasma, evidence shows that insulin may be synthesized de novo by brain cells. Moreover, insulin may enter the cerebrospinal fluid in one of two ways. First, insulin may cross the blood-brain barrier by a receptor-mediated transport system in the endothelial cells of brain microvessels. Another possible route of entry is via the circumventricular organs. These organs are specialized areas of the brain which are in contact with the cerebrospinal fluid, but lack a well developed restrictive blood-brain barrier. Insulin is then transported from the circumventricular area into deeper sites within the brain (Frank, H.J. and Pardridge, W.M., 1983; Pardridge, W.M., et al., 1985; Van Houten, M., and Posner, B.I., 1983).

Even though insulin levels in the brain and cerebrospinal fluid are not acutely affected by variations in insulin levels in the plasma, they bear a close relationship to the latter. Indeed, an equilibrium of insulin in these three compartments can be achieved if insulin is introduced into the system for a long period of time (Yalow, R.S. and Eng, J., 1983; Figlewicz, D.P., et al., 1985). The brain-plasma insulin ratio of ultralente insulin administered subcutaneously into guinea pigs, does not change much within the first two days. Only after three or more days will the brain-plasma insulin ratio increase. Interestingly, on occasion when plasma levels of insulin are acutely
falling, the brain insulin level is not falling and the CSF/plasma ratios go even higher (Eng, J. and Yalow, R.S., 1981). Another documented parallel between changes in the cerebrospinal fluid and plasma is seen in the hyperinsulinemic obese zucker rats, who have a higher insulin level in the cerebrospinal fluid than that of their lean littermates (Figlewicz, D.P., et al., 1985). Human experimentation shows that CSF insulin concentrations increase during peripheral infusions of insulin. Alterations in the peripheral plasma insulin levels change the level of insulin in the central nervous system, which can be detected as early as 30 minutes after the infusion starts (Wallum, B.J., et al., 1987).

1.4.2 Insulin Receptors in the CNS

Descriptions of the insulin receptor in the central nervous system come from studies conducted on brain membrane preparations. These show that insulin binding characteristics in the central nervous system are similar to those of insulin in other tissues (Havrankova, J., et al., 1979). Immunocytochemical staining demonstrates that most insulin receptors are located on the surface membrane of nerve cell bodies as well as at postsynaptic terminals. Only a few insulin receptors are present on glial cells (Clarke, D.W., et al., 1985a; 1985b). These receptors are not equally distributed throughout the regions of the brain. Insulin receptors are found in high density in the limbic-hypothalamic system, numerous nuclei in the brainstem and cerebellum, and in the cerebellar cortex. The highest density of insulin receptors occurs in the subfornical organ, subcomissural organ, supraoptic nucleus, area postrema, choroid plexus, suprachiasmatic nucleus, medial habenula, paraventricular hypothalamic nucleus, periventricular hypothalamic region, arcuate nucleus, piriform cortex, olfactory bulb, hippocampus, amygdala, neocortex, caudate putamen, and globus pallidus. The
actions of insulin in these regions are not well understood, except that the presence of insulin in the choroid plexus suggests that the choroid plexus is the site of insulin transport across the blood-brain barrier and that the insulin receptor may be involved in this transport (Moss, A.M., et al., 1990).

There are four major differences between insulin receptors in the brain and insulin receptors in other parts of the body. First, the insulin receptor in the brain shows structural differences with respect to a lower molecular weight of the α-subunit (Sesti, G., et al., 1992). Secondly, brain insulin receptors do not show negative cooperativity, as is the case in the periphery. Thirdly, brain insulin receptors do not mediate insulin degradation. Finally, brain insulin receptors do not appear to undergo up- or downregulation. The one exception to this is that glial cell insulin receptors are downregulated in response to an elevated insulin concentration. However, there are many uncertainties regarding the kinetics of insulin receptors in the brain (Gammeltoft, S., 1984).

Relatively few studies have investigated the regulation of insulin receptors in the central nervous system. Several studies show that insulin receptors are not regulated by plasma insulin. However, intracerebroventricular infusions of insulin into the brain of newborn rabbits cause a decrease in insulin-receptor binding (Devaskar, S.U. and Holekamp, N., 1984). Interesting studies on brain insulin binding in genetically obese rats provides some insight into insulin receptor downregulation in the presence of the chronically elevated insulin levels in the plasma and cerebrospinal fluid in this species (Figlewicz, D.P., et al., 1985). There are five conclusions from the Figlewicz study. First, they showed that chronic hyperinsulinemia increases the level of insulin in the cerebrospinal fluid. Secondly, insulin levels in the cerebrospinal fluid correlate well with insulin levels in the plasma, but not in a linear fashion. Thirdly, chronic elevation of insulin in the cerebrospinal fluid will downregulate the insulin
receptor in the brain. Fourthly, a 50% elevation in insulin level in the cerebrospinal fluid causes maximum downregulation of the insulin receptor. Finally, the regions of the brain in closest contact with the cerebrospinal fluid suffer the greatest downregulation. For example, downregulation of the insulin receptor occurs in the olfactory bulb but not in the cerebral cortex. Downregulation of insulin receptors in the hypothalamus was not significant in this study, whereas another study showed that chronic hyperinsulinemia in obese rats reduced insulin receptor binding in the hypothalamus (Melnyk, R.B., 1987).

1.4.3 Effects of Insulin in the CNS

Observations on the actions of insulin in the central nervous system suggest that insulin may have wide ranging effects on electrolyte balance (Bernstein, H.G., et al., 1981), the kinetics of neurotransmitters both in the nerve terminal and in the synapse (Rhoads, D.E., et al., 1984; Sauter, A., et al., 1983), neuronal cell growth (Girbau, M., et al., 1987), metabolism (Mackenzie, R.G. and Trulson, M.E., 1978), nutrient transport across the blood-brain barrier (McCall, A.L., et al., 1982), and feeding behavior and body weight (Brief, D.J., and Davis, J.D., 1984; Woods, S.C., et al., 1979; Woods, S.C., et al., 1985). These findings suggest that insulin is a very important hormone in modulating the functions of the CNS.

Insulin is known to induce a net uptake of potassium by skeletal muscle, adipocytes, liver, and other cells through a mechanism that is separate from its effects on glucose transport in these tissues. Studies suggest insulin does this by stimulating Na⁺-K⁺-ATPase through a cAMP-dependent mechanism. Elevation of insulin levels above physiologic concentrations increases the pump activity thereby hyperpolarizing the cells and inhibiting their action (Sansom, S.C., and Giebisch,
G.H., 1993). It has also been suggested that insulin is capable of activating cerebral 
$\text{Na}^{+}$-$\text{K}^{+}$-ATPase in a dose-dependent manner (Bernstein, H.G et al., 1981). Indeed, 
several studies indicate that local application of insulin causes a dose-dependent 
inhibition of the suprachiasmatic nuclei (Shibata, S., et al., 1986) and hippocampal 
pyramidal neurons (Palovcik, R.A., et al., 1984). This suggests that insulin may act as 
an inhibitory peptide in some regions of the central nervous system.

Studies of the regulation of catecholamines by insulin during insulin-induced 
hypoglycemia provide further evidence for the important effects of insulin on the 
brain. Insulin has effects on brain catecholamine levels (Agardh, C.D., et al., 1979).
The level of catecholamines is higher in brain regions where insulin receptors are dense 
(e.g. in the hippocampus and hypothalamus), than that in other regions with a lower 
density of insulin receptors (Chu, P.C., et al., 1986). Insulin has been shown to 
increase the turnover of norepinephrine, independent of glucose levels (Trulson, M.E. 
and Himmel, C.D., 1985). The release of dopamine, norepinephrine and epinephrine is 
increased in rat hypothalamic slices (Sauter, A., et al., 1983), while insulin inhibits the 
reuptake of catecholamines into the pre-synaptic terminal (Boyd, F.T, Jr., et al., 
1985) in a dose-dependent fashion. However, the net concentration of catecholamine 
in the hypothalamus is not significantly changed by the administration of insulin. This 
implies that insulin causes a shift in the distribution of catecholamines from 
presynaptic storage into the synaptic gap. This may then cause increased activity at 
postsynaptic catecholamine receptors. The study also indicates that the effects of 
insulin on catecholamines are more obvious if insulin is administered intraventricularly 
than intravenously (Sauter, A et al., 1983).

The effect of insulin on central catecholamines occurs within 10 to 90 minutes 
after the introduction of insulin into the central nervous system. However, the effects 
of chronic hyperinsulinemia on the levels of catecholamines has not been studied.
Animal models of IDDM (insulin deficiency) show reductions in catecholamine synthesis within the brain due to a progressive decrease in the activity of tyrosine hydroxylase (Bitar, M., et al., 1986). This is supported by at least three additional findings. First, reduction of dopamine synthesis causes upregulation of (³H) spiroperidol binding to dopamine receptors in the brain of diabetic rats (Trulson, M.E., and Himmel C.D., 1983). Secondly, there is an increased sensitivity to amphetamine in diabetic rats that may point to the upregulation of norepinephrine in the central nervous system (Chu, P.C., et al., 1986). Finally, streptozotocin-treated rats have been reported to show upregulation of alpha-1 adrenergic receptors in the brain (Bitar, M., et al., 1986). Thus, CNS catecholamine activity is reduced if insulin availability is insufficient or absent such as in diabetics.

An investigation into the effects of insulin on amino acid neurotransmitters has been done on synaptosomes. The findings show that insulin stimulates synaptosomal uptake of glutamic acid, aspartic acid, proline and GABA in a dose-dependent fashion. The effects are not uniform across the brain in that elevation of GABA reuptake is most noticeable in the limbic-hypothalamic system and brainstem. This is congruent with the fact that these regions also show a high density of insulin receptors on autoradiography (Rhoads, D.E., et al., 1984)

The effects of insulin on synaptic neurotransmission is puzzling. Some studies show that insulin causes hyperpolarisation. On the other hand, there are studies showing that insulin increases catecholamines release, indicating a depolarized state. There must be other mechanisms involved or affected by insulin that leads to the release of catecholamines. There are two possible answers to this puzzle. First, there may be methodological weaknesses related to sampling in the various studies. Sauter, and coworkers (1983) concluded that increasing catecholamine levels in the extraneuronal environment were a measure of increasing catecholamines release.
Meanwhile, it is possible that increased catecholamines are caused by the inhibition of catecholamine reuptake. Second, a study conducted by Palovcik, et al. (1984) shows that only 63% of hippocampal neuronal cells are inhibited by the presence of insulin, while the rest show no response (15%), excitation (9%), or excitation and inhibition (13%). Thus, the increasing release of catecholamines may only occur in non-inhibited neuronal cells.

Catecholamine and amino acid neurotransmitters reuptake are sodium-dependent and can be blocked by inhibition of Na\(^+\)-K\(^+\)-ATPase. Indeed, GABA and norepinephrine transporters have notable homology in cDNA sequence (Cooper, J., et al., 1991). Since, insulin stimulates Na\(^+\)-K\(^+\)-ATPase in neuronal cells, catecholamine and amino acid neurotransmitter reuptake may be stimulated by insulin. Indeed, insulin has been shown to stimulate reuptake of amino acid neurotransmitters, and to inhibit catecholamine reuptake in the hypothalamus. There is no explanation for this rather ambiguous effect on neurotransmitter reuptake, although it is probably due to a complex reflex of neuronal circuitry in the brain.

The transport of certain nutrients across the blood-brain barrier may be regulated by insulin. Concentrations of tryptophan, serotonin and 5-hydroxyindoleacetic acid in the brain are increased following ingestion of a carbohydrate meal (Fernstrom, J.D. and Wurtman, R.J., 1971, 1972; Crandall, E.A., et al., 1981, Crandall, E.A. and Fernstrom, J.D., 1983), but are not increased following consumption of a high protein diet (Glanville, N.T. and Anderson, G.H., 1985). Since insulin is increased by carbohydrate but not protein suggests that insulin may be a factor increasing the transport of tryptophan from the plasma through the blood-brain barrier into the central nervous system. More careful studies show that insulin injections increase the concentration of tryptophan in brain in fasted rats independent from hypoglycemia stress (Sangiah, S. and Caldwell, D.F., 1988).
Correlations between elevated tryptophan concentrations and resultant serotonin synthesis with increased insulin levels in the brain are strengthened by the fact that tryptophan hydroxylase, the rate limiting enzyme in serotonin synthesis, is not saturable. Increasing concentrations of 5-hydroxyindoleacetic acid in the brain correlate with increasing serotonin synthesis and with increasing central serotonin metabolism in response to insulin in a dose dependent manner. Brain tryptophan is low in diabetics and insulin administration increases tryptophan levels to above normal (Mackenzie R.G. and Trulson, M.E., 1978). This means that insulin regulates the transport of tryptophan through the blood-brain barrier and that insulin also controls serotonin metabolism and possibly neurotransmission at serotonergic synapses in the central nervous system. The change in tryptophan transport into the brain of diabetics is due to insulin deficiency and is not a vascular complication of diabetes mellitus (Mackenzie R.G. and Trulson, M.E., 1978).

Glucose transporters regulate the transport of glucose from the periphery into the central nervous system. This mechanism is believed to be a protective mechanism against hyperglycemia and hypoglycemia. The central nervous system is understood to have a specific glucose transport protein; namely, GLUT1, which is selectively localized along the blood-brain barrier (Partridge, W.M., et al., 1990). GLUT1 regulation in the endothelial cells of the blood-brain barrier is dependent upon the blood glucose level. Glucose deprivation enhances the post-transcriptional gene expression of brain capillary endothelial GLUT1 glucose transporter (Boado, R.J., and Pardridge, W.M., 1993). Insulin-induced hypoglycemia, fasting in normal subjects and untreated diabetes mellitus, all up-regulate the expression of GLUT1 glucose transporter in the central nervous system (Koranyi, L., et al., 1991). Fasted normal subjects and patients with untreated diabetes mellitus have a lack of insulin in the body. Meanwhile, chronic hyperinsulinemia and insulin-induced hypoglycemia causes
a downregulation of the insulin receptor which reduces insulin post-receptor effects. Two hours of hyperinsulinemic-euglycemic clamping in rats leads a reduction in glucose utilization rates by 25-50% in the medial basal hypothalamus, locus ceruleus and motor cortex neurons. Glucose utilization correlates negatively with the level of insulin (Grunstein, H.S., et al., 1985). These seemingly different conditions relate to an increasing post-transcriptional genetic process and upregulation of GLUT1. Alternatively it is possible that insulin inhibits the synthesis of GLUT1 and that insulin deficiency or any reduction in insulin activity disinhibits the synthesis of GLUT1.

Therefore, we may conclude that a) glucose kinetics in the central nervous system are insulin sensitive; b) the GLUT1 blood-brain barrier glucose transporter gene is regulated by insulin and glucose levels; c) glucose utilization is negatively correlated with plasma insulin levels; and d) it is possible that GLUT3 in brain neurons is also acutely regulated by insulin. Other interesting findings are that insulin receptors mediate glucose uptake and stimulate synthesis of a number of macromolecules in cultured astrocytes as it does in adipocytes (Clarke, D.W., et al., 1985b). Insulin also stimulates glycogen synthesis and glucose metabolism in neurons (Herz, M.M. and Poulson, O.B., 1983).

Evidence shows that insulin is a growth-promoting substance in brain development. Insulin stimulates acetylcholine transferase activity in retinal neurons during embryonic chicken development and insulin receptor activity is reduced as acetylcholine transferase activity concomitantly decreases (Kyriakis, J. M., et al., 1987). Insulin is also believed to be responsible for promoting neuronal regeneration in cell cultures from embryonic mouse brain (Baskin, D.G., et al., 1987). Insulin and its receptors undergo changes in concentration and density during embryonic development with increasing activity during cell migration and differentiation. Insulin
binding in the central nervous system is highest in newborn rat brain then decreases and is stable throughout adult life (LeRoith, D. et al., 1983).

Insulin, infused into the cerebrospinal fluid of baboons and rats inhibits feeding and results in loss of body weight (Woods, S.C. and Porte, D.Jr., 1983). Inhibition of the satiety signal in the CNS is believed to be at work in obese Zucker rats where insulin binding is known to be low in the olfactory bulbs and hypothalamus (Figlewicz D.P., et al., 1985). CSF insulin also influences other metabolic events in the periphery. Infusion of insulin into the CSF stimulates vagal traffic to the pancreas and increases plasma insulin levels (Porte, D.Jr. and Woods, S.C., 1981). These results suggest that insulin in the cerebrospinal fluid interacts with central autonomic pathways involved in the regulation of peripheral nutrient homeostasis.

1.4.4 The CNS Complications of IDDM

Diabetes may affect the CNS in a variety of ways. Most of the abnormalities in the CNS of diabetic patients are secondary to vascular disease (Affolter, V., et al., 1986). Cerebral infarction and hemorrhage are more common in diabetic patients, and their outcomes are worsened by hyperglycemia (Mooradian, A.D., 1988). Microangiopathy plays a relatively minor role. Other acute or chronic CNS dysfunctions in diabetes may be related to 1) impaired blood-brain barrier functions; 2) repeated metabolic insults, such as hyperglycemia, hypoglycemia, ketoacidosis, cerebral electrolyte disturbances; 3) peripheral and autonomic neuropathy; 4) uremic and hypertensive encephalopathy; and 5) altered brain metabolism or other neurochemical changes (Mooradian, A.D., 1988). The focus of the subsequent discussion will be on these other, lesser known, disorders.
Glucose is responsible for 90% of the energy supply to the brain. Since the brain only has a very small glucose store, a consistent supply of glucose from the circulation is essential for normal cerebral metabolism. Indeed, acute hypoglycemia causes rapid changes in CNS function as well as initiates a number of counterregulating mechanisms, such as increasing autonomic activity, decreasing metabolic activity in the brain and increasing the release of growth hormone, glucagon, cortisol, adrenaline, and noradrenaline to try and normalize systemic glucose levels (Gerich, J.E., et al., 1991). The consequences of hypoglycemia counterregulation are recognized by patients in the form of hypoglycemic symptoms such as sweating, trembling, headache, nervousness, tiredness, shakiness, lack of concentration, rapid heart beat, blurred vision, and hunger. The CNS response to hypoglycemia depends upon the rate of fall of the blood glucose level, absolute change in blood glucose and the long-term glycemic control. Most of these symptoms occur when the blood sugar level decreases to 2.2 mM or lower (Lingenfelser, Th. et al., 1992).

Patients with insulin-dependent diabetes mellitus, who are treated intensively, may be at risk of hypoglycemia unawareness. Hypoglycemia unawareness is defined as a state of where autonomic warning symptoms do not occur or are not recognized before neuroglycopenia has developed (Gerich, J.E et al., 1991). The sensor to recognize hypoglycemia is located in the ventromedian hypothalamic nuclei. These nuclei identify hypoglycemia, and mediate the sympathoadrenal response, when the arterial blood glucose falls to approximately 75 mg/dl or equal to 4.2 mM (Boyle, P.J., et al., 1994; Schwartz, N.S., et al., 1987; Borg, W.P., et al., 1994). Thus, the awareness of hypoglycemia is initiated by the reduction of glucose uptake in the brain, which is followed by an increase in the secretion of counterregulatory hormones and presentation of hypoglycemia symptoms.
Diabetic patients with near normal glycosylated hemoglobin levels are at an increased risk of hypoglycemia unawareness. They have a normal uptake of glucose into the brain, so that during hypoglycemia, when the availability of glucose is diminished, these patients have neuroglycopenia before any mechanism to trigger autonomic hypoglycemia symptoms can be invoked. In Type-I diabetes mellitus with glycosylated hemoglobin levels higher than 8.9%, glucose uptake into the brain decreases, and the autonomic activation occurs before symptom of neuroglycopenia occurs (Boyle, P.J., et al., 1995). The DCCT reported that patients with IDDM, who had tight insulin/glycemic control, were more likely to encounter severe hypoglycemia and require assistance to recover from hypoglycemia, than patients with conventional therapy (The DCCT Research Group, 1993). Meanwhile, IDDM patients with near normal levels of glycosylated hemoglobin are able to maintain glucose uptake in the face of hypoglycemia. Interestingly, IDDM patients with glycosylated hemoglobin levels higher than 7.9%, and non-diabetic patients, do not have this ability and show significant impairment of glucose uptake in the brain during hypoglycemia (Boyle, P.J et al., 1995). It is likely that hypoglycemia unawareness is one of the largest challenges and probably the limiting factor in insulin therapy. Intensive therapy has been shown to reduce and delay the onset of diabetic complications, however, at the same time, it also puts the patient at increased risk of life-threatening hypoglycemia.

Cognitive impairment in diabetic patients, has been recognized by Miles and Root in 1922. Other studies have also concluded that diabetic patients have neuropsychological impairment on tasks that require visual and motor efficiency, and somatosensory discrimination. Cognitive skills and memory remain intact (Skenazy, J.A. and Bigler, E.D., 1984). A population study also shows that young adult subjects with IDDM perform as well as age- and social economy status-matched non-diabetics, in learning and memory processes. However, diabetic population shows
psychomotor slowing (Ryan, C.M. and Williams, T.M., 1993). Comprehensive neuropsychological testing on adolescent diabetic patients (n=125) and non-diabetic controls (n=83) show that cognitive function is reduced in diabetes mellitus (Ryan, C., et al., 1985). Type-I diabetics with early onset of diabetes (diagnosis before age of 5 years) performed significantly poorer than Type-I diabetics with later onset of disease, and significantly poorer than non-diabetic controls. The same study also found that there was no difference between Type-I diabetics with later onset of disease and non-diabetic controls.

Cognitive performance is reduced when the blood glucose level falls to less than 2 mM (Pramming, S et al., 1986). When the blood sugar of a patient with Type-I diabetes mellitus is clamped at 3.3 mM, the patients' attention ability and fine motor skills decline and hence, patients require a longer time to solve problems. Normalization of blood glucose level normalizes the psychometric performance (Holmes, C.S., et al., 1983). It is likely that cognitive function in Type-I diabetes mellitus patients is also regulated by long term glycemic-insulin control, which will affect the ability to escape from cellular hypoglycemia. This ability also relates to the downregulation of BBB glucose transport carriers in Type-I diabetes mellitus patients with chronic hyperglycemia. Downregulation of glucose transporter in Type-I diabetes mellitus patients will reduce the availability of glucose in the neuronal cells when blood glucose levels are in the normal range. The glycemic thresholds and the magnitude of cognitive dysfunction in tightly-controlled Type-I diabetes mellitus patients, during hypoglycemia, are lower compared to poorly controlled Type-I diabetes mellitus patients (Ziegler, D., et al., 1992).

Maintaining hyperglycemia at 16.7 mM in Type-I diabetes mellitus slows down reaction time and causes some impairment in memory (Holmes, C.S., et al., 1983). However, other studies show that hyperglycemia with blood glucose up to 10 mM
enhances memory and learning capacities. These data show that the dose-response relationship between cognitive function and blood glucose levels is an inverted U-shaped curve (Gold, P.E., 1986; Gold, P.E., et al., 1986). Another study on non-diabetic volunteers shows that rapid elevation of blood glucose to 16.7-22.2 mM has no effect at all on CNS processing mechanisms. Therefore, cognitive impairment in diabetes mellitus may be modulated by the level of glucose in the circulation, and related to acute and chronic changes that alter neurotransmitter function, BBB transport systems and insulin sensitivity (Mooradian, A.D., 1988).

The latest publication by Joslin Diabetic Center, Boston, Massachusetts, (Draelos, M.T., et al., 1995) shows that cognitive function of IDDM individuals is generally well-preserved. Neuropsychological functions are diminished only at blood glucose level 2.2 mM or less, and only small differences in cognitive functions are observed between blood glucose levels of 5.4, 8.9 (baseline), 14.4, and 21.1 mM. Prior glycemic control as measured by HbA1C do not affect cognitive function. Other relevant parameters such as age, duration of diabetes, IQ or counterregulatory hormone levels do not appear to have significant influence on cognitive function in IDDM. Several interesting points observed in Joslin’s diabetic study are that, first, the diabetic subjects have a poor insulin/glycemic control (HbA1C = 10.6±2.3%) according to the DCCT population study (the DCCT Research Group, 1993). This HbA1C value expresses that Joslin’s study used diabetic subjects suffered from insulin resistance. The assumption of insulin resistance is strengthened by the reason of why they used baseline cognitive function at blood glucose levels of 8.9 mM, which is because they found out that at glucose level between 5.0 M and 6.1 mM near-hypoglycemia condition occurred in many subjects with poor insulin/glycemic control. Another interesting study is reported by The Pittsburgh Epidemiology of Diabetes Complications. The study concluded that the best predictor of cognitive
performance is not the number of severe hypoglycemic attacks but the diagnosis of
The conclusion above is justified because peripheral neuropathy is usually
accompanied by chronic hyperglycemia and decreasing Na⁺/K⁺-ATPase activity. This
means that polyneuropathy may reflect the occurrence of hyperglycemia and
hyperinsulinemia or precisely the occurrence of insulin resistance.

CNS processing is impaired during severe hyperglycemia in patients with
IDDM. This may be secondary to changes in plasma osmolality and electrolyte
balance in the brain, or due to a direct effect of glucose on neurotransmission. Blood
glucose levels modulate animal responses to opioid. It has been shown that
hypoglycemia potentiates the antinociceptive effects of morphine, and that
hyperglycemia decreases these effects. Naloxone sensitivity on streptozotocin-
induced diabetic is also decreased during hyperglycemia (Simon, G.S. and Dewey,
W.L., 1981). In animals with streptozotocin-induced diabetes, of less than 3 weeks
duration, there is no alteration in BBB choline transport. However, when the
hyperglycemia is extended until nine weeks, BBB choline transport is reduced
significantly. Five day hyperinsulinization does not normalize choline transport. It
was observed that choline transport through the blood-brain barrier in aged rats is also
reduced (Mooradian, A. D., 1987; 1988a). Chronic hypoinsulinemia is not related to
the reduction of choline acetyltransferase (Bitar, M., et al., 1986). Chronic
hyperglycemia will down-regulate insulin binding in the BBB (Frank, H.J.L et al.,
1986). This leads to the loss of BBB sensitivity to insulin, so that insulinization will
not be able to normalize choline transport.
1.4.5 IDDM, Insulin, Wakefulness and Sleep

There is some evidence that sleep may be impaired in patients with diabetes. Chronic sleep impairment has been reported in non insulin-dependent diabetes mellitus (NIDDM) where it is thought to be related to nocturia, sleep apnea and disturbances in insulin/glycemic control. In these patients, insulin resistance appears to be the strongest determinant of sleep apnea episodes, and not body mass index as might be expected (Tiihonen, M., et al., 1993). This may also be a cause of excessive daytime sleepiness (Feinberg, I., 1993). Sleepiness has also been reported in patients with IDDM. In this case, nocturnal hypoglycemia which occurs in about 25-60% of patients is believed to be the cause (Bendtson, I., et al., 1992). Insulin is another factor that influences sleep quality. Intraperitoneal injection of insulin, immediately before sleep time in rats, significantly increased the amount of SWS in the first 2 hours of sleep. However, as insulin levels decreased so also the amount of SWS returned to normal. Indeed, there were no differences in the overall amount of SWS in an 8 hour sleep period between insulin-treated streptozotocin diabetic rats and untreated controls. Studies of the effects of insulin on REM sleep show that insulin completely abolishes REM sleep in the first 3 hours of sleep in rats, and reduces the total amount of REM sleep by about 50% (Sangiah, S., et al., 1982). The effects of insulin on REMS and NREM sleep were independent of the blood glucose level (Sangiah S., and Caldwell D.F., 1988). Insulin also caused a decrease in sleep time in rats during the light phase. Due to less sleep time, the circadian rhythmicity of NREM sleep were attenuated in diabetic rats (Kapás, L., et al., 1991). Other studies suggest that insulin affects the duration of SWS, whereas the duration of REMS remains unchanged (Danguir, J. and Nicolaidis, S., 1984).
1.5 Insulin, IDDM, Sleep and Quality of Life

Conventional measures of health outcome have focused on disease, illness and other negative aspects of health care. In this approach, quality of health is measured by biochemical data, histopathology, morbidity, and mortality. This has led to important medical discoveries and resulted in a decrease in the morbidity and mortality rate in developed countries, and especially an increase in life expectancy among new-born babies. However, this measurement of health outcome is not complete in that health status has been defined as total social, psychological and physical well being (World Health Organization, 1958). Thus, in addition to the above variables, the state of health measurement is now changing to reflect the assessment of the ability of an individual to perform in his or her daily activities, and their feelings of and evaluation of their own personal health. This means that health outcome is reflected by the evaluation of clinical status, self-functioning and well being.

The medical outcome of insulin treatment in IDDM populations was reported by The Diabetes Control And Complications Trial Research Group in the September, 1993 issue of The New England Journal of Medicine (the DCCT Research Group, 1993). Herein it was stated that tight metabolic control is the guiding principle towards achieving a longer, healthier life among diabetic patients. Thus, the quality of clinical status in patients with IDDM has been related to the level of metabolic control, freedom from complications, flexibility of insulin administration and freedom from insulin-induced hypoglycemia (Hornquist, J.O., et al., 1990; Tallroth, G., et al., 1989).

As a disease, IDDM affects the overall life situation of the individual. In fact, self-reported questionnaire-based research by Stewart et al., (1994) pointed out that a decrease in physical functioning, fatigue, pain, psychological distress, anxiety, and
depression are common problems among patients with IDDM. Thus, the measurement of health status which includes only longevity, prognosis, and functional potential of the body organs, is no longer adequate. An ideal measurement should include performance in daily activities, social role, physical and cognitive performance, mood, feelings, affection, and each individual's expectation of her/his view about a good life. Interestingly, it is found that the quality of life satisfaction is similar between IDDM individuals and the general population (Hanestad, B.R., 1989).

Sleep has been exempted from most measurements of health status (Stewart, A.L. and Hays, R.D., 1992). Of seven comprehensive health status assessments, only one included sleep problems as an indicator of health status (Bergner, M., et al., 1981). Sleep problems are prevalent in the general population (Kales, A. et al., 1987) and frequently rated by subjects as a significant health problem (Urponen, H., et al., 1988). Verbal questioning of 184 patients with diabetes and 99 control subjects indicates that sleep problems are more common in diabetic subjects than controls (Sridhar, G.R., and Madhu, K., 1994). The most common sleep disorders in diabetics is difficulty in initiating sleep, and difficulty in maintaining sleep, while only 2 out of 184 IDDM patients complained of excessive daytime sleepiness.

Daytime sleepiness is a product of inadequate nighttime sleep, which sometimes is missreported as a problem in lack of energy or fatigue. Indeed, the pathogenesis of IDDM is related to the existence of specific sleep factors IL-1 and TNF (Eisenbarth, G.S., et al., 1994). Therefore, long before the establishment of diabetes, individuals may be experiencing excessive sleepiness. In addition to that, a study on animals shows that insulin inhibits the formation of REM sleep (Sangiah, S. and Caldwell, D.F, 1988). In fact, one third of diabetic patients complain about sleep problem (Sridhar, G.R., and Madhu, K., 1994). Another study indicates that insulin therapy causes iatrogenic hyperinsulinemia in patients with IDDM (Galloway, J.A.
and Chance, R.E., 1994). Thus, it is possible that current insulin therapy has a role in the genesis of sleep problems in diabetic patients. It is likely that IDDM and insulin therapy may reinforce each other in the formation of sleep problems.

A report released by the National Commission on Sleep Disorders Research (Leger, D., 1994) indicated that in 1988 the overall cost of total accidents that related to sleep disorders was between $43.15 to $56.02 billion. Furthermore, 41.6% of total fatal accidents that occur between 2:00 and 7:00 a.m. and 36.1% of those that occur between 2:00 to 5:00 p.m., are related to sleep disorders. If diabetic subjects are reported as more likely to have sleep problems than the general population, then patients with diabetes mellitus are at a higher risk in having an accident related to their sleep disorder. The risk of accidents, social embarrassment, fatigue, academic decline and even only slight impairment in other daily performances, due to their sleep related problems are, in fact, important issues in the life of patients with diabetes mellitus. Therefore, the measurement of the quality of life of patients with diabetes mellitus must also include the measurement of the sleep profile.
1.6 Hypotheses

The discovery of insulin receptors in the central nervous system, particularly in the brain stem and limbic regions, has raised questions regarding their physiological significance. Insulin appears to be an endogenous sleep-regulating substance in some species such as the rat, where chronic intracisternal administration increases slow-wave activity (Danguir, J. and Nicolaidis, S., 1980; 1980a; 1984) and suppresses REM sleep (Sangiah, S., et al., 1982; Sangiah, S. and Caldwell, D.F., 1988). Insulin infusion into the brains of baboons (Woods, S.C et al., 1979) and rats (Brief, D.C. and Davis, J.D., 1984) has also been shown to cause reductions in feeding and weight loss, presumably through its action on the satiety center. Unfortunately, the indirect effects of glucose were not examined in these models of human diabetes. Also, it is difficult to know whether high doses of porcine insulin given to rats or other animals mimics the effects of physiological concentrations of endogenous insulin and whether these findings have any relevance to humans.

Insulin is actively transported into the central nervous system from the peripheral circulation across the blood-brain barrier. It also enters the cerebrospinal fluid by passive diffusion in areas of the brain where BBB is less well developed, such as the circumventricular organs (Frank, H.J. and Pardridge, W.M., 1983; Pardridge, W.M., et al., 1985; Van Houten, M. and Posner, B.I., 1983). The concentration of insulin in the central nervous system is in equilibrium with the insulin level in the peripheral circulation (Figlewicz, D.P., et al., 1985; Wallum, B.J., et al., 1987; Yalow, R.S. and Eng, J., 1983). Insulin receptors in the central nervous system are regulated by the level of insulin in the central nervous system (Figlewicz, D.P., et al., 1985; Melnyk, R.B., 1987). Several animal studies have confirmed that insulin modulates neurotransmitters and neuronal activities in the central nervous system, in a dose-

Evidence that insulin may be having an effect on the human brain is somewhat more circumspect. A number of recent studies report that hypoglycemia unawareness is more common in patients treated with human insulin than beef/pork insulin (Egger, M., et al., 1991; 1991a; Teuscher, A. and Berger, W.G., 1987), although other studies refute these data (Colagiuri, S., et al., 1992; Lingenfelser, Th. et al., 1992; Maran, A., et al., 1993). One highly disputed postmortem analysis of unexplained death in IDDM goes so far as to suggest that human insulin-treated diabetic patients may be at risk of sudden death during sleep as a result of hypoglycemia unawareness (Tattersall, R.B., and Gill, G.V., 1991). There is also a body of literature showing that cognitive performance declines in diabetic individuals (Ryan, C., et al., 1985; Ryan, C.M. and Williams, T.M., 1993; Skenazy, J.A. and Bigler, E.D., 1984). While it is generally accepted that these deficits are due to fluctuations in blood glucose (Gold, P.E., et al., 1986) rather than insulin per se, this latter possibility has not been specifically examined. Clinical interviews also indicate that sleep problems are more common in diabetic patients than in non-diabetics (Sridhar, G.R. and Madhu, K., 1994).

Based on the above considerations we hypothesized that insulin may have effects on the human central nervous system and that these may be influenced by insulin species, the type of insulin delivery, and the kinetics of the specific insulin preparations. To test this, we examined four working hypotheses:

1. There are differences between individuals with insulin-dependent diabetes mellitus and non-diabetic controls with respect to: a) sleep pattern, b) daytime sleepiness, and c) daytime cognitive performance

2. There are differences between the effects of human and beef/pork insulins with respect to: a) sleep pattern, and b) daytime sleepiness
3. There are differences between lispro and beef/pork insulins in respect to: a) sleep pattern, b) daytime sleepiness, and c) daytime cognitive performance.

4. There are differences between twice daily intermittent subcutaneous insulin injection and continuous subcutaneous insulin infusion with respect to: a) sleep pattern, b) daytime sleepiness, and c) daytime cognitive performance, in patients with Type-I diabetes mellitus.
2. METHODS AND MATERIALS

2.1 Methods

2.1.1 Research Design

Dr. Herman’s research has been in the area of clinical pharmacokinetics and the use of drugs as probes of physiological processes and drug action in humans. Several years ago a study was undertaken to examine the effects of glucose and insulin on hepatic glucuronidation. Young healthy subjects with IDDM were chosen because their insulin levels could be controlled and lorazepam was the study drug since its metabolism and elimination occurs via a single pathway, namely glucuronidation in the liver, and therefore, its clearance is a measure of the rate and extent of the conjugative process. The design was a crossover from human insulin to beef/pork insulin and vice versa, as there was some indication that these insulins may have different effects. In the course of the study, it was observed that while patients were randomized to the human insulin treatment arm, they rapidly attained sleep following intravenous administration of lorazepam and remained markedly sedated for 4-6 hours, thereafter. Conversely, on beef/pork insulin they attained sleep much later and slept only briefly or intermittently. This led to a pilot study, confirming these observations, and then to the studies described herein.

My thesis includes two separate studies; namely, 1) a crossover comparison of sleep, daytime sleepiness and daytime cognitive performance in patients with IDDM randomized to treatment with human or beef/pork insulin (as in the original observation), and 2) a double blind study of the same parameters in patients crossed over from beef/pork to lispro/human ultralente insulin. This latter study contained a
group of non-diabetic controls and a blinded, but uncrossed, treatment arm where patients were randomized to either lispro insulin or beef/pork insulin administered using an insulin pump. However, for purposes of clarity in the presentation of our data, we will discuss four comparisons: 1) a comparison of sleep, daytime sleepiness, and daytime cognitive performance in patients with insulin-dependent diabetes mellitus and non-diabetic controls, 2) a comparison of the effects of intermittent twice daily injection of human R/N insulin versus beef/pork R/N insulin on sleep, daytime sleepiness, daytime cognitive performance in patients with IDDM, 3) blinded comparison of the effects of intermittent twice daily injection of lispro/human ultralente insulin versus beef/pork R/N insulin on the same sleep, and daytime cognitive parameters and 4) a blinded comparison of the effects of intermittent twice daily subcutaneous insulin injection versus continuous subcutaneous infusion of insulin using an insulin pump. The sequence and timing of these four studies are simplified in figures 2.1.1, 2.12, 2.1.3, and 2.1.4. Overnight Polysomnography (PSG) was used to measure quantitative and qualitative aspects of the nighttime sleep. Daytime sleepiness was measured using the Multiple Sleep Latency Testing (MSLT) at four separate time points two hours after waking from the PSG and at two hour intervals, thereafter. A panel of four psychometric tests was used to measure daytime cognitive performance. The distribution of the insulin receptors in behavioral geography of the brain, which is in high density in the limbic system, brain stem, and cerebellum, leads to the choice of tests that measure short-term memory, sustained attention, response speed, visuomotor coordination, tactile identification and discrimination. The psychometric tests were the Digit Symbol Substitution Test (DSST), the Symbol Digit Modalities Test (SDMT), the Manual Finger Tapping Test (MFTT) and the Grooved Peg Board Test (GPBT). Detailed explanations of all of the measurements will be presented later in this section.
MEASUREMENTS

1. Sleep: 7.5 hour Overnight Polysomnography (MSTT)
2. Daytime sleepiness: Multiple Sleep Latency Test (MSLT)
3. Daytime Cognitive Performance:
   - Digit Symbol Substitution Test (DSSST)
   - Symbol Digit Modalities Test (SDMT)
   - Grooved Pegboard Test (GPBT)
   - Manual Finger Tapping Test (MFTT)

8 healthy diabetic subjects
8 healthy non-diabetic subjects

Figure 2.1.1. Study 1 compared 8 healthy diabetic subjects to 8 healthy non-diabetic subjects in sleep, daytime sleepiness and daytime cognitive performance. The data for the 8 healthy diabetic subjects was derived from Studies 2,3 and 4.
STUDY 2

Figure 2.1.2. Study 2 was a crossover study comparing the effect of beef/pork R/NPH with human R/N on healthy IDDM subjects, in sleep, daytime sleepiness and daytime cognitive performance.
Figure 2.1.3. Study 3 was a double blind crossover study comparing the effect of beef/pork R/N with Lispro/human ultralente on healthy IDDM subjects, in sleep, daytime sleepiness and daytime cognitive performance.
Figure 2.1.4. Study 4 was a crossover study comparing the effect of twice daily subcutaneous insulin injection versus continuous subcutaneous insulin infusion using an insulin pump healthy IDDM subjects, in sleep, daytime sleepiness and daytime cognitive performance. The data for the subcutaneous intermittent insulin injection was derived from Study 3.
2.1.2 Recruitment of Study Subjects

The study cohort was drawn from the Saskatoon community based on the following inclusion criteria:

a. Healthy, non-diabetic males were selected for the control group, and healthy males with insulin-dependent diabetes mellitus for the experimental group

b. Age between 18 and 30 years

c. Currently enrolled or a graduate of a post-secondary education program

d. Non-smoker

e. Drug free, including prescription and non-prescription drugs.

For subjects with insulin-dependent diabetes mellitus, the following requirements also applied:

f. No clinical or biochemical evidence of retinopathy, nephropathy, neuropathy or cardiovascular disease. Patients must be normotensive and have no protein in their urine on dipstick analysis.

g. On twice daily insulin therapy for at least 6 months preceding the study

h. Age of onset of diabetes was at 6 years or older

The sample size was calculated on the basis of equation 2.1 (Pope, J.E. and Bellamy, N., 1995), where:

\[
n = 2 \times (Z_\alpha + Z_\beta)^2 \times SD^2 / \Delta^2
\]

(2.1)

\[
Z_\alpha = Z \text{ value for Type I error at } \alpha = 0.05
\]

\[
Z_\beta = Z \text{ value for Type II error at } \beta = 0.20
\]
SD = standard deviation
Δ = the magnitude of the difference being sought

The number of subjects required to detect a clinically significant difference of 20% between treatments, with a level of significance (α) of 0.05, and a degree of certainty (1 - β) of 80%, given a standard deviation of each observation of 15%, based on our initial pilot study, the minimum number of each treatment group was found to be 7. We elected to enroll eight subjects in each group to account for dropouts or data lost through analysis.

Subjects were invited to participate through posted advertisement at the University of Saskatchewan and Royal University Hospital, and an advertisement in the Saskatchewan Diabetes Education News Letter. People would call me and we would arrange an interview. At the first meeting, the entire research protocol and the potential risks and benefits of participating in the study were explained. If an interest was expressed, they then filled out and signed the consent form (APPENDIX 1) which had been approved by the University Advisory Committee on Ethics in Human Experimentation and an appointment was arranged with Dr. R. J. Herman for a physical examination and education regarding switching of insulins and management of the insulin pump.

2.1.3 Measurements
2.1.3.1 Preparations

Each phase of the study began with a 2 week observational period. Subjects, regardless of group, were asked to wake up in the morning and to go to bed at night at
approximately the same time every day and to maintain an overall sleep duration of at least 7.5 hours. Sleep behavior was recorded in a sleep diary (APPENDIX 2). Diabetic subjects were also required to monitor insulin/glycemic control at least four times daily twice a week. Blood sugar was measured using a glucose oxidase test strip and a glucometer (Companion™2, Medisense) both provided to patients without charge over the course of the study. Measurement were taken before breakfast, before lunch, before dinner, and before retiring for sleep, and recorded in the sleep diary. Advice was given to subjects in order to avoid hypoglycemic episodes during the study interval. If hypoglycemia occurred, patients were to call Dr. Herman to obtain specific instructions on diet and insulin dose adjustment. Finally, three days prior to study, subjects were asked to avoid all napping, and to reduce their consumption of alcohol and caffeine-containing beverages. These instructions were accompanied by a page-long written summary (APPENDIX 3) given to the patient with his insulins and test kit at the beginning of the study.

2.1.3.2 Overnight Polysomnography

Subjects came to the sleep research laboratory at Royal University Hospital in Saskatoon at 9:00 p.m. on a weekend evening for the measurement of Overnight Polysomnography (PSG). The sleep diary of each subject was analyzed. If there was any indications that sleep or behavior deviated from that outlined above, the sleep study would be postponed and the patient asked to prepare himself better for the next sleep study.

Six channel montages were used to monitor sleep stages. Those channels were: two electroencephalogram (EEG) channels, namely C3/A2 and C4/A1; two electrooculogram (EOG) channels on the right (ROC/A1) and left outer canthus
(LOC/A₂); one submentalis electromyography (EMG) channel; and one electrocardiograph (ECG) channel. Sleep was recorded on paper with a paper speed of 10 mm/second and a pen deflection of 10 mm per 50 microvolts. The C₃, C₄, ROC, LOC, A₁, and A₂ low filter frequencies were 0.3 Hz, the high filter frequencies were 35 Hz, and a sensitivity of 50 μV/cm. The EMG low filter frequency was 10.0 Hz, the high filter frequency was 70 Hz, and the sensitivity was set at 20 μV/cm. The ECG low filter frequency was 1 Hz, the high filter frequency was 15 Hz, and the sensitivity was 70 μV/cm (Carskadon, M.A., 1982a).

Sleep monitoring started with a biocallibration procedure. During biocallibration, subjects were asked to relax and lie quietly on their backs in bed. We asked them to open their eyes for 60 seconds, and then to close their eyes, but to remain awake for another 60 seconds. Next, we asked them to open their eyes, to look straight ahead, to the left, right, straight ahead again, up, down, to blink 5 times, and finally, to grit their teeth. The purpose of this was to determine if there were any problems so that they could be resolved prior to the PSG recording. Biocallibration was also very important as a reference for the person doing the sleep scoring, particularly if there were any questionable epochs of wakefulness or movement. Fifteen minutes before “lights out”, subjects were asked to measure their blood glucose levels, encouraged to use the restroom, and to fill in a pre-sleep questionnaire (see APPENDIX 4). At exactly 23:00, the lights were turn out and sleep recording commenced. The duration of the PSG was seven and a half hours, during which time the subject’s sleep pattern was continuously monitored.

At 6:30 am, the lights in the room were turned on and subjects were wakened. A fasting blood sugar and a post-sleep questionnaire (see APPENDIX 5) were obtained. If the morning blood glucose level was 3.0 mM or below, the recording was
rejected and the subject asked to repeat the examination on another day. If everything was satisfactory, the patients proceeded to MSLT and psychometric testing.

The sleep stages, awake, stage-1, 2, 3, 4, and REMS, were scored manually according to the Rechtschaffen and Kales standard manual (Rechtschaffen, A. and Kales, A., 1968). The scoring was done blinded to the subjects and to their insulin treatment. Movement arousal was diagnosed when there was a change in the EEG activity accompanied by an increase in the amplitude of EMG for no longer than one half of the sleep epoch.

Other parameters and their definitions were (Williams R.L., et al., 1974):

a. Sleep onset latency - time elapsed from “lights out” until the first two consecutive epochs of stage-1 sleep
b. Latency to stage-2 sleep - time to the first epoch of stage-2 sleep
c. Latency to SWS - time to the first epoch of stage-3 sleep
d. REM onset latency - time to the first epoch of REMS
e. Number of awakenings - awakenings after the first onset of sleep
f. Number of stage shifts - Number of changes from sleep stage one to another stages during the PSG.
g. Number of REM sleep periods - number of REMS cycles during the PSG.
h. Total amount of time (minutes) spent in stage-1, 2, 3 and 4
i. Total amount of time (minutes) spent in SWS - total time spent in stage-3 sleep plus stage-4 sleep
j. Total amount of time (minutes) spent in SWS in the first sleep cycle
k. Total amount of time (minutes) of each sleep stage during the PSG
l. Total amount of time (minutes) spent in SWS in the first four hours of sleep
m. Total amount of time (minutes) spent in REMS
n. Total amount of time (minutes) spent in REMS from hours five to seven and a half

o. Wake time after sleep onset (WASO) - time awake after the onset of sleep

p. Total amount of time (minutes) of movement arousal during the PSG

q. Total bed time (TBT) - time period when the subject is settled in bed with the electrodes attached, from the time of “lights out” until the time of “lights on”. For the purposes of this study TBT was set to seven and one half hours.

r. Sleep period time (SPT) - the time in bed minus the time awake prior to sleep onset.

s. Total sleep time (TST) - SPT minus the time awake after sleep onset.

t. Sleep efficiency - percentage of TST to TBT

These measurements emphasize the ease of falling asleep, the distribution and quantity of SWS, the distribution and quantity of REMS, REM onset latency, and wakefulness following sleep onset.

2.1.3.3 Multiple Sleep Latency Test

The Multiple Sleep Latency Test (MSLT) was performed on the day after Overnight Polysomnography. The procedure and analysis followed the guidelines recommended by the American Sleep Disorders Association (The American Sleep Disorders Association, 1992). We conducted four stages of MSLT with individual tests repeated at two hourly intervals. The first stage of the MSLT was carried out at 9:00 a.m. which was two and a half hours after the end of nocturnal recording. Subsequent MSLTs were at 11:00, 13:00 and 15:00 hours. The MSLT was set in a dark, quiet, sleep-inducing environment with a room temperature of about 22°C.
Between MSLTs, the subjects activities were monitored and napping was strictly prohibited.

Six channel montages were used to monitor sleep during the MSLT. These included: two electroencephalograph (EEG) channels which were C3/A2 and C4/A1, two electrooculography (EOG) channels, on the right (ROC/A1) and left (LOC/A2) outer canthus, one submentalis electromyography (EMG) channel, and one electrocardiography (ECG) channel. Sleep was recorded on paper with a speed of 15 mm/sec. The pen deflection was 10 mm for 50 microvolt. The C3, C4, ROC, LOC, A1, and A2, low filter frequency was 0.3 Hz, the high filter frequency was 35 Hz, and the sensitivity was 50 μV/cm. The submentalis EMG low filter frequency was 10.0 Hz, the high filter frequency was 70 Hz, and the sensitivity was 20 μV/cm. The ECG low filter frequency was 1 Hz, the high filter frequency was 15 Hz, and the sensitivity was 70 μV/cm.

On the day of the MSLT study, all subjects dressed in daytime clothing. All activities were suspended fifteen minutes before each test period. At this time subjects were encouraged to use the restroom, and then to measure a blood glucose level and to fill out the pre-MSLT questionnaire (APPENDIX 6). In the remaining time, subjects were asked to relax in bed while biocallibration was performed. The MSLT recording was started at exactly 9:00, 11:00, 13:00 and 15:00. Subjects were instructed to close their eyes, and to lie quietly and not resist falling asleep while the lights were turned off. The subject’s polygraph was carefully observed over the next 20 minutes. If two consecutive epochs of sleep appeared after five minutes, the observation period would be extended for a further 15 minutes to observe the nature of the sleep. At the end of this time, the room light was turned on and subjects were asked to complete a post-MSLT questionnaire (APPENDIX 7). Immediately
thereafter, subjects were required to leave the bedroom and to entertain themselves
with their preferred activities.

The parameters measured during the MSLT were:

a. Sleep onset latency defined as the time from “lights out” until the first appearance
   of two consecutive epochs of stage-1 sleep.

b. Latency to stage-2 sleep, SWS and REMS (minutes)

c. Number of sleep episodes in the four test periods

d. Number of REMS episodes in the four test periods

e. Clinical state before and after sleep, including fatigue, sleepiness and pain, as
   measured on the pre- and post-sleep questionnaires

Sleepiness was estimated by the average sleep onset latency on the four MSLT stages.

2.1.3.4 Psychometric Testing

There were four psychometric tests that we administered to all subjects to assess
the effects of insulin on daytime cognitive performance. They were: 1) the Digit
Symbol Substitution Test (DSST), 2) the Symbol Digit Modalities Test (SDMT), 3)
the Grooved Peg Board Test (GPBT), and 4) the Manual Finger Tapping Test
(MFTT). These tests were administered under basal conditions on the day of, and at
times separating, the four stages of the MSLT. To control for the effects of learning
subjects were required to practice the tests three times on the day prior to the study
date, in the hour before initiation of the overnight PSG recording. Each time the test
battery was administered, the test sequence was randomized and numeric/symbol
pairing were changed.
The purpose of the testing under basal conditions was to see the influence of insulin on cognitive performance and the variability of cognitive performance throughout the day. The tests were administered at 8:00, 10:00, 12:00, and 14:00. Before each sequence of psychometric tests, a blood sugar level was measured.

The Digit Symbol Substitution Test is a paper and pencil speed test, which measures a subject’s ability to concentrate and maintain attention on a memory related task. Motor persistence, sustained attention, response speed, visuomotor coordination and short-term memory play important roles in determining an individuals performance on this test. In addition, performance has been found to be influenced by the level of education, gender, type of job and age of the subject. Intellectual prowess, memory, or learning generally do not affect a person’s score (Lezak, M.D., 1995). The test consists of 113 blank squares, each of which is paired with a randomly assigned number from one to nine. On the top of the page is a key, which matches each number with a nonsense symbol. The task is to fill in the blank squares as quickly as possible with the correctly paired nonsense symbol with the score being the number of correct responses within a ninety second test period (Lezak, M.D., 1995). False responses are recorded separately. A sample and test instructions are shown in APPENDIX 8 and 9.

The SDMT is also a paper and pencil speed test. The task is similar to the DSST, only here the subject is asked to fill in 110 blank squares with numbers that have been previously paired to nonsense symbols. Again, the key is shown at the top of the page and the score is the number correct responses within a 90 second test period (Lezak, M.D., 1995). The test instructions and a sample test are shown in APPENDIX 9 and 10. The SDMT assesses complex scanning, short term memory, and visual tracking. Manual speed and agility contribute significantly to the SDMT performance. As with the DSST, performance is influenced by the level of education,
gender, type of job and age of the subject (Lezak, M.D., 1995). Repeat testing shows a 7% fluctuation in scores with a correlation coefficient of 0.80. The SDMT score is generally slightly lower than the DSST, but shows a high correlation to the latter. The SDMT is usually more sensitive than the DSST for detecting acute or chronic cerebral dysfunction (Lezak, M.D., 1995).

The Manual Finger Tapping Test (MFTT) is a manual dexterity test. It consists of a tapping key with a device to record the number of taps. Each subject is asked to tap the index finger of the dominant hand on the tapping key as fast as possible, while the other fingers and palm of the hand stay motionless on the tapping board. Test instruction is shown in APPENDIX 11. The score is the average number of taps on three 10-second trials with a brief rest period between trials, and is recorded on the score sheet (APPENDIX 12). The MFTT is affected by age, education, gender, and type of job (Lezak, M.D., 1995). The test is also sensitive to the effects of sedatives and anticonvulsant medications. It has been suggested that repeated trials cause fatigue and that the scores on test performed at later times will be lower than the scores obtained earlier. However, a study of normal subjects showed that there was no fatigue after ten consecutive administrations of the test, although a small, but significant, increase in tapping frequency was observed after weekly repeated testing over a period of ten weeks. The MFTT appears to be highly reliable on repeat testing with correlation coefficient of 0.94 (Gill, D.M., et al., 1986).

The Grooved Pegboard Test (GPBT) is also a manual dexterity test. It consists of a small board of 5 by 5 slotted holes angled in different directions and a well containing 25 pegs. Each peg has a ridge along one side requiring it to be rotated into the correct position for insertion. The task is to take the pegs, one at a time using the dominant hand, and to insert them into the slotted holes as quickly as possible in a predetermined sequence of left to right. Falling pegs are considered an error and can
not be taken and reinserted into the holes. The score is the time of completion of the board. Errors are reported separately on a score sheet (APPENDIX 12). The GPBT is a complex test that is sensitive to the effect of sedating medication. GPBT performance is also affected by age, education, gender, and type of job (Lezak, M.D., 1995). Test-retest reliability for the GPBT is high with a correlation coefficient of 0.82. The instructions of the test are shown in APPENDIX 13.

2.1.4 Statistics

The first study was a parallel comparison of a treated and untreated control group. The other three studies employed a crossover design where the subject was his own control. The two treatments to be compared in each of the four studies were: 1) patients with IDD on insulin versus non-diabetic controls, 2) human R/N insulin versus beef/pork R/N insulin, 3) lispro/human UL insulin versus beef/pork R/N insulin, and 4) intermittent twice daily insulin injection versus continuous subcutaneous insulin infusion using an insulin pump. Other factors considered to influence the treatment outcome were the first-night sleep effect, sequence of insulin treatments, and between subject variability. The three non-treatment conditions, were, thus, quantified using a three factor-factorial design. The first factor was Group. Two groups were assigned according to the type of insulin received in the first treatment session (e.g., human insulin in the first session versus beef/pork insulin in the first session). The second factor was Subject (Group), where the variability between the eight subjects was controlled within each group. The third factor was Session. There were two sessions, the first session and the second session, regardless of insulin treatment. Statistical analysis employed two way Analysis of Variance for a repeated measurement design with a four-factor factorial: Group (df = 1), Subject(Group) (df =
6), Session (df = 1) and Insulin (df = 1). The statistical analysis was computerized using the SAS statistical software Version 6.08 (SAS Institute Inc., Cary, NC). The probability of a Type I error of 0.05 was accepted as the level of significance.

2.2 Materials

2.2.1 Human Subjects

<table>
<thead>
<tr>
<th>Subjects</th>
<th>Mean ± SE</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Diabetics</td>
<td></td>
</tr>
<tr>
<td>Age (years)</td>
<td>21.78 ± 1.06</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>25.23 ± 0.99</td>
</tr>
<tr>
<td>Age at the onset of diabetes (years)</td>
<td>13.33 ± 2.35</td>
</tr>
<tr>
<td>Duration of diabetes (years)</td>
<td>8.78 ± 1.92</td>
</tr>
<tr>
<td>Daily insulin dose (Unit/Kg Body Weight)</td>
<td></td>
</tr>
<tr>
<td>Human R/N (n = 7)</td>
<td>0.83 ± 0.09</td>
</tr>
<tr>
<td>Beef/Pork Connaught Novo Nordisk R/NPH (n = 7)</td>
<td>0.83 ± 0.09</td>
</tr>
<tr>
<td>Beef/Pork Iletin I R/N (n = 8)</td>
<td>0.77 ± 0.06</td>
</tr>
<tr>
<td>Lispro /Human Ultralente (n = 8)</td>
<td>0.77 ± 0.06</td>
</tr>
<tr>
<td>Continuous Subcutaneous Insulin Infusion (n = 8)</td>
<td>0.76 ± 0.07</td>
</tr>
</tbody>
</table>

2. Control Subjects (n = 8)

<table>
<thead>
<tr>
<th>Subjects</th>
<th>Mean ± SE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>21.25 ± 0.80</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>23.40 ± 0.74</td>
</tr>
</tbody>
</table>
2.2.2 Insulin, Insulin Pump and Blood Glucose Testing System

2.2.2.1 Insulin

The types of insulin used in this study are abstracted in the Table 2.2.

Table 2.2.2. Insulin species, trade names, manufacturers, preparations, administration, and related studies.

<table>
<thead>
<tr>
<th>Species</th>
<th>Trade name</th>
<th>Manufacturer</th>
<th>Preparation</th>
<th>Administration</th>
<th>Stud</th>
</tr>
</thead>
<tbody>
<tr>
<td>Human</td>
<td>Humulin R</td>
<td>Eli Lilly&lt;sup&gt;*)&lt;/sup&gt;</td>
<td>Regular</td>
<td>Subcutaneous injection</td>
<td>2</td>
</tr>
<tr>
<td>Human</td>
<td>Humulin N</td>
<td>Eli Lilly&lt;sup&gt;*)&lt;/sup&gt;</td>
<td>NPH</td>
<td>Subcutaneous injection</td>
<td>2</td>
</tr>
<tr>
<td>Human</td>
<td>Humulin U</td>
<td>Eli Lilly&lt;sup&gt;*)&lt;/sup&gt;</td>
<td>Ultralente</td>
<td>Subcutaneous injection</td>
<td>3</td>
</tr>
<tr>
<td>Beef/Pork</td>
<td>Insulin-Toronto</td>
<td>Connaught Novo Nordisk&lt;sup&gt;**)&lt;/sup&gt;</td>
<td>Regular</td>
<td>Subcutaneous injection</td>
<td>2</td>
</tr>
<tr>
<td>Beef/Pork</td>
<td>Regular</td>
<td>Eli Lilly&lt;sup&gt;*)&lt;/sup&gt;</td>
<td>Regular</td>
<td>Subcutaneous injection</td>
<td>3</td>
</tr>
<tr>
<td>Beef/Pork</td>
<td>Iletin I</td>
<td>Eli Lilly&lt;sup&gt;*)&lt;/sup&gt;</td>
<td>Regular</td>
<td>CSII&lt;sup&gt;***)&lt;/sup&gt;</td>
<td>4</td>
</tr>
<tr>
<td>Beef/Pork</td>
<td>NPH Insulin</td>
<td>Connaught Novo Nordisk&lt;sup&gt;**)&lt;/sup&gt;</td>
<td>NPH</td>
<td>Subcutaneous injection</td>
<td>2</td>
</tr>
<tr>
<td>Beef/Pork</td>
<td>NPH Iletin I</td>
<td>Eli Lilly&lt;sup&gt;*)&lt;/sup&gt;</td>
<td>NPH</td>
<td>Subcutaneous injection</td>
<td>3</td>
</tr>
<tr>
<td>Lispro</td>
<td>Ly275585</td>
<td>Eli Lilly&lt;sup&gt;*)&lt;/sup&gt;</td>
<td>Regular</td>
<td>Subcutaneous injection</td>
<td>3</td>
</tr>
<tr>
<td>Lispro</td>
<td>Ly275585</td>
<td>Eli Lilly&lt;sup&gt;*)&lt;/sup&gt;</td>
<td>Monomeric</td>
<td>CSII&lt;sup&gt;***)&lt;/sup&gt;</td>
<td>4</td>
</tr>
</tbody>
</table>

*) Eli Lilly, Indianapolis, Indiana.

**) Connaught Novo Nordisk, Mississauga, Ontario.

***) Continuous Subcutaneous Insulin Injection
2.2.2.2 Insulin Pump for Continuous Subcutaneous Insulin Infusion

a) H-TRON® V100 (Disentronic Medical Systems, Plymouth, Minnesota)

b) 6C U100 syringe (AutoSyringe, Hooksett, New Hampshire)

c) 42" Sub-Q-Set subcutaneous Infusion Sets (AutoSyringe, Hooksett, New Hampshire)

2.2.2.3 Glucose Oxidase Test Strip Blood Sugar Testing

a) MediSense Companion™2 Blood Glucose Tester (MediSense Inc. Waltham, Massachusetts)

b) MediSense Companion™2 Blood Glucose Sensor Electrodes (MediSense Incorporation Waltham, Massachusetts)

c) MediSense Companion™2 Glucose Calibrator (MediSense Waltham, Massachusetts)

d) MediSense Companion™2 Glucose Test Solution (MediSense Incorporation Waltham, Massachusetts)

e) MediSense Lancing device (MediSense Incorporation Waltham, Massachusetts)

f) Monolet Sterile Lancets (Sherwood Medical, St. Louis, Missouri)
2.2.3 Equipment

2.2.3.1 Electroencephalograph and Accessories

a) *Grass Model 8 (Grass Medical Instrument, Quincy, Massachusetts)
b) Electrode Impedance Meter Model EZM (Grass Medical Instrument, Quincy, Massachusetts)
c) Medi-Trace EEG Recording Charts (Graphic Controls Canada Limited, Gananoque, ON)
d) Silver Disc Electrodes with 60 inch long cable (Grass Medical Instrument, Quincy, Massachusetts)
e) Elefix EEG Electrode Paste (Nihon Kohden America Inc., Irvine, California)
f) SkinPure skin preparation cream (Nihon Kohden America Inc., Irvine, California)

2.2.3.2 Software

3. RESULTS

3.1 Patients with Type-I Diabetes Mellitus vs. Control Subjects

This study involved eight healthy subjects with insulin-dependent diabetes mellitus and eight gender-, age- and BMI-matched, healthy non-diabetic controls. They were males, non-smokers, drug free, and between 18 to 26 year old of age. Among diabetic subjects, age at the onset of diabetes were between 6 to 24 years old, and duration of diabetes were between 2 to 18 years.

Figure 3.1.1 shows the duration of sleep as recorded in the daily sleep diaries obtained by diabetic and control subjects during the week of preparation preceding the overnight sleep studies. The data presented are the mean ± standard error. As can be seen, diabetic and control subjects had a similar duration of sleep during the night. Also, there were no significant differences in duration of sleep during weekday nights and weekend nights in either of the groups. Self-scored sleepiness as measured using the Stanford Sleepiness Scale is shown in Figure 3.1.2. Diabetic patients tended to report slightly less sleepiness immediately prior to and immediately following Overnight Polysomnography. However, none of these differences were significant.

Table 3.1.1 shows the sleep onset latency and stage shifts during Overnight Polysomnography in patients with IDDM versus normal controls. There were no differences between the two groups with respect to the latency to stage-1 and stage-2 sleep, latency to slow-wave sleep, and number of awakenings. However, the latency to REM sleep, number of stage shifts and number of REMS periods were significantly different. The mean latency to REM sleep in subjects with IDDM was 77.82±3.84 min., which is about 30% shorter than that of non-diabetic controls at
111.25±11.77 min. (p = 0.05). The diabetic subjects also had a significantly greater number of stage shifts (83.03±4.90 vs. 61.00±2.69 times per night, p = 0.002) and a significantly greater number of REM sleep periods than non-diabetic controls (8.88±0.71 vs. 5.75±0.77, p = 0.05).

Table 3.1.2 shows the duration of each sleep stage and overall sleep efficiency during Overnight Polysomnography in subjects with IDDM and non-diabetic controls. As can be seen, there are no differences between the two groups in the time spent in stage-1 sleep, REM sleep, REM sleep in the second half of the night, and total sleep time. However, there were differences in that subjects with insulin-dependent diabetes mellitus spent 25% more time in stage-2 sleep (219.45±7.91 min.) than non-diabetic controls (182.06±7.12 min., p = 0.02). Also, the mean duration of slow-wave sleep in diabetic subjects was 82.28±5.01 min., which is 26% shorter than that of non-diabetic controls (111.44±10.42 min., p = 0.02) and the duration of slow-wave sleep in the first sleep cycle was 40.36±3.55 min. also 25% shorter than that of control subjects (53.50±10.82 min. p = 0.05). Non-diabetic controls spent more time awake after sleep onset (49.19±10.10 min.) than diabetic subjects (20.06±4.12 min. p = 0.05) and had a slightly poorer sleep efficiency, although with the latter the differences were only of borderline significance (86.11±1.84% vs. 91.96±1.56%, p = 0.08).

Figure 3.1.2 shows the results of the self-assessment of sleepiness according to the Stanford Sleepiness Scale as administered before each nap of the MSLT in patients with insulin-dependent diabetes mellitus and control subjects. The results of the MSLT, itself, are shown in Table 3.1.3. As can be seen, control subjects reported their sleepiness to be greater than that reported by IDDM subjects at every time point. However, diabetic subjects fell asleep on nearly every nap of the four designated
sleep times in the MSLT, whereas non-diabetic individuals fell asleep only about half the time (3.42±0.22 vs. 2.13±0.35 of a total of four possible sleep periods, p = 0.005). Thus, diabetic subjects had a significantly higher probability of falling asleep during the MSLT than non-diabetic individuals. Moreover, the sleep onset latency for diabetic subjects was 9.96±1.32 minutes compared to 15.09±1.42 minutes for non-diabetic individuals (p = 0.02). The sleep onset latency in the four potential sleep periods is shown in Figure 3.1.3. Sleepiness decreased towards the late afternoon in diabetic patients, while in control subjects, sleepiness was greatest at the midday. Patients with IDDM had shorter sleep onset latencies than normal control in all sleep periods with differences reaching statistical significance level at 9:00 and 13:00 hours. Also, 5 of 8 diabetic subjects experienced an episode of REM sleep during MSLT, whereas no non-diabetic individual had REM sleep during the test. Figure 3.1.4 indicates the regression and correlation between the Stanford Sleepiness Scale and Sleep Onset Latency during the four stages of the MSLT. Again, diabetics had shorter sleep onset latencies for their level of expressed sleepiness, although correlations for the two groups were very weak.

Daytime cognitive performance as measured by the Digit Symbol Substitution Test (DSST), Symbol Digit Modalities Test (SDMT), Grooved Pegboard Test (GPBT) and Manual Finger Tapping Test (MFTT) at 8:00, 10:00, 12:00 and 14:00 hours in patients with IDDM and control subjects are shown in Table 3.1.4 and Figures 3.1.5, 3.1.6, 3.1.7 and 3.1.8. None of the differences between diabetic and non-diabetic subjects were significant, although diabetics had significantly greater variability in their performance throughout the day. Moreover, when examined for the different insulin species and modes of insulin administration, diabetic subjects consistently scored better than non-diabetic controls on most tests of vigilance, short-term memory, visual-motor coordination, and response speed (i.e., the SDMT and
DSST) at most time points. Specifically, the DSST baseline score, as shown on Figure 3.1.5, was significantly better in patients with diabetes mellitus at 10:00 and 12:00 hours. Similarly, IDDM subjects performed significantly better than control subjects on the SDMT (Figure 3.1.6) at 10:00, 12:00, and 14:00 hours. On tests of motor coordination, speed and dexterity, such as the GPBT and MFTT, there were no differences between controls and diabetics (Figures 3.1.7 and 3.1.8).
Figure 3.1.1. The time spent in sleep as recorded in the daily sleep diaries of diabetic and control subjects during the whole week, weekday nights and weekend nights in the days preceding the overnight sleep studies. Data are means ± S.E.. None of the differences between control and diabetic groups or between weekdays and weekends reached the level of significance at $p \leq 0.05$. 
Figure 3.1.2. Stanford Sleepiness Scores measured immediately before bed time and immediately after wake time during Overnight Polysomnography, and immediately before every nap during the four stages of the MSLT. The data are means ± S.E. * indicates that differences were significant at $p \leq 0.05$. 
Table 3.1.1. Sleep onset latencies and stage shifts during Overnight Polysomnography in subjects with insulin-dependent diabetes mellitus versus normal controls. Data are mean ± S.E.

<table>
<thead>
<tr>
<th></th>
<th>DIABETICS</th>
<th>CONTROLS</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>n = 8</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Latency to Stage-1 Sleep (min.)</td>
<td>16.03±4.18</td>
<td>14.81±4.97</td>
<td>0.74</td>
</tr>
<tr>
<td>Latency to Stage-2 Sleep (min.)</td>
<td>2.81±0.44</td>
<td>3.56±0.65</td>
<td>0.40</td>
</tr>
<tr>
<td>Latency to SWS (min.)</td>
<td>17.92±1.73</td>
<td>24.06±8.91</td>
<td>0.52</td>
</tr>
<tr>
<td>Latency to REM Sleep (min.)</td>
<td>77.82±3.84</td>
<td>111.25±11.77</td>
<td>0.05</td>
</tr>
<tr>
<td>Number of Awakenings</td>
<td>10.38±1.41</td>
<td>8.88±1.13</td>
<td>0.28</td>
</tr>
<tr>
<td>Number of Stage Shifts</td>
<td>83.03±4.90</td>
<td>61.00±2.69</td>
<td>0.002</td>
</tr>
<tr>
<td>Number of REMS Periods</td>
<td>8.88±0.71</td>
<td>5.75±0.77</td>
<td>0.05</td>
</tr>
</tbody>
</table>
Table 3.1.2. Duration of each stage and overall sleep efficiency during Overnight Polysomnography in subjects with IDDM versus normal controls. Data are mean ± S.E.

<table>
<thead>
<tr>
<th>n = 8</th>
<th>DIABETICS</th>
<th>CONTROLS</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Duration of Stage-1 Sleep (min.)</td>
<td>17.18±2.17</td>
<td>14.13±2.07</td>
<td>0.37</td>
</tr>
<tr>
<td>Duration of Stage-2 Sleep (min.)</td>
<td>219.45±7.91</td>
<td>182.06±7.12</td>
<td>0.02</td>
</tr>
<tr>
<td>Duration of SWS (min.)</td>
<td>82.28±5.01</td>
<td>111.44±10.42</td>
<td>0.02</td>
</tr>
<tr>
<td>Duration of SWS in the 1st Cycle (min.)</td>
<td>40.36±3.55</td>
<td>53.50±10.82</td>
<td>0.05</td>
</tr>
<tr>
<td>Duration of SWS in the 1st 4 hours (min)</td>
<td>67.54±4.86</td>
<td>73.88±9.22</td>
<td>0.41</td>
</tr>
<tr>
<td>Duration of REMS (min.)</td>
<td>84.62±4.33</td>
<td>75.81±4.64</td>
<td>0.28</td>
</tr>
<tr>
<td>Duration of REMS at the end of Sleep (min.)</td>
<td>54.24±2.78</td>
<td>50.38±3.95</td>
<td>0.53</td>
</tr>
<tr>
<td>Duration of Wakefulness After Sleep Onset (min.)</td>
<td>20.06±4.12</td>
<td>49.19±10.10</td>
<td>0.05</td>
</tr>
<tr>
<td>Sleep Efficiency (%)</td>
<td>91.96±1.56</td>
<td>86.11±1.84</td>
<td>0.08</td>
</tr>
<tr>
<td>Total Sleep Time (min.)</td>
<td>410.42±8.00</td>
<td>387.50±8.30</td>
<td>0.16</td>
</tr>
</tbody>
</table>
Figure 3.1.3. Mean sleep onset latency on the MSLT at 9:00, 11:00, 13:00 and 15:00 hours in insulin-dependent diabetic subjects and normal controls. * indicates differences were significant at $p \leq 0.05$. 
Figure 3.1.4. Regression between sleep onset latency and the score on the Stanford Sleepiness Scale taken immediately before each stage of the MSLT. The regression equation and the correlation coefficient for the diabetic groups were: beef/pork R/NPH, $y = -0.74x + 11.66$, $r = 0.12$; lispro/human ultralente, $y = -2.86x + 14.80$, $r = 0.40$; insulin pump, $y = -1.29x + 12.44$, $r = 0.19$. For the control group, this was $y = -0.87x + 17.56$, and $r = 0.15$. 
Table 3.1.3. Sleep onset latencies, number of sleep periods and number of subjects with REMS during the MSLT in subjects with IDDM versus normal controls. Data are mean ± S.E.

<table>
<thead>
<tr>
<th>n = 8</th>
<th>DIABETICS</th>
<th>CONTROLS</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Latency to Stage-1 Sleep (min.)</td>
<td>9.96±1.32</td>
<td>15.09±1.42</td>
<td>0.02</td>
</tr>
<tr>
<td>Latency to Stage-2 Sleep (min.)</td>
<td>7.61±1.69</td>
<td>12.83±1.47</td>
<td>0.08</td>
</tr>
<tr>
<td>Number of Sleep periods</td>
<td>3.42±0.22</td>
<td>2.13±0.35</td>
<td>0.005</td>
</tr>
<tr>
<td>Number of Patients with REMS</td>
<td>5</td>
<td>0</td>
<td></td>
</tr>
</tbody>
</table>

Table 3.1.4. Daytime cognitive performance and its variability as measured by the Digit Symbol Substitution Test (DSST), the Symbol Digit Modalities Test (SDMT), the Manual Finger Tapping Test (MFTT), and the Grooved Pegboard Test (GPBT) in subjects with IDDM versus normal controls. Data are mean ± S.E.

<table>
<thead>
<tr>
<th>n = 8</th>
<th>DIABETICS</th>
<th>CONTROLS</th>
<th>p-value MEANS</th>
<th>p-value VARIABILITY</th>
</tr>
</thead>
<tbody>
<tr>
<td>DSST SCORE</td>
<td>84.43±4.56</td>
<td>79.06±1.72</td>
<td>0.29</td>
<td>0.006</td>
</tr>
<tr>
<td>SDMT SCORE</td>
<td>73.01±3.57</td>
<td>67.19±1.73</td>
<td>0.16</td>
<td>0.03</td>
</tr>
<tr>
<td>MFTT SCORE</td>
<td>55.30±1.99</td>
<td>54.14±0.78</td>
<td>0.59</td>
<td>0.008</td>
</tr>
<tr>
<td>GPBT SCORE</td>
<td>51.15±1.64</td>
<td>52.19±1.82</td>
<td>0.68</td>
<td>0.39</td>
</tr>
</tbody>
</table>
Figure 3.1.5. Mean daytime cognitive performance on the Digit Symbol Substitution Test (DSST) in patients with IDDM versus control subjects. * indicates that differences were significant at $p \leq 0.05$. 
Figure 3.1.6. Mean daytime cognitive performance on the Symbol Digit Modalities Test (SDMT) in diabetic versus control subjects. * indicates that differences were significant at $p \leq 0.05$. 
Figure 3.1.7. Mean daytime cognitive performance on the Grooved Pegboard Test (GPBT) in diabetic versus control subjects. * indicates that differences were significant at p ≤ 0.05.
Figure 3.1.8. Mean daytime cognitive performance on the Manual Finger Tapping Test (MFTT) in IDDM versus control subjects. None of the differences were significant at $p \leq 0.05$. 
3.2 Human R/N vs. Beef/Pork R/NPH Insulin

Eight healthy male subjects with insulin dependent diabetes mellitus, participated in the study. However, one of them was subsequently withdrawn when it was learned that he also had narcolepsy. Thus, the final analyses examined only seven subjects. Inclusion or exclusion of this latter individual did not alter the results of the study. The mean age of the study group (n=7) was 22.0±3.1 years. Age at onset of diabetes was 14.43±2.82 years, and the duration of diabetes was 7.8±1.09 years. Biochemical laboratory data of the seven subjects with IDDM showed that the blood protein, albumin, AST, ALP, Gamma GT, creatinine, urea, uric acid, cholesterol, triglycerides and other blood test and urinalysis were normal. Table 3.2.1 displays the blood glucose level, as measured by glucometer, pre-breakfast, pre-lunch, pre-supper and at bed-time, while on treatment with intermittent twice daily injections of beef/pork R/NPH and human R/N insulin, in the week prior to the study and during the study, itself. In general, blood sugar control was acceptable for patients with Type-I diabetes mellitus and was similar on randomization to human R/N and beef/pork R/NPH insulins. Indeed, blood sugars differed at only one time point during “at home” blood glucose testing.

The results of Overnight Polysomnography in diabetic subjects while on intermittent human R/N versus beef/pork R/NPH insulin are shown in Tables 3.2.2 and 3.2.3 and Figures 3.2.1 and 3.2.2. As can be seen, the sleep onset latency was significantly shorter while on treatment with human R/N insulin (19.93±4.65 min.) than beef/pork R/NPH insulin (31.00±6.96 min. p = 0.02). Latency to stage-2 sleep, slow-wave sleep, REM sleep, and number of awakenings, stage shifts and REM sleep periods were not significantly different between treatments. The number of stage
shifts and REM periods were relatively high compared to the previous non-diabetic controls, but there were no differences between insulin cohorts. There were no differences in the duration of stage-1 sleep, stage-2 sleep, slow-wave sleep, time awake after sleep onset, and overall sleep efficiency. However, diabetic subjects on human R/N insulin spent 76.00±7.19 minutes in REM sleep, which is 25% longer than that on beef/pork R/NPH insulin (60.63±10.68 min.). This was significant at \( p = 0.01 \). Moreover, the distribution of SWS and REMS varied considerably between the two treatments. While on human R/N insulin, diabetic subjects spent about twice as much time in SWS in the first hour of sleep (\( p = 0.05 \)) and about twice as much as time in REMS in the last three and a half hours of sleep (\( p = 0.05 \)) compared to similar measurements on beef/pork R/NPH insulin. REM sleep normally appears at the end of the night, much like the pattern in human R/N insulin-treated diabetic subjects. On beef/pork insulin, on the other hand, the distribution of REM sleep appeared to be more random, and to lack a specific pattern. Moreover, while diabetics were on beef/pork R/NPH insulin, they had a higher propensity for EEG arousal appearing as sporadic short bursts of alpha activity, especially during stage-2 and slow-wave sleep. However, this finding is difficult to interpret, given methodological problems we encountered in quantifying the power of the alpha activity.

Table 3.2.4 presents the sleep onset latencies, number of sleep periods and number of subjects with REM sleep during the MSLT. While on treatment with human R/N insulin, diabetic subjects were more likely to sleep (3.29±0.42 vs. 2.43±0.57 of the four possible sleep periods during MSLT, \( p = 0.01 \)) and required significantly less time to fall asleep (10.88±2.05 min. vs. 13.72±2.19 min., \( p = 0.02 \)) than on treatment with beef/pork R/NPH insulin. Diabetic subjects were also more likely to exhibit REMS during the MSLT nap while on beef/pork insulin.
Table 3.2.1. Blood glucose level (mM) as measured by glucometer in subjects with IDDM while on treatment with intermittent beef/pork R/NPH and human R/N insulin. Data are mean ± S.E.

<table>
<thead>
<tr>
<th>Time (n = 7)</th>
<th>Human R/N</th>
<th>Beef/Pork R/NPH</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>A. Week Preceding the Study</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pre-Breakfast</td>
<td>5.89±0.47</td>
<td>5.50±0.37</td>
<td>0.25</td>
</tr>
<tr>
<td>Pre-Lunch</td>
<td>6.88±0.47</td>
<td>5.18±0.48</td>
<td>0.04</td>
</tr>
<tr>
<td>Pre-Supper</td>
<td>6.44±0.16</td>
<td>6.11±0.49</td>
<td>0.45</td>
</tr>
<tr>
<td>Bed Time</td>
<td>7.51±0.57</td>
<td>6.76±0.88</td>
<td>0.29</td>
</tr>
<tr>
<td><strong>B. Sleep Study</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bed Time</td>
<td>9.64±1.54</td>
<td>11.37±1.36</td>
<td>0.08</td>
</tr>
<tr>
<td>Wake Time</td>
<td>8.67±1.03</td>
<td>8.40±1.10</td>
<td>0.94</td>
</tr>
<tr>
<td>Pre-MSLT 09:00</td>
<td>13.07±1.08</td>
<td>12.80±0.96</td>
<td>0.74</td>
</tr>
<tr>
<td>Pre-MSLT 11:00</td>
<td>10.62±1.26</td>
<td>9.91±1.06</td>
<td>0.66</td>
</tr>
<tr>
<td>Pre-MSLT 13:00</td>
<td>9.87±0.71</td>
<td>8.53±0.54</td>
<td>0.12</td>
</tr>
<tr>
<td>Pre-MSLT 15:00</td>
<td>10.87±1.27</td>
<td>10.30±1.25</td>
<td>0.66</td>
</tr>
</tbody>
</table>
Table 3.2.2. Sleep onset latencies and stage shifts during Overnight Polysomnography in subjects with insulin-dependent diabetes mellitus while on treatment with human R/N versus beef/pork R/NPH insulin. Data are mean ± S.E.

<table>
<thead>
<tr>
<th></th>
<th>Human R/N</th>
<th>Beef/Pork R/NPH</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>n = 7</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Latency to Stage-1 Sleep (min.)</td>
<td>19.93±4.65</td>
<td>31.00±6.96</td>
<td>0.02</td>
</tr>
<tr>
<td>Latency to Stage-2 Sleep (min.)</td>
<td>2.50±0.33</td>
<td>2.71±0.49</td>
<td>0.47</td>
</tr>
<tr>
<td>Latency to SWS (min.)</td>
<td>16.64±2.34</td>
<td>19.79±2.92</td>
<td>0.64</td>
</tr>
<tr>
<td>Latency to REM Sleep (min.)</td>
<td>103.44±18.11</td>
<td>103.64±22.17</td>
<td>0.79</td>
</tr>
<tr>
<td>Number of Awakenings</td>
<td>7.71±2.04</td>
<td>9.57±1.45</td>
<td>0.34</td>
</tr>
<tr>
<td>Number of Stage Shifts</td>
<td>74.29±6.01</td>
<td>79.29±10.43</td>
<td>0.77</td>
</tr>
<tr>
<td>Number of REM Sleep Periods</td>
<td>8.14±1.12</td>
<td>6.00±0.87</td>
<td>0.14</td>
</tr>
</tbody>
</table>
Table 3.2.3. Duration of each stage, time awake after sleep onset and overall sleep efficiency during Overnight Polysomnography in subjects with IDDM while on treatment with human R/N versus beef/pork R/NPH insulin. Data are mean ± S.E.

<table>
<thead>
<tr>
<th></th>
<th>Human R/N</th>
<th>Beef/Pork R/NPH</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>n = 7</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Duration of Stage-1 Sleep (min.)</td>
<td>19.29±2.98</td>
<td>21.86±3.16</td>
<td>0.53</td>
</tr>
<tr>
<td>Duration of Stage-2 Sleep (min.)</td>
<td>193.14±13.86</td>
<td>217.47±15.33</td>
<td>0.30</td>
</tr>
<tr>
<td>Duration of Total SWS (min.)</td>
<td>81.64±8.72</td>
<td>77.21±8.90</td>
<td>0.88</td>
</tr>
<tr>
<td>Duration of SWS in the 1st Cycle (min.)</td>
<td>42.64±6.53</td>
<td>40.64±5.35</td>
<td>0.96</td>
</tr>
<tr>
<td>Duration of SWS in the 1st 4 hours (min)</td>
<td>66.50±12.02</td>
<td>52.29±6.48</td>
<td>0.87</td>
</tr>
<tr>
<td>Duration of Total REMS (min.)</td>
<td>76.00±7.19</td>
<td>60.63±10.68</td>
<td><strong>0.01</strong></td>
</tr>
<tr>
<td>Duration of REMS at the end of Sleep (min.)</td>
<td>60.58±8.82</td>
<td>31.50±6.33</td>
<td><strong>0.05</strong></td>
</tr>
<tr>
<td>Duration of Wakefulness After Sleep Onset (min.)</td>
<td>47.93±24.01</td>
<td>24.99±9.39</td>
<td>0.17</td>
</tr>
<tr>
<td>Sleep Efficiency (%)</td>
<td>88.29±2.82</td>
<td>83.77±5.92</td>
<td>0.14</td>
</tr>
<tr>
<td>Total sleep Time (min.)</td>
<td>371.21±27.90</td>
<td>392.80±11.71</td>
<td>0.38</td>
</tr>
</tbody>
</table>
Figure 3.2.1. The distribution of SWS throughout the night as recorded during Overnight Polysomnography while patients were on treatment with human R/N insulin and beef/pork R/NPH insulin. Data are means ± S.E.. * denotes that the difference between the two treatments was significant at p ≤ 0.05.
Figure 3.2.2. Distribution of REMS as recorded during Overnight Polysomnography while patients were on treatment with human R/N insulin or beef/pork R/NPH insulin. Data are means ± S.E.. * indicates that the difference between treatments was significant at p ≤ 0.05.
Table 3.2.4. Sleep onset latencies, number of sleep periods and number of subjects with REMS during the MSLT in subjects with IDDM while on treatment with human R/N versus beef/pork R/NPH insulin. Data are mean ± S.E.

<table>
<thead>
<tr>
<th></th>
<th>Human R/N</th>
<th>Beef/Pork R/NPH</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Latency to Stage-1 Sleep (min.)</td>
<td>10.88±2.05</td>
<td>13.72±2.19</td>
<td>0.02</td>
</tr>
<tr>
<td>Latency to Stage-2 Sleep (min.)</td>
<td>10.04±1.47</td>
<td>13.33±2.25</td>
<td>0.16</td>
</tr>
<tr>
<td>Number of Sleep periods</td>
<td>3.29±0.42</td>
<td>2.43±0.57</td>
<td>0.009</td>
</tr>
<tr>
<td>Number of Patients with REMS</td>
<td>0</td>
<td>2</td>
<td>-</td>
</tr>
</tbody>
</table>
3.3 Intermittent Lispro/Human Ultralente Insulin Injection vs. Beef/pork Iletin R/N Insulin Injection

Home blood glucose measurements obtained by diabetic subjects in the week preceding each of the study periods are shown in Table 3.3.1. As can be seen, these indicate a reasonable level of glycemic control. Glucose levels were similar at all time points between all treatment groups. Moreover, no episodes of hypoglycemia were reported.

Blood glucose measurements before and after Overnight Polysomnography, and before each phase of the MSLT at 9:00, 11:00, 13:00 and 15:00 hours are shown in Table 3.3.2. These levels are higher than the blood glucose levels obtained in pre-study monitoring, and are explained by the fact that the majority of them were not fasting or pre-meal measurements and that patients were relatively inactive during the testing period. The wake time fasting and 11:00 hour pre-MSLT blood glucose levels are not dissimilar from those reported in Table 3.3.1. Pre-MSLT blood glucose measurements at 13:00 while on treatment with beef/pork Iletin R/N insulin were 12.41±1.90 mM, which was significantly lower than that of lispro/human ultralente insulin at 14.84±1.87 mM. However, as a rule, blood glucose measurements did not differ between beef/pork Iletin R/N and lispro/human ultralente insulin treatment arms. Hypoglycemia was found in one patient on arising from an overnight PSG study. The experiment was subsequently canceled and repeated at a later date. Long term glucose control, as measured by the HbA$_{1c}$ and Glycated Protein, is shown in Table 3.3.3. The HbA$_{1c}$ level for beef/pork Iletin R/N insulin was 7.70±0.64% and for lispro/human ultralente insulin it was 7.81±0.60%. Glycated Protein for beef/pork
Iletin R/N was 362.5±26.3 μM, and for lispro/human ultralente insulin it was 377.1±24.5 μM. These measurements were not significantly different between treatments.

Table 3.3.5 shows the sleep onset latencies and stage shifts during Overnight Polysomnography of diabetic subjects while on treatment with beef/pork Iletin R/N and lispro/human ultralente insulins. In this table, intermittent injection denotes the average of the corresponding beef/pork Iletin R/N and lispro/human ultralente measurements. As can be seen, there were no differences between treatments with respect to the sleep onset latency, latency to stage-2 sleep, latency to SWS, latency to REM sleep, number of awakenings, number of stage shifts or number of REMS periods. Moreover, these measurements were similar to those observed for diabetic subjects in the control study (Table 3.1.1) with the only exception being that the latency to stage-1 sleep tended to be shorter while subjects were on beef/pork Iletin R/N compared to the Connaught Novo Nordisk R/NPH formulation (13.44±4.83 min. vs. 31.00±6.96 min. p = 0.11). Sleep latencies were even shorter on lispro/human ultralente insulin (7.88±2.57 min.). Also, we continued to see a large number of stage shifts and REM periods in these individuals. Sleep efficiencies were good on both insulin treatments.

The time spent in the various sleep stages and the overall sleep efficiency during Overnight Polysomnography are presented in Table 3.3.9. The amount of time spent on stage-1 sleep was significantly greater in patients while on lispro/human ultralente insulin compared to beef/pork Iletin R/N insulin (p=0.02). The time spent in stage-2 sleep and other sleep stages were similar for both insulin treatments. However, the duration of stage-2 sleep, again, was very high and the overall duration of SWS, the duration of SWS in the first sleep cycle and the duration of SWS in first four hours of sleep on both insulin treatments were even shorter than those seen in the
control study. The duration of REM sleep was about 10% greater and the duration of REM sleep in the last three and a half hours of the sleep was about 20% greater while on beef/pork Iletin R/N than on lispro/human ultralente insulin (55.44±6.06 min. vs. 45.19±8.42 min. p=0.03). However, the REM times seen in this study for beef/pork Iletin R/N insulin-treated patients were greater than the REMS times seen with the Connaught Novo Nordisk beef/pork R/NPH insulin formulation used in the previous human insulin-beef/pork insulin study (Table 3.2.3), although neither of these measurements were significantly different from controls (Table 3.1.2). Beef/pork insulin-treated diabetics appeared to spend less time awake after sleep onset and this was also seen in the control and human insulin-beef/pork insulin comparisons. However, the variability in this parameter was quite large and the differences were not significant. Sleep efficiency and total sleep time were equal on both insulin treatments.

Table 3.3.13 shows the sleep onset latencies and number of sleep periods during daytime napping on the 4 stage MSLT (9:00, 11:00, 13:00 and 15:00 hours) in diabetic patients, while on treatment with lispro/human ultralente and beef/pork Iletin R/N insulins. There were no differences between treatments on the latency to stage-1 sleep, stage-2 sleep and number of sleep periods. However, as in the former studies, sleep onset latencies tended to be very short in diabetic subjects and most attained sleep in three or more of the potential sleep periods. It is interesting to note that none of the diabetic subjects had REM sleep during the daytime napping while on treatment with lispro/human ultralente insulin. Nevertheless, one of them did have a REM sleep period during treatment with beef/pork Iletin R/N insulin. This was also the case in the beef/pork-human insulin study.
Daytime cognitive performance as measured by the DSST, SDMT, MFTT, GPBT at 8:00, 10:00, 12:00 and 14:00 are shown on Table 3.3.17. These indicate that there were no differences between beef/pork Iletin R/N and lispro/human ultralente insulin in any of the composite performance scores. Also, variability in the test performances at the different testing times were not significantly different. However, there were differences between treatments at certain times during the day. For example, diabetic subjects on lispro/human ultralente insulin showed a slight decrement in baseline performance on the SDMT early in the day compared to the insulin pump (Figure 3.3.4). On the other hand, performance was worse on the GPBT on beef/pork Iletin R/N insulin (Figure 3.3.5). The DSST and MFTT (Figure 3.3.6) were too variable to draw conclusions.
Table 3.3.1. Pre-study (at home monitoring) blood glucose level (mM) as measured by glucometer in patients with insulin-dependent diabetes mellitus while on treatment with intermittent beef/pork R/N insulin, intermittent lispro/human ultralente insulin and intermittent injection matched for insulin species with continuous subcutaneous insulin using an insulin pump. Data are mean ± S.E.

<table>
<thead>
<tr>
<th></th>
<th>n = 8</th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th>p-values for Contrasts</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Beef/Pork R/N</td>
<td>Lispro/Hu. UL</td>
<td>Intermittent Injection</td>
<td>Insulin Pump</td>
<td>A</td>
<td>B</td>
<td>C</td>
<td>D</td>
</tr>
<tr>
<td>Pre-Breakfast</td>
<td>6.76±0.97</td>
<td>7.63±0.51</td>
<td>6.94±0.66</td>
<td>7.74±0.63</td>
<td>0.45</td>
<td>0.27</td>
<td>0.85</td>
<td>0.25</td>
</tr>
<tr>
<td>Pre-Lunch</td>
<td>8.26±1.00</td>
<td>7.46±1.12</td>
<td>7.72±0.89</td>
<td>7.80±1.09</td>
<td>0.30</td>
<td>0.51</td>
<td>0.77</td>
<td>0.92</td>
</tr>
<tr>
<td>Pre-Supper</td>
<td>7.81±0.82</td>
<td>8.59±1.24</td>
<td>7.50±0.59</td>
<td>6.77±0.65</td>
<td>0.55</td>
<td>0.21</td>
<td>0.18</td>
<td>0.36</td>
</tr>
<tr>
<td>Bed Time</td>
<td>8.78±1.02</td>
<td>8.52±0.63</td>
<td>8.25±0.66</td>
<td>8.90±1.03</td>
<td>0.73</td>
<td>0.93</td>
<td>0.61</td>
<td>0.45</td>
</tr>
<tr>
<td>2:00 am</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>7.54±0.87</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

Note: Contrast A: Beef/Pork R/N versus Lispro/Hu. UL. Contrast C: Lispro versus the Insulin Pump. Hu. UL = Human Ultralente Insulin

Contrast B: Beef/Pork R/N versus the Insulin Pump
Contrast D: Intermittent Injection versus the Insulin Pump
Table 3.3.2. Blood glucose level (mM) during sleep study as measured by glucometer in patients with IDDM while on treatment with intermittent beef/pork R/N insulin, intermittent lispro/human ultralente insulin, and intermittent injection matched for insulin species with continuous subcutaneous insulin using an insulin pump. Data are mean ± S.E.

<table>
<thead>
<tr>
<th>Time</th>
<th>n = 8</th>
<th>R/N</th>
<th>Hu. UL</th>
<th>Injection</th>
<th>Pump</th>
<th>p-value for Contrasts</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bed Time</td>
<td>13.13±2.18</td>
<td>10.48±1.50</td>
<td>12.54±2.38</td>
<td>9.64±0.86</td>
<td>0.16</td>
<td>0.18 0.45 0.25</td>
</tr>
<tr>
<td>Wake Time</td>
<td>8.68±1.03</td>
<td>7.99±1.67</td>
<td>8.76±1.51</td>
<td>8.92±1.02</td>
<td>0.55</td>
<td>0.84 0.59 0.91</td>
</tr>
<tr>
<td>Pre-MSLT 09:00</td>
<td>13.44±1.12</td>
<td>11.54±1.72</td>
<td>13.44±1.45</td>
<td>10.38±1.12</td>
<td>0.23</td>
<td>0.03 0.52 0.05</td>
</tr>
<tr>
<td>Pre-MSLT 11:00</td>
<td>8.61±1.15</td>
<td>10.66±1.57</td>
<td>9.33±1.53</td>
<td>8.68±1.31</td>
<td>0.23</td>
<td>0.95 0.32 0.75</td>
</tr>
<tr>
<td>Pre-MSLT 13:00</td>
<td>12.41±1.90</td>
<td>14.84±1.87</td>
<td>13.33±2.07</td>
<td>8.85±1.18</td>
<td><strong>0.004</strong></td>
<td>0.06 0.007 0.04</td>
</tr>
<tr>
<td>Pre-MSLT 15:00</td>
<td>11.93±1.89</td>
<td>14.53±1.25</td>
<td>13.65±1.90</td>
<td>9.06±0.83</td>
<td>0.08</td>
<td>0.11 <strong>0.01</strong> 0.03</td>
</tr>
</tbody>
</table>

**Note:**
- Contrast A: Beef/Pork R/N versus Lispro/Hu.UL
- Contrast B: Beef/Pork R/N versus the Insulin Pump
- Contrast C: Lispro versus the Insulin Pump
- Contrast D: Intermittent Injection versus the Insulin Pump
- Hu. UL = Human Ultralente Insulin
Table 3.3.3. Hemoglobin A1c (%) and Glycated Proteins (µM) in patients with IDDM, while on treatment with beef/pork R/N insulin, intermittent lispro/human ultralente insulin and intermittent injection matched for insulin species with continuous subcutaneous insulin using an insulin pump. Data are mean ± S.E.

<table>
<thead>
<tr>
<th></th>
<th>n = 8</th>
<th>Beef/Pork R/N</th>
<th>Lispro/Hu. UL.</th>
<th>Intermitt. Injection</th>
<th>Insulin Pump</th>
<th>p-values for Contrasts</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>A</td>
</tr>
<tr>
<td>Hemoglobin A1c</td>
<td>8</td>
<td>7.70±0.64</td>
<td>7.81±0.60</td>
<td>7.73±0.66</td>
<td>8.44±0.63</td>
<td>0.50</td>
</tr>
<tr>
<td>Glycated Protein</td>
<td>362.5±26.3</td>
<td>377.1±24.5</td>
<td>372.1±24.7</td>
<td>393.5±27.0</td>
<td></td>
<td>0.18</td>
</tr>
</tbody>
</table>

Note:
A: Contrast Beef/Pork R/N versus Lispro/Hu. UL.
C: Contrast Lispro/Hu. UL. versus the Insulin Pump
B: Contrast Beef/Pork R/N versus the Insulin Pump
D: Contrast Intermittent Injection versus the Insulin Pump
Normal values for Hemoglobin A1c are 3.0 - 5.5 %
Normal values for Glycated Protein are ≤ 285 µM

Hu. UL. = Human Insulin Ultralente.
Table 3.3.4. Sleep onset latencies and stage shifts during Overnight Polysomnography of patients with IDDM while on treatment with intermittent insulin injection versus continuous subcutaneous insulin using an insulin pump (matched for insulin species). Data are mean ± S.E.

<table>
<thead>
<tr>
<th></th>
<th>Intermittent Injection</th>
<th>Insulin Pump</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Latency to Stage-1 Sleep (min)</td>
<td>11.44±4.66</td>
<td>12.50±3.93</td>
<td>0.80</td>
</tr>
<tr>
<td>Latency to Stage-2 Sleep (min)</td>
<td>2.50±0.42</td>
<td>3.13±0.72</td>
<td>0.51</td>
</tr>
<tr>
<td>Latency to SWS (min)</td>
<td>18.63±3.72</td>
<td>20.69±4.24</td>
<td>0.41</td>
</tr>
<tr>
<td>Latency to REM Sleep (min)</td>
<td>86.44±15.81</td>
<td>77.00±4.77</td>
<td>0.55</td>
</tr>
<tr>
<td>Number of Awakenings</td>
<td>11.88±1.79</td>
<td>12.75±2.39</td>
<td>0.44</td>
</tr>
<tr>
<td>Number of Stage Shifts</td>
<td>80.38±4.60</td>
<td>85.13±5.69</td>
<td>0.42</td>
</tr>
<tr>
<td>Number of REMS Periods</td>
<td>7.63±1.22</td>
<td>10.75±1.98</td>
<td>0.04</td>
</tr>
</tbody>
</table>
Table 3.3.5. Sleep onset latencies and stage shifts during Overnight Polysomnography of patients with IDDM while on treatment with intermittent beef/pork R/N insulin, lispro/human ultralente insulin and continuous subcutaneous insulin using an insulin pump (not matched for insulin species). Intermittent injection is the average of beef/pork R/N and lispro/human ultralente measurements. Data are mean ± S.E.

<table>
<thead>
<tr>
<th></th>
<th>n = 8</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Beef/Pork R/N</td>
</tr>
<tr>
<td>Latency to Stage-1 Sleep</td>
<td>13.44±4.83</td>
</tr>
<tr>
<td>Latency to Stage-2 Sleep</td>
<td>2.63±0.52</td>
</tr>
<tr>
<td>Latency to SWS</td>
<td>20.94±2.69</td>
</tr>
<tr>
<td>Latency to REM Sleep</td>
<td>99.19±23.30</td>
</tr>
<tr>
<td>Number of Awakenings</td>
<td>13.13±2.23</td>
</tr>
<tr>
<td>Number of Stage Shifts</td>
<td>83.38±5.85</td>
</tr>
<tr>
<td>Number of REMS Periods</td>
<td>9.00±1.02</td>
</tr>
</tbody>
</table>

Note: Contrast A: Beef/Pork R/N versus Lispro/Hu. UL.  
Contrast C: Lispro/Hu. UL. versus the Insulin Pump.  
Hu. UL. = Human Ultralente Insulin  
Contrast B: Beef/Pork R/N versus the Insulin Pump,  
Contrast D: Intermittent Injection versus Insulin Pump
Table 3.3.6. Sleep onset latencies and stage shifts during Overnight Polysomnography of the 4 diabetic subjects randomized to the beef/pork pump. Intermittent injection is the average of beef/pork R/N and lispro/human ultralente measurements. Data are mean ± S.E.

<table>
<thead>
<tr>
<th></th>
<th>Beef/Pork R/N</th>
<th>Lispro/Hu. UL.</th>
<th>Intermit. Inject.</th>
<th>Beef/Pork Pump</th>
<th>p-values for Contrasts</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>A</td>
</tr>
<tr>
<td>Latency to Stage-1 Sleep</td>
<td>16.50±9.10</td>
<td>9.38±5.29</td>
<td>12.94±7.19</td>
<td>10.38±6.55</td>
<td>0.11</td>
</tr>
<tr>
<td>Latency to Stage-2 Sleep</td>
<td>2.25±0.66</td>
<td>4.38±1.84</td>
<td>3.31±1.23</td>
<td>2.25±0.60</td>
<td>1.00</td>
</tr>
<tr>
<td>Latency to SWS</td>
<td>16.50±1.74</td>
<td>20.63±5.05</td>
<td>18.56±3.35</td>
<td>14.38±1.11</td>
<td>0.29</td>
</tr>
<tr>
<td>Latency to REM Sleep</td>
<td>69.13±4.11</td>
<td>75.50±5.11</td>
<td>72.31±3.20</td>
<td>77.63±9.19</td>
<td>0.42</td>
</tr>
<tr>
<td>Number of Awakenings</td>
<td>9.25±1.44</td>
<td>13.75±1.31</td>
<td>11.50±0.89</td>
<td>11.00±1.78</td>
<td>0.28</td>
</tr>
<tr>
<td>Number of Stage Shifts</td>
<td>72.00±5.37</td>
<td>85.00±12.62</td>
<td>78.50±6.36</td>
<td>78.50±3.23</td>
<td>0.30</td>
</tr>
<tr>
<td>Number of REMS Periods</td>
<td>8.75±1.65</td>
<td>8.50±1.5</td>
<td>8.63±1.14</td>
<td>10.50±1.99</td>
<td>0.45</td>
</tr>
</tbody>
</table>

Note: Contrast A: Beef/Pork R/N versus Beef/Pork Pump
Contrast B: Contrast Lispro/Hu. UL. versus Beef/Pork Pump.
Contrast C: Contrast Intermittent Injection versus Beef/Pork Pump
Hu. UL. = Human Ultralente Insulin
Table 3.3.7. Sleep onset latencies and stage shifts during Overnight Polysomnography of the 4 diabetic subjects randomized to the lispro pump. Intermittent injection is the average of all beef/pork R/N and lispro/human ultralente measurements. Data are mean ± S.E.

<table>
<thead>
<tr>
<th></th>
<th>n=4</th>
<th>Beef/Pork R/N</th>
<th>Lispro/ Hu. UL.</th>
<th>Intermittent Inject.</th>
<th>Lispro Pump</th>
<th>P-values for Contrasts</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>A</td>
</tr>
<tr>
<td>Latency to Stage-1 Sleep</td>
<td>4</td>
<td>10.38±4.44</td>
<td>6.38±1.16</td>
<td>8.38±1.95</td>
<td>14.63±5.13</td>
<td>0.63</td>
</tr>
<tr>
<td>Latency to Stage-2 Sleep</td>
<td></td>
<td>3.00±0.84</td>
<td>2.75±0.60</td>
<td>2.88±0.53</td>
<td>4.00±1.24</td>
<td>0.42</td>
</tr>
<tr>
<td>Latency to SWS</td>
<td>4</td>
<td>25.38±4.19</td>
<td>20.75±7.64</td>
<td>23.06±5.18</td>
<td>27.00±7.49</td>
<td>0.76</td>
</tr>
<tr>
<td>Latency to REM Sleep</td>
<td></td>
<td>129.25±43.74</td>
<td>103.75±30.81</td>
<td>116.50±37.18</td>
<td>76.38±4.65</td>
<td>0.28</td>
</tr>
<tr>
<td>Number of Awakenings</td>
<td>4</td>
<td>17.00±3.34</td>
<td>14.50±2.87</td>
<td>15.75±2.98</td>
<td>14.50±4.63</td>
<td>0.45</td>
</tr>
<tr>
<td>Number of Stage Shifts</td>
<td></td>
<td>97.75±6.69</td>
<td>88.75±4.80</td>
<td>91.75±4.99</td>
<td>91.75±10.55</td>
<td>0.56</td>
</tr>
<tr>
<td>Number of REMS Periods</td>
<td></td>
<td>9.25±1.44</td>
<td>6.50±1.85</td>
<td>7.88±1.36</td>
<td>11.00±0.91</td>
<td>0.28</td>
</tr>
</tbody>
</table>

Note: Contrast A: Beef/Pork R/N versus Lispro Pump
Contrast B: Contrast Lispro/Hu. UL. versus Lispro Pump.
Contrast C: Contrast Intermittent Injection versus Lispro Pump
Hu. UL. = Human Ultralente Insulin
Table 3.3.8. Duration of sleep stages, time awake after sleep onset, and sleep efficiency during Overnight Polysomnography of patients with diabetes mellitus while on treatment with intermittent insulin injection versus continuous subcutaneous insulin using an insulin pump (matched for insulin species). Data are mean ± S.E.

<table>
<thead>
<tr>
<th></th>
<th>Intermittent Injection</th>
<th>Insulin Pump</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Duration of Stage-1 Sleep (min.)</td>
<td>18.50±2.80</td>
<td>16.31±2.12</td>
<td>0.11</td>
</tr>
<tr>
<td>Duration of Stage-2 Sleep (min.)</td>
<td>225.31±8.92</td>
<td>220.81±13.76</td>
<td>0.80</td>
</tr>
<tr>
<td>Duration of Total SWS (min.)</td>
<td>75.06±5.71</td>
<td>79.75±8.86</td>
<td>0.60</td>
</tr>
<tr>
<td>Duration of SWS in the 1st Cycle (min.)</td>
<td>36.13±5.80</td>
<td>38.13±6.78</td>
<td>0.80</td>
</tr>
<tr>
<td>Duration of SWS in the 1st 4 hours (min)</td>
<td>56.13±8.54</td>
<td>64.81±7.02</td>
<td>0.24</td>
</tr>
<tr>
<td>Duration of Total REMS (min.)</td>
<td>87.19±7.20</td>
<td>98.75±9.41</td>
<td>0.23</td>
</tr>
<tr>
<td>Duration of REMS at the End of Sleep (min.)</td>
<td>50.06±6.02</td>
<td>65.50±7.60</td>
<td>0.12</td>
</tr>
<tr>
<td>Duration of Wakefulness After Sleep Onset (min.)</td>
<td>27.56±10.48</td>
<td>22.69±10.13</td>
<td>0.19</td>
</tr>
<tr>
<td>Sleep Efficiency (%)</td>
<td>91.95±2.46</td>
<td>93.68±1.15</td>
<td>0.36</td>
</tr>
<tr>
<td>Total Sleep Time (min.)</td>
<td>409.94±10.79</td>
<td>414.88±9.93</td>
<td>0.23</td>
</tr>
<tr>
<td></td>
<td>Insulin Pump</td>
<td>Lasp/</td>
<td>Intermittent Injection</td>
</tr>
<tr>
<td>------------------</td>
<td>--------------</td>
<td>-------</td>
<td>------------------------</td>
</tr>
<tr>
<td><strong>n = 8</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dur. of Stage-1 Sleep (min.)</td>
<td>15.7±2.36</td>
<td>22.0±4.25</td>
<td>18.9±2.12</td>
</tr>
<tr>
<td>Dur. of Stage-2 Sleep (min.)</td>
<td>217.1±34.22</td>
<td>226.1±15.0</td>
<td>221.6±10.42</td>
</tr>
<tr>
<td>Dur. of Total SWS (min.)</td>
<td>81.7±9.75</td>
<td>72.0±13.31</td>
<td>76.9±5.98</td>
</tr>
<tr>
<td>Dur. of SWS in the 1st Cycle (min.)</td>
<td>36.4±5.95</td>
<td>30.6±4.21</td>
<td>35.6±2.40</td>
</tr>
<tr>
<td>Dur. of REMS (min.)</td>
<td>88.6±8.34</td>
<td>57.4±7.45</td>
<td>72.9±6.42</td>
</tr>
<tr>
<td>Dur. of REMS at the end of Sleep (min.)</td>
<td>55.4±8.06</td>
<td>45.1±3.59</td>
<td>55.4±8.06</td>
</tr>
<tr>
<td>Sleep Efficiency (%)</td>
<td>29.1±15.16</td>
<td>40.7±10.74</td>
<td>34.9±12.10</td>
</tr>
<tr>
<td>Total Sleep Time (min.)</td>
<td>408.5±17.36</td>
<td>401.7±11.20</td>
<td>405.1±13.69</td>
</tr>
</tbody>
</table>

**Note:**

Contrast A: Beef/Pork R/N versus Lispro/Hu. UL.
Contrast B: Lispro/Hu. UL. versus the Insulin Pump.
Contrast C: Human Ultralente Insulin versus the Insulin Pump.
WASO = Wakefulness After Sleep Onset.

**Table 3.3.9:** Duration of sleep stages, time awake after sleep onset and sleep efficiencies during Overnight Polysomnography in patients with IDDM while on treatment with intermittent beef/pork R/N insulin, lispro/human ultralente insulin versus continuous subcutaneous insulin using an insulin pump (not matched for insulin species). Intermittent injection is the average of all beef/pork R/N and lispro/human ultralente measurements. Data are mean±S.E.
Table 3.3.10. Duration of sleep stages, time awake after sleep onset and sleep efficiencies during Overnight Polysomnography in the 4 diabetic subjects randomized to the beef/pork pump. Intermittent injection is the average of beef/pork R/N and lispro/human ultralente measurements. Data are mean ± S.E.

<table>
<thead>
<tr>
<th></th>
<th>Beef/Pork R/N</th>
<th>Lispro/ Hu. UL.</th>
<th>Intermit. Inject.</th>
<th>Beef/Pork Pump</th>
<th>p-values for Contrasts</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>A</td>
</tr>
<tr>
<td>Dur. of Stage-1 Sleep (min.)</td>
<td>12.88±2.95</td>
<td>20.00±3.74</td>
<td>16.44±3.07</td>
<td>12.88±2.19</td>
<td>1.00</td>
</tr>
<tr>
<td>Dur. of Stage-2 Sleep (min.)</td>
<td>226.63±10.29</td>
<td>228.25±27.99</td>
<td>227.44±17.50</td>
<td>206.00±25.76</td>
<td>0.50</td>
</tr>
<tr>
<td>Dur. of Total SWS (min.)</td>
<td>76.63±9.64</td>
<td>70.63±8.52</td>
<td>73.63±6.81</td>
<td>85.88±18.08</td>
<td>0.57</td>
</tr>
<tr>
<td>Dur. of SWS in the 1st Cycle (min.)</td>
<td>33.63±7.38</td>
<td>34.25±7.99</td>
<td>33.94±5.63</td>
<td>49.38±9.43</td>
<td>0.23</td>
</tr>
<tr>
<td>Dur. of SWS in the 1st 4 hrs. (min.)</td>
<td>59.75±10.70</td>
<td>55.88±10.58</td>
<td>57.81±3.47</td>
<td>69.75±13.18</td>
<td>0.44</td>
</tr>
<tr>
<td>Dur. of Total REMS (min.)</td>
<td>99.75±10.28</td>
<td>79.88±6.93</td>
<td>89.81±6.00</td>
<td>117.25±10.55</td>
<td>0.37</td>
</tr>
<tr>
<td>Dur. of REMS at the end of Sleep (min.)</td>
<td>59.00±8.99</td>
<td>49.25±3.76</td>
<td>54.13±3.20</td>
<td>77.13±9.84</td>
<td>0.32</td>
</tr>
<tr>
<td>Dur. of WASO (min.)</td>
<td>13.13±3.97</td>
<td>39.50±13.39</td>
<td>26.31±7.19</td>
<td>13.25±2.73</td>
<td>0.97</td>
</tr>
<tr>
<td>Sleep Efficiency (%)</td>
<td>93.38±2.53</td>
<td>89.63±3.58</td>
<td>91.50±2.91</td>
<td>94.88±2.01</td>
<td>0.10</td>
</tr>
<tr>
<td>Total Sleep Time (min.)</td>
<td>420.38±11.48</td>
<td>404.00±15.58</td>
<td>412.19±12.80</td>
<td>426.38±8.94</td>
<td>0.18</td>
</tr>
</tbody>
</table>

Note: Contrast A: Beef/Pork R/N versus Beef/Pork Pump
Contrast B: Lispro/Hu. UL. versus Beef/Pork Pump
Contrast C: Intermittent Injection versus Beef/Pork Pump
WASO = Wakefulness After Sleep Onset
Hu. UL. = Human Ultralente Insulin
Table 3.3.11. Duration of sleep stages, time awake after sleep onset and sleep efficiencies during Overnight Polysomnography of the 4 diabetic subjects randomized to the lispro pump. Intermittent injection is the average of beef/pork R/N and lispro/human ultralente measurements. Data are mean ± S.E.

<table>
<thead>
<tr>
<th>n = 4</th>
<th>Beef/Pork R/N</th>
<th>Lispro/Hu. UL.</th>
<th>Intermitt. Inject.</th>
<th>Lispro Pump</th>
<th>p-values for Contrasts</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dur. of Stage-1 Sleep (min.)</td>
<td>18.63±3.44</td>
<td>24.13±2.61</td>
<td>21.38±2.74</td>
<td>19.75±2.87</td>
<td>0.43  0.25  <strong>0.03</strong></td>
</tr>
<tr>
<td>Dur. of Stage-2 Sleep (min.)</td>
<td>207.63±15.20</td>
<td>224.00±16.25</td>
<td>215.81±13.33</td>
<td>235.63±8.59</td>
<td>0.38  0.50  0.41</td>
</tr>
<tr>
<td>Dur. of Total SWS (min.)</td>
<td>86.88±13.03</td>
<td>73.50±7.59</td>
<td>80.19±7.88</td>
<td>73.63±3.83</td>
<td>0.26  0.99  0.33</td>
</tr>
<tr>
<td>Dur. of SWS in the 1st Cycle (min.)</td>
<td>37.75±5.76</td>
<td>38.63±9.92</td>
<td>38.19±6.92</td>
<td>26.88±6.44</td>
<td><strong>0.03</strong>  0.13  <strong>0.01</strong></td>
</tr>
<tr>
<td>Dur. of SWS in the 1st four hrs. (min.)</td>
<td>61.50±16.57</td>
<td>52.50±14.74</td>
<td>57.00±12.93</td>
<td>59.88±6.35</td>
<td>0.91  0.48  0.72</td>
</tr>
<tr>
<td>Dur. of Total REMS (min.)</td>
<td>77.50±11.35</td>
<td>74.63±5.57</td>
<td>76.06±8.31</td>
<td>80.13±5.54</td>
<td>0.81  0.56  0.46</td>
</tr>
<tr>
<td>Dur. of REMS at the end of Sleep (min.)</td>
<td>51.88±9.05</td>
<td>41.13±5.93</td>
<td>46.50±7.73</td>
<td>53.63±9.04</td>
<td>0.91  0.30  0.43</td>
</tr>
<tr>
<td>Dur. of WASO (min.)</td>
<td>45.13±29.76</td>
<td>42.00±18.91</td>
<td>43.56±24.14</td>
<td>32.13±20.30</td>
<td>0.26  0.15  0.08</td>
</tr>
<tr>
<td>Sleep Efficiency (%)</td>
<td>86.43±7.23</td>
<td>90.53±4.52</td>
<td>88.48±5.80</td>
<td>92.48±1.08</td>
<td>0.41  0.64  0.47</td>
</tr>
<tr>
<td>Total Sleep Time (min.)</td>
<td>396.63±34.36</td>
<td>399.50±18.41</td>
<td>398.06±26.03</td>
<td>403.38±17.09</td>
<td>0.73  0.63  0.65</td>
</tr>
</tbody>
</table>

Note: Contrast A: Beef/Pork R/N versus Lispro Pump  
Contrast B: Lispro/Hu. UL. versus Lispro Pump  
Contrast C: Intermittent Injection versus Lispro Pump  
Hu. UL. = Human Ultralente Insulin  
WASO = Wakefulness After Sleep Onset
Figure 3.3.1. Regressions and correlation between blood glucose level and latency to Stage-1 sleep during the MSLT while diabetic subjects were on treatment with intermittent lispro/human ultralente insulin, intermittent beef/pork R/N insulin or continuous subcutaneous insulin using an insulin pump. The regression equation and the coefficient of correlation for lispro/human ultralente insulin was \( y = -0.24 x + 12.46, r = 0.21 \); for beef/pork R/N insulin it was \( y = 0.01 x + 9.55, r = 0.005 \), and for the insulin pump it was \( y = 0.16 x + 8.97, r = 0.09 \).
Table 3.3.12. Sleep onset latencies, number of sleep periods and number of subjects with REMS during the MSLT in patients with IDDM while on treatment with intermittent insulin injection versus continuous subcutaneous insulin using an insulin pump (matched for the insulin species). Data are mean ± S.E.

<table>
<thead>
<tr>
<th></th>
<th>Intermittent Injection</th>
<th>Insulin Pump</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>n = 8</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Latency to Stage-1 Sleep (min.)</td>
<td>8.25±1.10</td>
<td>10.75±1.44</td>
<td>0.02</td>
</tr>
<tr>
<td>Latency to Stage-2 Sleep (min.)</td>
<td>4.08±0.82</td>
<td>4.55±1.34</td>
<td>0.67</td>
</tr>
<tr>
<td>Number of Sleep periods</td>
<td>3.5±0.19</td>
<td>3.63±0.26</td>
<td>0.60</td>
</tr>
<tr>
<td>Number of Patients with REMS</td>
<td>1</td>
<td>2</td>
<td>-</td>
</tr>
</tbody>
</table>
Table 3.3.13. Sleep onset latencies, number of sleep periods and number of subjects with REMS during the MSLT in patients with IDDM while on treatment with intermittent beef/pork R/N insulin, lispro/human ultralente insulin and continuous subcutaneous insulin using an insulin pump (not matched for insulin species). Intermittent injection is the average of beef/pork R/N and lispro/human ultralente measurements. Data are mean ± S.E.

<table>
<thead>
<tr>
<th></th>
<th>Beef/Pork R/N</th>
<th>Lispro/ Hu. UL.</th>
<th>Intermitt. Injection</th>
<th>Insulin Pump</th>
<th>p-values for Contrasts</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>A</td>
</tr>
<tr>
<td>Lat. to Stage-1 Sleep (min.)</td>
<td>9.99±1.84</td>
<td>9.47±1.58</td>
<td>9.73±1.51</td>
<td>10.75±1.44</td>
<td>0.77</td>
</tr>
<tr>
<td>Lat. to Stage-2 Sleep (min.)</td>
<td>7.09±2.27</td>
<td>4.96±1.70</td>
<td>6.03±1.53</td>
<td>4.55±1.34</td>
<td>0.43</td>
</tr>
<tr>
<td>No. of Sleep Periods</td>
<td>3.25±0.49</td>
<td>3.375±0.375</td>
<td>3.32±0.35</td>
<td>3.63±0.26</td>
<td>0.82</td>
</tr>
<tr>
<td>No. of Patients with REMS</td>
<td>1</td>
<td>0</td>
<td>1</td>
<td>2</td>
<td></td>
</tr>
</tbody>
</table>

Note: Contrast A: Beef/Pork R/N versus Lispro/Hu. UL.  Contrast B: Beef/Pork R/N versus the Insulin Pump, Contrast C: Lispro/Hu. UL. versus the Insulin Pump. Contrast D: Intermittent Injection versus the Insulin Pump. Hu. UL. = Human Ultralente Insulin
Table 3.3.14. Sleep onset latencies, number of sleep periods and number of subjects with REMS during the MSLT in the 4 diabetic subjects randomized to the beef/pork pump. Intermittent injection is the average of beef/pork R/N and lispro/human ultralente measurements Data are mean ± S.E.

<table>
<thead>
<tr>
<th>n = 4</th>
<th>Beef/Pork R/N</th>
<th>Lispro/Hu. UL.</th>
<th>Intermit. Inject.</th>
<th>Beef/Pork Pump</th>
<th>p-values for Contrasts</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>A</td>
</tr>
<tr>
<td>Lat. to Stage-1 Sleep (min.)</td>
<td>7.90±1.54</td>
<td>10.34±2.81</td>
<td>9.11±2.14</td>
<td>11.55±2.27</td>
<td><strong>0.05</strong></td>
</tr>
<tr>
<td>Lat. to Stage-2 Sleep (min.)</td>
<td>4.63±1.26</td>
<td>6.39±3.27</td>
<td>5.51±0.348</td>
<td>6.96±2.10</td>
<td>0.13</td>
</tr>
<tr>
<td>No. of Sleep Periods</td>
<td>3.5±0.29</td>
<td>3.25±0.75</td>
<td>3.75±0.47</td>
<td>3.25±0.48</td>
<td>0.39</td>
</tr>
<tr>
<td>No. of Patients with REMS</td>
<td>1</td>
<td>-</td>
<td>-</td>
<td>1</td>
<td>-</td>
</tr>
</tbody>
</table>

Note: Contrast A: Beef/Pork R/N versus Beef/Pork Pump
Contrast B: Contrast Lispro/Hu. UL. versus Beef/Pork Pump
Contrast C: Contrast Intermittent Injection versus Beef/Pork Pump
Hu. UL. = Human Ultralente Insulin
Table 3.3.15. Sleep onset latencies, number of sleep periods and number of subjects with REMS during the MSLT in the 4 diabetic subjects randomized to the lispro pump. Intermittent injection is the average of beef/pork R/N and lispro/human ultralente measurements. Data are mean ± S.E.

<table>
<thead>
<tr>
<th></th>
<th>Beef/Pork R/N</th>
<th>Lispro/ Hu. UL.</th>
<th>Intermitt. Inject.</th>
<th>Lispro Pump</th>
<th>p-values for Contrasts A</th>
<th>B</th>
<th>C</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lat. to Stage-1 Sleep (min.)</td>
<td>12.09±3.25</td>
<td>8.60±1.79</td>
<td>9.98±1.95</td>
<td>9.94±2.01</td>
<td>0.34</td>
<td>0.20</td>
<td>0.73</td>
</tr>
<tr>
<td>Lat. to Stage-2 Sleep (min.)</td>
<td>9.56±4.28</td>
<td>3.53±1.18</td>
<td>6.55±2.54</td>
<td>2.15±0.41</td>
<td>0.20</td>
<td>0.38</td>
<td>0.22</td>
</tr>
<tr>
<td>No. of Sleep Periods</td>
<td>3.00±1.00</td>
<td>3.50±0.29</td>
<td>3.25±0.60</td>
<td>4.00±0.00</td>
<td>0.39</td>
<td>0.18</td>
<td>0.30</td>
</tr>
<tr>
<td>No. of Patients with REMS</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

Note: **Contrast A**: Beef/Pork R/N versus Lispro Pump  
**Contrast B**: Lispro/Hu. UL. versus Lispro Pump.  
**Contrast C**: Intermittent Injection versus Lispro Pump  
Hu. UL. = Human Ultralente Insulin
Table 3.3.16. Daytime cognitive performance and its variability in subjects with IDDM while on treatment with intermittent insulin injection versus continuous subcutaneous insulin using an insulin pump (matched for insulin species). Daytime cognitive performance was measured by the Digit Symbol Substitution Test (DSST), the Symbol Digit Modalities Test (SDMT), the Manual Finger Tapping Test (MFTT), and the Grooved Pegboard Test (GPBT), at 8:00, 10:00, 12:00, and 14:00 hours. Data are mean ± S.E.

<table>
<thead>
<tr>
<th>n = 8</th>
<th>Intermittent Injection</th>
<th>Insulin Pump</th>
<th>Mean p-value</th>
<th>Variability p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>DSST SCORE</td>
<td>83.79±4.74</td>
<td>86.22±4.30</td>
<td>0.04</td>
<td>0.39</td>
</tr>
<tr>
<td>SDMT SCORE</td>
<td>72.02±3.72</td>
<td>75.09±3.35</td>
<td>0.05</td>
<td>0.39</td>
</tr>
<tr>
<td>GPBT SCORE</td>
<td>52.25±1.99</td>
<td>49.16±1.02</td>
<td>0.03</td>
<td>0.04</td>
</tr>
<tr>
<td>MFTT SCORE</td>
<td>55.31±1.93</td>
<td>55.27±2.15</td>
<td>0.77</td>
<td>0.38</td>
</tr>
</tbody>
</table>
Table 3.3.17. Daytime cognitive performance and its variability in subjects with IDDM while on treatment with intermittent beef/pork R/N insulin, intermittent lispro/human ultralente insulin and continuous subcutaneous insulin using an insulin pump. Performance was measured using the Digit Symbol Substitution Test (DSST), the Symbol Digit Modalities Test (SDMT), the Manual Finger Tapping Test (MFTT), and the Grooved Pegboard Test (GPBT) at 8:00, 10:00, 12:00, and 14:00 hours. Intermittent injection is the average of beef/pork R/N and lispro/human ultralente measurements. Data are mean ± S.E.

<table>
<thead>
<tr>
<th></th>
<th>n = 8</th>
<th>Beef/Pork R/N</th>
<th>Lispro/Hu. UL.</th>
<th>Intermittent Injection</th>
<th>Insulin Pump</th>
<th>Contrasts for Means</th>
<th>Contrasts for Variability</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>A</td>
<td>B</td>
</tr>
<tr>
<td>DSST</td>
<td>83.53±5.14</td>
<td>84.03±4.49</td>
<td>83.78±4.92</td>
<td>86.22±4.30</td>
<td>0.80</td>
<td>0.53</td>
<td>0.61</td>
</tr>
<tr>
<td>SDMT</td>
<td>73.09±4.23</td>
<td>70.94±3.34</td>
<td>72.02±3.72</td>
<td>75.09±3.35</td>
<td>0.18</td>
<td>0.25</td>
<td>0.02</td>
</tr>
<tr>
<td>GPBT</td>
<td>52.91±1.63</td>
<td>51.59±2.45</td>
<td>52.25±1.99</td>
<td>49.19±1.01</td>
<td>0.26</td>
<td>0.01</td>
<td>0.18</td>
</tr>
<tr>
<td>MFTT</td>
<td>55.56±1.99</td>
<td>55.06±1.96</td>
<td>55.31±2.15</td>
<td>55.22±2.16</td>
<td>0.58</td>
<td>0.69</td>
<td>0.78</td>
</tr>
</tbody>
</table>

Note: Contrast A: Beef/Pork R/N versus Lispro/Hu. UL.
Contrast C: Lispro/Hu. UL. versus the Insulin Pump.
Hu. UL. = Human Ultralente Insulin

Contrast B: Beef/Pork R/N versus the Insulin Pump.
Contrast D: Intermittent Injection versus the Insulin Pump
Table 3.3.18. Daytime cognitive performance and its variability in subjects with IDDM randomized to the beef/pork pump. Performance was measured using the Digit Symbol Substitution Test (DSST), the Symbol Digit Modalities Test (SDMT), the Manual Finger Tapping Test (MFTT), and the Grooved Pegboard Test (GPBT), at 8:00, 10:00, 12:00, and 14:00 hours. Intermittent injection is the average of beef/pork R/N and lispro/human ultralente measurements. Data are mean ± S.E.

<table>
<thead>
<tr>
<th></th>
<th>Beef/Pork R/N</th>
<th>Lispro/Hu. UL.</th>
<th>Intermittent Inject.</th>
<th>Beef/Pork Pump</th>
<th>A</th>
<th>B</th>
<th>C</th>
<th>A</th>
<th>B</th>
<th>C</th>
<th>A</th>
<th>B</th>
<th>C</th>
</tr>
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<tbody>
<tr>
<td>n = 4</td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>DSST SCORE</td>
<td>87.00±7.20</td>
<td>88.19±3.20</td>
<td>87.59±2.65</td>
<td>90.25±4.95</td>
<td>0.65</td>
<td>0.79</td>
<td>0.70</td>
<td>0.15</td>
<td>0.12</td>
<td>0.05</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SDMT SCORE</td>
<td>75.13±4.78</td>
<td>72.91±3.40</td>
<td>73.72±3.90</td>
<td>77.69±4.10</td>
<td>0.35</td>
<td>0.04</td>
<td>0.06</td>
<td>0.34</td>
<td>0.30</td>
<td>0.45</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>GPBT SCORE</td>
<td>50.81±1.12</td>
<td>50.06±1.86</td>
<td>50.44±1.40</td>
<td>48.56±0.28</td>
<td>0.14</td>
<td>0.51</td>
<td>0.56</td>
<td>0.0004</td>
<td>0.0001</td>
<td>0.0001</td>
<td></td>
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</tr>
<tr>
<td>MFTT SCORE</td>
<td>58.50±2.41</td>
<td>57.63±2.24</td>
<td>58.08±2.11</td>
<td>58.31±2.11</td>
<td>0.92</td>
<td>0.54</td>
<td>0.77</td>
<td>0.36</td>
<td>0.43</td>
<td>0.5</td>
<td></td>
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</tr>
</tbody>
</table>

Note: Contrast A: Beef/Pork R/N versus Beef/Pork Pump
Contrast B: Lispro/Hu. UL. versus Beef/Pork Pump
Contrast C: Contrast Intermittent Injection versus Beef/Pork Pump
Hu. UL. = Human Ultralente Insulin
Table 3.3.19. Daytime cognitive performance and its variability in subjects with IDDM randomized to the lispro pump. Performance was measured using the Digit Symbol Substitution Test (DSST), the Symbol Digit Modalities Test (SDMT), the Manual Finger Tapping Test (MFTT), and the Grooved Pegboard Test (GPBT), at 8:00, 10:00, 12:00, and 14:00 hours. Intermittent injection is the average of beef/pork R/N and lispro/human ultralente measurements. Data are mean ± S.E.

<table>
<thead>
<tr>
<th></th>
<th>Beef/Pork R/N</th>
<th>Lispro/ Hu. UL.</th>
<th>Intermittent Inject.</th>
<th>Lispro Pump</th>
<th>Contrasts for Means</th>
<th>Contrasts for Variability</th>
</tr>
</thead>
<tbody>
<tr>
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<td></td>
<td></td>
<td></td>
<td></td>
<td>A</td>
<td>B</td>
</tr>
<tr>
<td>DSST</td>
<td>80.06±9.88</td>
<td>79.88±8.51</td>
<td>79.97±8.83</td>
<td>82.19±7.15</td>
<td>0.75</td>
<td>0.70</td>
</tr>
<tr>
<td>SDMT</td>
<td>71.06±7.61</td>
<td>69.56±6.26</td>
<td>70.32±6.88</td>
<td>72.50±5.57</td>
<td>0.60</td>
<td>0.03</td>
</tr>
<tr>
<td>GPBT</td>
<td>55.00±2.29</td>
<td>53.13±4.80</td>
<td>54.07±3.78</td>
<td>49.81±2.11</td>
<td>0.04</td>
<td>0.31</td>
</tr>
<tr>
<td>MFTT</td>
<td>52.63±2.63</td>
<td>52.50±2.92</td>
<td>52.54±2.79</td>
<td>52.13±3.29</td>
<td>0.52</td>
<td>0.41</td>
</tr>
</tbody>
</table>

Note: Contrast A: Beef/Pork R/N versus Lispro Pump
Contrast C: Intermittent Injection versus Lispro Pump

Contrast B: Lispro/Hu. UL. versus Lispro Pump
Hu. UL. = Human Ultralente Insulin
Figure 3.3.2. Performance on the DSST in diabetic patients as measured under basal conditions at 8:00, 10:00, 12:00 and 14:00 hours. (I) indicates the performance of patients while receiving insulin by intermittent injection and (P) indicates performance while receiving insulin by continuous subcutaneous insulin pump. * indicates that differences were significant at \( p \leq 0.05 \).
Figure 3.3.3. Mean daytime cognitive performances on the Digit Symbol Substitution Test (DSST) in patients with insulin-dependent diabetes mellitus while on intermittent beef/pork R/N insulin, intermittent lispro/human ultralente insulin, and the insulin pump. None of the differences were significant at $p \leq 0.05$. 
Figure 3.3.4. Mean daytime cognitive performances on the Symbol Digit Modalities Test (SDMT) of patients with IDDM while on intermittent beef/pork R/N insulin, intermittent lispro/human ultralente insulin, and the insulin pump. * indicates that the differences between lispro/human ultralente and the insulin pump were significant at $p \leq 0.05$. 
Figure 3.3.5. Mean daytime cognitive performances on the Grooved Pegboard Test (GPBT) in patients with IDDM while on intermittent beef/pork R/N insulin, intermittent lispro/human ultralente insulin and continuous subcutaneous insulin using an insulin pump. * indicates that the differences between the insulin pump and beef/pork R/N insulin were significant at p ≤ 0.05.
Figure 3.3.6. Mean daytime cognitive performances on the Manual Finger Tapping Test (MFTT) in patients IDDM while on intermittent beef/pork R/N insulin, intermittent lispro/human ultralente insulin and continuous subcutaneous insulin using an insulin pump. None of the differences were significant at $p \leq 0.05$. 
Figure 3.3.7. The regressions and correlations between blood glucose level and daytime DSST scores at 8:00, 10:00, 12:00 and 14:00 hours in patients with diabetes mellitus while on intermittent beef/pork R/N insulin, intermittent lispro/human ultralente insulin and continuous subcutaneous insulin using an insulin pump. The regression equation and coefficient of correlation for beef/pork R/N insulin were $y = -0.29 x + 76.02$ and $r = 0.11$, for lispro/human ultralente were $y = -0.51 x + 63.84$ and $r = 0.24$, and for the insulin pump were $y = -0.11 x + 86.98$ and $r = 0.03$. 
3.4 Intermittent Insulin Injection vs. Continuous Subcutaneous Insulin Infusion Administered by an Insulin Pump

"At home" blood glucose measurements in the week preceding each phase of the study are shown in Table 3.3.1. As before, no differences were seen between daytime blood glucose control when insulin was administered by continuous subcutaneous infusion or by intermittent insulin injection. A spot 2:00 a.m. glucose obtained 2-3 days preceding the PSG indicated that blood sugars were stable and that the possibility of nocturnal hypoglycemia was appropriately low on the pump. Also on the pump, the blood glucose levels at bed time and wake time, during Overnight Polysomnography, were nearly identical (Table 3.3.2). Throughout the four stages of the MSLT study, blood glucose levels were generally lower on the insulin pump, independent of insulin species. The reason this occurred was because glycemic control was relaxed in order to avoid hypoglycemia during testing, and this was more readily facilitately by ongoing adjustments in insulin dosage on the pump. Long-term glycemic control as indicated by the hemoglobin A₁C and glycated protein levels (Table 3.3.3) was actually better when diabetic subjects were on intermittent insulin. The explanation for this was that basal insulin infusion rates were intentionally reduced during the night to exaggerate the differences in insulin kinetics between the insulin pump (low nocturnal insulin levels) and twice daily intermittent insulin injection (high nocturnal insulin levels). The night time interval is an important determinant of overall glycemic control and, indeed, better correlation are seen between the HbA₁C and fasting (nocturnal) blood glucose than preprandial (daytime) levels.

Table 3.3.4 shows the effects of insulin administration on the sleep onset latency and number of stage shifts and REMS periods during Overnight
Polysomnography. As can be seen, the latency to stage-1 sleep, latency to stage-2 sleep, and latency to SWS and REM sleep were not significantly different in subjects randomized to intermittent insulin injection or continuous subcutaneous insulin infusion when matched for insulin species. Indeed, the only differences were that diabetic subjects had significantly more REM sleep periods while on continuous subcutaneous insulin pump than on intermittent insulin injection.

Tables 3.3.5, 3.3.6 and 3.3.7 demonstrate the effects of pumping Iletin beef/pork R or lispro insulin on sleep latencies and number of stage shifts and REMS periods in diabetic subjects. Again, regardless of the species of insulin used in the pump, there were no significant differences, except for the number of REMS periods which were increased on the insulin pump. The frequency of awakening and stage shifting was virtually identical for both insulin types as administered by intermittent injection or insulin pump.

Table 3.3.8 shows the duration of each sleep stage, the time awake after sleep onset and the overall sleep efficiency during Overnight Polysomnography of diabetic subjects while on intermittent insulin injection or continuous subcutaneous insulin, matched for insulin species. There were no significant differences between any of the measurements on the two insulin treatments. However, in respect of subsequent discussions, it is important to note that during insulin pump therapy diabetic subjects had about 15% more SWS in the first 4 hours of sleep (64.81±7.02 min. compared to 56.13±8.54 min., p = 0.24). Also, insulin pumping was associated with a 15% increase in the overall duration of REM sleep (p = 0.23) and a 30% increase in the time spent in REM sleep in the second half of the night (p = 0.12). Sleep efficiency was about the same for the two insulin regimens.

Tables 3.3.9, 3.3.10, and 3.3.11 show the effects of insulin administration on the duration of sleep stages and sleep efficiencies during Overnight Polysomnography.
for the various insulin species. In this case, the use of the insulin pump was associated with a shorter duration of stage-1 sleep (16.31±2.12 min. vs. 18.91±2.12 min., p = 0.04), a longer time spent in REM sleep (98.69±9.42 min. vs. 82.94±5.41 min., p = 0.05), a longer duration of REMS at the end of the night (65.38±7.62 min. vs. 50.31±3.01 min., p = 0.06), and less time awake after sleep onset (22.69±10.13 min. vs. 34.94±12.10 min., p = 0.01) than intermittent insulin injection. This was even more apparent when patients randomized to pumping with beef/pork Iletin R insulin or pumping with lispro insulin were considered separately (Tables 3.3.10 or 3.3.11). Indeed the use of beef/pork Iletin R insulin in the insulin pump was associated with a 12% increase in the duration of SWS (p=0.28), a 47% and 17% increase in the duration of SWS in the first sleep cycle (p = 0.05) and the first 4 hours of sleep (p = 0.03), an 18% increase in the duration of REMS (p = 0.01) and a 31% increase in the duration of REMS in the last three and a half hours of sleep (p= 0.03) compared to intermittent lispro/human ultralente insulin. Pumping beef/pork Iletin R insulin showed smaller, but consistent increases over intermittent beef/pork Iletin R/N insulin, although the numbers were too small to approach statistical significance. On the other hand, pumping lispro insulin did not improve the sleep profile of patients with Type-I diabetes mellitus.

Diabetic subjects on the insulin pump took more time to fall asleep on day time napping on the MSLT (p = 0.02) than on treatment with intermittent injection matched for insulin species (Table 3.3.12). This was particularly true for patients randomized to the beef/pork Iletin R insulin pump (Table 3.3.14) where the increase was in the order of 46% (p=0.05). Again, there were no differences between patients randomized to the lispro pump and intermittent insulins and no differences in the number of sleep periods or number of subjects attaining REMS on the MSLT. The
regression and correlation between blood glucose level and latency to stage-1 sleep during the MSLT are shown in Figure 3.3.1 and are quite weak.

Baseline daytime cognitive performance with respect to the mode of insulin administration and insulin species are shown in Figures 3.3.2 to 3.3.6 and Tables 3.3.16 and 3.3.17. As can be seen, performance on the DSST, SDMT, and GPBT were slightly, but significantly, improved on the insulin pump. The nature of the improvement was a decrease in the time to completion with no change in the frequency of incorrect responses. The magnitude of the differences was small (3 - 6%), but consistent throughout the day (shown for the DSST in Figure 3.3.2). Also, the variability in test scores throughout the day were significantly less (p < 0.01) on the insulin pump, particularly for the GPBT. There were no differences or only minor differences at some time points between insulins. Figure 3.3.7 shows the regression and correlation between blood glucose level and the baseline psychometric scores of diabetic subjects on the insulin pump. As with intermittent insulin injection, correlations were weak and not significant.
4. Discussion

4.1 Patients with Type-I Diabetes Mellitus vs. Control Subjects

Considering the results, particularly Tables 3.1.1 to 3.1.4, and Figures 3.1.1 to 3.1.8, the first hypothesis of the study is accepted that there are significant differences between patients with IDDM and non-diabetic controls, in their pattern of night time sleep, daytime sleepiness and daytime cognitive performance. Indeed, there are six important realities faced by diabetic patients. First, diabetics have a disrupted night sleep. Secondly, they have a short REM onset latency. Thirdly, diabetic subjects spend significantly more of the night in “light” (stage-1 and stage-2) sleep and significantly less time in SWS and REMS than non-diabetic controls. Fourthly, during daytime MSLT, diabetics have a shortened sleep onset latency and are more likely to sleep than non-diabetic controls. Fifthly, diabetic subjects also have periods of REM sleep during daytime napping. Lastly, their performance on various psychometric tests fluctuates throughout the day and this fluctuation is significantly greater than that seen in non-diabetic controls.

Evidence for a chronic disrupted sleep pattern in patients with IDDM is provided in the frequent stage shifts and numbers of awakenings on Overnight Polysomnography. A fragmented sleep pattern is also suggested by the disturbed REM sleep which is shown by the significantly higher number of REM sleep periods in patients with IDDM. This usually indicates that subjects are unable to maintain sleep either because of frequent periods of arousal during sleep or because of an intrinsic abnormality in sleep, itself (Williams, R.L., et al., 1974).

The most common causes of sleep arousal are the sleep apnea syndrome, periodic limb movement disorders, the restless leg syndrome and psychiatric-
associated sleep disorders. A sleep history was taken in each of our subjects and none of them reported, or was made aware by someone close living to them, that they ever snored loudly, stopped breathing, or experienced gasps, grunts, or chokes accompanied by tachycardia during sleep. None of them admitted to having night terrors, morning headaches or confusion or dry mouth upon awakening, and none noticed or had ever been told that they had repetitive limb movement during sleep, unpleasant sensations in the legs or discomfort that was relieved by movement of the legs. Subjects also denied being depressed or bored with life and none of them had been diagnosed or hospitalized with a psychiatric disease. When we inquired as to the likelihood of these subjects dozing off or falling asleep in eight hypothetical situations, such as listed in the Epworth Sleepiness Scale, they believed that the chance was very small or non existent. Indeed, our subjects were young, lean and healthy and their sleep history, likewise, indicated that they did not suffer from any of the above sleep-related disorders.

Anamnestic data are valuable, however, objective data are needed to confirm that sleep fragmentation is not caused by sleep apnea, periodic limb movements or the restless leg syndrome. This can be obtained quite simply by measuring abdominal and thoracic respiratory efforts, oral and nasal air flow, oxymetry, intercostal EMG, anterior tibial muscle EMG and limb movement during Overnight Polysomnography. Unfortunately, we did not have a definitive sleep laboratory in Saskatoon at the time these studies were completed. Thus, our data are deficient in this regard. However, these services are now available and in sleep studies presently underway of diabetic and non-diabetic subjects on insulin/glycemic clamping, we have seen fragmented sleep not accompanied by sleep apnea, periodic leg movements or the restless leg syndrome. Thus, it appears that fragmented sleep in these individuals is caused by EEG arousal that is intrinsic to the sleep abnormality, itself.
Further evidence for EEG arousal comes from observations that diabetic subjects have frequent bouts of alpha activity during sleep. Alpha EEG sleep is a well known phenomenon and is a diagnostic criterion for the chronic fatigue syndrome. It appears as episodic bursts of alpha activity during stage-2 and SWS and they do not interrupt the underlying sleep stage. It can be analyzed manually using Moldofsky's criteria (Flanigan, M.J., et al., 1995) or by power spectral analysis. We tried to analyze alpha EEG using a computerized method. Unfortunately, we experienced some methodological problems and are not able to report on the alpha EEG data during sleep in our patients. Suffice it to say, we saw frequent and prolonged episodes of alpha EEG sleep in our diabetic subjects, but not in normal controls. These analyses are recommended in all future sleep studies in diabetes to help elucidate the cause of sleep fragmentation in these individuals.

REM onset latency in patients with IDDM is significantly shorter than non-diabetic controls, even though the REM onset latency in diabetics is still within the normal range (70 - 110 minutes). There are two possible explanations for this; namely, 1) a first-night effect, and 2) increased sleep pressure causing increased REMS during NREM sleep. The presence of a first-night effect (Agnew, H.W.Jr., et al., 1966) is suggested by the observation that the non-diabetic control subjects experienced a slight delay in the appearance of REM sleep, a significantly longer time awake after sleep onset and a shorter REM sleep duration than diabetic individuals. However, they also had less frequent awakening and less stage shifting than diabetics. The second possibility, that being the sleep pressure hypotheses is that the chronic sleep disturbance seen in diabetic subjects actually causes the occurrence of a bizarre distribution of REM sleep. Chronic sleep disruption is known to increase sleep propensity during the wake periods and increases REM sleep pressure in NREM sleep. This condition is distinguishable by an early onset of REM sleep (Boberly,
A.A., 1994). Since patients with IDDM appear to have a chronic disrupted sleep pattern, we feel this latter hypothesis is a better explanation for the shortened REM onset latency in these individuals. If there is a first-night effect, it is only apparent in non-diabetic controls.

Diabetic subjects spend less time in SWS than non-diabetic controls. They also manifest less SWS in the first cycle of sleep. Moreover, diabetics subjects fail to gain a sufficient quantity of REM sleep (19.66±5.12% of sleep compared to 28.00±5.66% for age and gender matched normative controls) (Williams, L.R., et al., 1974). This is further evident in the fact that the duration of REM sleep was restored to normal ranges on reducing the rate of nocturnal insulin administration either by choosing a PM intermediate-acting insulin with a shorter time-effects profile such as Humulin N (shown in the comparison with beef/pork R/NPH) or by switching to an insulin pump with a low night-time infusion rate. Comparisons with non-diabetic controls failed to show a difference in REMS duration. This may relate to a first-night effect in non-diabetic controls, which did not occur in diabetic subjects because they were already quite sleepy due to their pre-existing sleep abnormality and because they had multiple sleep recordings and were, thus, more acclimatized to the laboratory.

Our finding of specific sleep disorder in patients with insulin-dependent diabetes mellitus is the first report of such abnormality. Indeed, the only reference to sleep in these individuals focuses on the relatively common occurrence of nocturnal hypoglycemia resulting from the unopposed effects of exogenous insulin (Bendston, I., et al., 1992). We did not measure blood glucose levels during overnight PSG, so that the possibility that nocturnal hypoglycemia caused EEG arousal and fragmented sleep pattern in our diabetic patients cannot be completely excluded. However, there are several reasons why we reject this explanation. First, save for the one individual whose study was repeated, there was no indication on the basis of clinical symptoms
or pre- and post-PSG blood glucose determinations, that hypoglycemia occurred. If it did, unbeknownst to us, it would be expected to be a random event, whereas the sleep abnormality in diabetic subjects was pervasive and consistent on repeated measurements. Secondly, the disturbances in sleep and daytime performance were clearly related to insulin (specifically insulin pharmacokinetics/insulin pharmacodynamics) and only weakly related to blood glucose levels. Thirdly, insulin has shown similar effects on SWS and REMS in animal models (Sangiah, S., et al., 1982; Sangiah, S., and Caldwell, D.F., 1988; Kápas, L., et al., 1991) as we observed in humans. Finally, patients with NIDDM are reported to have a chronic disrupted sleep pattern due to the sleep apnea syndrome, and this is strongly related to the level of insulin resistance (Feinberg, I., 1993). Indeed one must ask whether EEG arousal in these individuals is consequence of apnea alone, or whether hyperinsulinemia is also playing a role.

The biochemical or psychological abnormalities that accompany the diabetic sleep disorder are not addressed in this study. It would appear from tight relationship between CNS response and insulin pharmacokinetics, and its hour-to-hour variability over the course of the night, that insulin has a direct neuromodulatory effect on the brain. Insulin increases the transport of L-tryptophan through the blood-brain barrier (Sangiah, S. and Caldwell, D.F., 1988). Secondly, insulin receptors on brain endothelial cells are involved in the regulation of amino acid transporters including L-tryptophan transporters on the blood-brain barrier (Ermish, A., et al., 1993). Meanwhile, patients with IDDM suffer from iatrogenic hyperinsulinemia (Galloway, J.A. and Chance, R.E., 1994) and down regulation of insulin receptors in the CNS has been observed with long-standing hyperinsulinemia (Figlewicz, D.P., et al., 1985). Thus, it is possible that insulin-treated diabetic patients lack L-tryptophan in the brain due to impairment of its transporter caused by down regulation of insulin receptors.
L-tryptophan is believed to have hypnotic effects in the brain (Gillin, J.C., et al., 1995) in that it has been shown to shorten the sleep onset latency and augment SWS. Also, L-tryptophan is a precursor of serotonin and serotonin is known to be involved in generating SWS and in the synthesis and accumulation of other sleep factors (Jones, B.E., 1994).

In addition to (and possibly resulting from) chronic night-time sleep fragmentation and REMS deprivation, diabetic subjects appear to have increased daytime sleepiness characterized by a short sleep onset latency and a greater tendency to sleep during the MSLT. Indeed, the latency to stage-1 sleep was in the gray-zone between normal ($\geq 15$ min.) and abnormal ($\leq 5$ min.) indicating that these individuals suffer from daytime sleepiness with moderate social impact (The International Classification of Sleep Disorders, 1990). Non-diabetic controls have a normal sleep onset latency on the MSLT. Diabetic subjects, also fall asleep on 85% of the potential sleep periods during the MSLT compared to 50% for normal controls. This means that they easily attain sleep in almost every napping environment. Fragmentation of night-time sleep is known to cause an increase in daytime sleep tendency (Carskadon, M.A., and Dement, W.C., 1982). This is believed to be a homeostatic process to maintain sufficient quantity of sleep so that the restorative function of sleep can be accomplished.

Subjective self-assessment questionnaires administered prior to each stage of the MSLT show that, at the time of testing, diabetic subjects were generally functioning at a high level, and were able to concentrate, although not at peak efficiency. However, this self-assessment tool is not very reliable since diabetic subjects consistently scaled themselves significantly more alert than controls despite having a shorter sleep onset latency. In addition, regression analyses suggest that diabetic subjects have a lower intercept and steeper regression line than non-diabetic
controls. This leads to the conclusion that diabetics are less aware of their state of sleepiness. This is not a new finding. Indeed, it has been reported patients tend to deny the extent of their sleepiness and to rate themselves as alert on the Stanford Sleepiness Scale (Hartse, K.M., et al., 1982). Sleepy patients often are not able to recognize their daytime impairment, perhaps because they do not have a good frame of reference in assessing their own wakefulness.

Another interesting finding in this study is the occurrence of REM sleep during a significant number of MSLT measurements in diabetic subjects. Selective REMS deprivation has been associated with an increasing attempt by affected subjects to enter REM sleep (Bonnet, M.H., 1994). Since diabetics have REM sleep fragmentation as shown by their high number of REM periods during Overnight Polysomnography, and this is accompanied by chronic REM sleep deprivation, it is possible that these individuals have increased REMS pressure. Thus, increased REMS pressure could be responsible for the appearance of REMS on daytime napping during the MSLT. Non-diabetic control subjects have only acute minor REM sleep deprivation, and in this case, their REMS pressure would not be high enough to cause REM intrusion during daytime napping.

The appearance of REM sleep during the MSLT raises the question of whether or not our diabetic subjects also suffered from narcolepsy. While it is true that one of our diabetics was suspected of having narcolepsy, he was withdrawn from the study and all of his data were not included in the analysis. Subjective sleepiness measurements on the pre-MSLT Stanford Sleepiness Scale showed that diabetic subjects were not aware of their sleepiness (see Figure 3.1.2). Moreover, none of them experienced recurrent irresistible daytime sleep attacks and none of them had ever had sudden loss of muscle tone provoked by intense emotion. Indeed, referring to The International Classification of Sleep Disorders (The American Sleep Disorders
Association, 1990), the only feature suggestive of narcolepsy in our diabetic subjects was sleep onset REM periods during the MSLT. Other characteristics of narcolepsy did not exist. Overnight Polysomnography showed that diabetics had a sleep onset latency longer than 10 minutes and a REM onset latency much longer than 20 minutes. Also, none of the diabetic subjects had a sleep onset latency on the MSLT less than 5 minutes. Finally, insulin-dependent diabetics generally do not have HLA DR2, simply because this HLA phenotype has been shown to protect them from the occurrence of Type-I diabetes (Palmer, J.P., and Lernmark, A., 1990). HLA DR2, on the other hand, is an important genetic marker for the development of narcolepsy (Guilleminault, C., 1994).

The MSLT, itself, may be questioned as a cause of the shortened sleep onset latency in diabetic subjects. It is possible that repeated MSLT increases the comfort for subjects to take a nap in the sleep laboratory and since our diabetic patients took the test on multiple occasions, they fell asleep more readily. However, our data shows that the sleep onset latency was shortened in IDDM subjects from the very first session of the study (See Table 3.2.4), and that sleep onset latency in the last treatment session; namely, treatment with the insulin pump, was significantly longer than earlier measurements. Furthermore, the test-retest reliability of MSLT has been confirmed ($r = 0.97$ and $p < 0.001$) (Zwyghuizen-Doorenbos, A., et al., 1988). Therefore, the differences in daytime sleep onset latency between IDDM and normal controls are likely due to the disease and not due to repeated testing in the study.

Hypotheses that explain the increased daytime sleepiness in diabetic subjects include chronic iatrogenic hyperinsulinemia and its effects on adrenergic pathways in the central nervous system. Chronic hyperinsulinemia is a known accompaniment of insulin-treated diabetic subjects and is associated with an increased availability of insulin in the central nervous system. Since insulin increases the amount of
catecholamines at the synaptic cleft (Sauter, A., et al., 1983), long term hyperinsulinemia may down-regulate catecholamine receptors. Indeed, up regulation of catecholamine receptors has been observed in untreated diabetic animals (Trulson, M.E., and Himmel, C.D., 1983) and loss of sensitivity to amphetamine reported to occur in hyperinsulenic rats (Chu, P.C., Lin, M.T., Shian, L.R., and Leu, S.Y., 1986). Thus, down-regulation of catecholamine receptors lessens the central nervous system sensitivity to catecholamine stimulation which could lead to decreased wakefulness and an increased tendency to fall asleep during daytime napping in diabetic subjects.

Insulin has also been shown to reduce the amount of GABA, glutamic acid and other amino acid neurotransmitters in the synaptic cleft (Rhoads, D.E., et al., 1984). Glutamate is an excitatory amino acid which normally causes arousal in the human central nervous system. During sleep, less glutamate is released (Cooper, J.R., et al., 1991). It is possible that hyperinsulinemia causes up-regulation of glutaminergic pathways in diabetic individuals. Thus, glutamate hypersensitivity may be related to the inability of diabetics to maintain sleep during the night. Decreased glutamate stimulation during wakefulness may also cause increased daytime sleepiness.

Measurements of learned daytime performance on psychometric testing of diabetic and non-diabetic individuals yield other interesting observations. It has been previously reported that cognitive performance is impaired in patients with diabetes mellitus (Miles, W.R. and Root, H.F., 1922; Ryan, C.M., and Williams, T.M., 1993; Skenazy, J.A. and Bigler, E.D., 1984). However, our data suggest that there are no differences in cognitive performance between young healthy patients with IDDM and age-matched non-diabetic controls. Indeed, diabetics actually appeared to perform better on some tests at certain times during the day (Figures 3.1.5, 3.1.6 and 3.1.7).
Performance in diabetic subjects showed large fluctuations throughout the day, and that time-of-day variability was significantly greater in diabetic than non-diabetic individuals. Finally, alterations in psychometric performance have been shown to be reliable measures of the sedative effects of a number of drugs (Friedman, H., et al., 1992). If the increased daytime sleepiness in patients with insulin-dependent diabetes mellitus is related to a sedative effect of insulin, itself, a difference in daytime cognitive performance should have been apparent on our testing.

There are three possible explanations for our failure to detect differences in psychometric performance between diabetic and non-diabetic control. First, the sample size in our study was small and so the possibility exists that there was a Type II error. The sample size of our study was calculated to reach a power of 80% to detect what was deemed to be a clinically relevant difference of 20% with an alpha less than 0.05. Post-hoc analysis shows that the power of the study was quite good, in the range of 85-99% for the four tests. This means that sample size is not the explanation for the failure to detect differences in psychometric performance.

Secondly, both groups experienced REM sleep deprivation. Diabetic had long-term, cumulative REMS deprivation, while normal controls had acute REMS deprivation, possibly due to the first-night effect. Studies in animals have shown that selective REM sleep deprivation causes learning and memory impairment. This is also supported by the theory of the function of dreams in the restoration, processing, and consolidation of memory. However, the relation between REM sleep deprivation and reduced cognitive function has been recently challenged. Human studies have shown that even after seven nights of REM sleep deprivation, the decline of daytime cognitive performance is not significant (Bonnet, M.H., 1994). Thus, it is unlikely that REM sleep deprivation is the cause of a low psychometric performance in diabetic and non-diabetic individuals. Furthermore, it appears that chronic sleep
fragmentation and not selective REM sleep deprivation is the main problem in IDDM. This statement is supported by a study that indicates that it is the continuity of sleep, not sleep efficiency, amount of slow-wave sleep nor REM sleep, itself, that determines the restorative effects of sleep (Bonnet, M.H., 1986). Moreover, restoration of sleep is very important in determining daytime performance and sleepiness (Bonnet, M.H., 1985). Performance after every one minute of sleep disruption correlates well with performance after total loss of sleep. However, arousal for 10 minutes after every onset of stage-2 sleep or REM sleep, is good enough to earn a normal restorative outcome of sleep. Thus, even though diabetic subjects have disrupted sleep, it is possible that restorative sleep is still good enough to build a normal daytime performance. It is also possible that restorative sleep is formed by the amount of SWS coupled with REM sleep or even sleep efficiency.

Finally, the MSLT and psychometric testing may be measuring different parameters. MSLT is a test for assessing the likelihood of sleep to occur, while psychometric tests assess the performance of a subject on a variety life skills and/or types of behavior that is studied. To demonstrate sleepiness on performance testing, the completion time of the task must be long enough to be able to indicate the impairment. Tasks that require less than ten minutes to complete are rarely able to detect sleepiness. On the other hand, tasks requiring completion times between ten to twenty minutes may detect performance-related sleepiness (Roth, T., et al., 1992). All of the psychometric tasks that we administered in our study to diabetic and control subjects were completed in a relatively short time interval, i.e., three times ten seconds for the MFTT, 90 seconds for each of the DSST and SDMT, and a maximum of three minutes for completion of the GPBT. Thus, the time to complete all tasks was less than 5 minutes. Consequently, even though diabetic subjects were significantly sleepier during the day than non-diabetic controls, the psychometric
performances of the two groups were the same. Our psychometric testing may simply not have been long enough to detect the sedative effects of insulin.

There are two major factors intrinsic to IDDM that may account for the oscillations in cognitive performance. First, blood glucose levels fluctuate to a much greater extent in diabetic subjects than non-diabetic controls. While it is true that performance has been linked to glucose, it appears that the relation is strongest when glucose levels are in the extreme range (Pramming, S., et al., 1986; Holmes, C.S., et al., 1983). Indeed, the correlation between blood glucose and cognitive function in subjects with IDDM is not linear, but rather an inverted U-shaped curve (Gold, P.E., 1986). Moreover, the latest publication from the Joslin Diabetes Center (Draelos, M.T., et al., 1995) suggests that cognitive function in patients with IDDM is generally well-preserved. Neuropsychological functions diminished only at blood glucose levels of 2.2 mM or below, and only small insignificant differences were observed between levels of 5.4, 8.9 (baseline), 14.4, and 21.1 mM. Blood glucose levels in our studies fell in the flatter regions of this relation. Moreover, correlations between performance and blood glucose were quite weak and there were no systematic variations throughout the day to explain the differences in psychometric performance.

Second, insulin levels in the circulation of diabetic subjects oscillate as the function of the absorption of short- and/or longer-acting insulin from subcutaneous depots. The absorption of insulin may be influenced by many factors. However, these oscillations, for the most part, are predictable and are consistent over time within an individual subject. The fluctuations in psychometric performance in patients with IDDM showed this type of variability. Moreover, they could be readily linked to differences in insulin kinetics/administration. Thus, the explanation may be insulin. However, as mentioned, glucose is an important substrate for normal neuronal function, and it may be that both insulin and glucose are involved. For example, we
know that insulin-treated diabetic patients are hyperinsulinemic (Galloway, J.A. and Chance R.E., 1990), and that hyperinsulinemia leads to a down-regulation of insulin receptors in the CNS. We also know that glucose transporters in the central nervous system are insulin dependent. Down-regulation of insulin receptors may decrease neuronal sensitivity to glucose. Thus, it is possible that fluctuations of insulin levels in the CNS may regulate glucose supply to neuronal cells such that performance varies according to insulin levels.
4.2 Human R/N vs. Beef/Pork R/NPH Insulin

Based on the results shown in Tables 3.2.1 to 3.2.5 and Figures 3.2.1 to 3.2.7, the second hypothesis is also accepted. Indeed, there are four interesting findings from this study. First, diabetic subjects exhibit a fragmented sleep pattern on both human R/N and beef/pork R/NPH insulins, similar to the former observations in the diabetic/control study. Secondly, diabetic subjects initiate sleep earlier on treatment with human R/N insulin than beef/pork R/NPH insulin. Thirdly, diabetic subjects spend a greater percentage of the night in REMS and have a better distribution of REM sleep on human R/N insulin. And finally, human R/N insulin-treated diabetic subjects have shorter sleep onset latencies and a greater tendency to fall asleep during the day on the MSLT.

Irrespective of insulin, patients with IDDM spend a high percentage of night in stage-1 and 2 sleep (60%), a low percentage of the night in SWS (18%) and REMS (15%), especially on beef/pork R/NPH insulin. These latter are below normative standards for age and gender (SWS 20.76±4.78%, REMS 28.00±5.66%) (Williams, R.L., et al., 1974). Thus, the observations in the diabetic-control study are confirmed. Also, in this study, we observed a high number of stage shifts and REM periods. There were no differences in this regard between human and beef/pork insulins. However, while on human R/N insulin, diabetic subjects fell asleep faster both on the Overnight Polysomnography and MSLT, and achieved sleep in a greater number of MSLT sleep periods. Also, human R/N insulin treatment was associated with a more normal sleep profile. There were no differences in cognitive performance before or during lorazepam sedation on the two insulin treatments. Finally, since subjects were assigned to the various treatments randomly, we analyzed the data in a factorial format to examine the effects of the order of treatments and the assessment sequence.
on outcome. This showed that the differences in sleep parameters were due to insulin treatment, human versus beef/pork, and not due to a first-night effect or the order of randomization in the study.

At first hand, the second study appears to be a balanced comparison between two insulin regimes differing mainly in their component insulin species. Not only are the dosages and combination of insulin preparations similar, but they produced equivalent levels of blood sugar control at all measurement times. However, there are important differences between human and beef/pork insulins. Human insulin more readily dissociates into its monomeric form and is more hydrophilic than beef/pork insulin. Thus, peak concentrations of human insulin are achieved earlier and are generally higher than with corresponding doses of beef/pork insulin. Moreover, its time-effect profile is significantly shorter (Brogden, R.N. and Heel, R.C., 1987). On the other hand, beef/pork insulin is more lipophylic than human insulin, and may distribute more readily into the circumventricular regions of the brain where the blood-brain barrier is less well developed.

On the day of the Overnight Polysomnography, subjects were advised to administer their evening insulin dose at 5:00 p.m. It would be expected, therefore, that at the initiation of sleep at approximately 11:00 p.m., the effects of regular human and beef/pork insulins will have largely dissipated (Figure 4.2.1). However, the intermediate-acting component of human N insulin would be at or near peak levels and beef/pork NPH insulin would be on the rise. Moreover, the last three or four hours of sleep, the overall effects of human N insulin would be decreasing, whereas beef/pork NPH insulin would likely remain fairly high. Indeed, it has been claimed that human insulin-treated diabetics are deficient in insulin in the early morning hours. Thus, there are important kinetic as well as species differences between insulins in this study.
Figure 4.2.1. The estimated time-concentration profile of regular insulin and NPH insulin in patients with Type-I diabetes mellitus while on human R/N or beef/pork R/NPH intermittent insulin injection (Howey, D.C., et al., 1994). The horizontal bar represents the fluctuation of insulin level related to the meal, in the healthy, non-diabetic population. The vertical bar represents the sleep period while the patterned part represents the REM sleep time.
The abnormalities in the sleep profile of diabetic subjects at the beginning of the night occur while both human and beef/pork insulin levels are relatively high. In this case, the shortened sleep onset latency for human insulin treatment suggests either a soporific effect of human insulin, presumably a species-related difference, or a greater inhibitory effect of beef/pork insulin on sleep, possibly because of its greater distribution into the CNS, a kinetic-related difference. The fact that the differences in the sleep latency between insulins is carried into the MSLT during the subsequent day and that the MSLT abnormalities, in general, suggest chronic sleep deprivation for both insulin species, leads us to favor the kinetic hypothesis. Also, as mentioned, there were no effects of insulin on cognitive performance, and these tests are fairly sensitive to the sedating properties of drugs on the brain.

The effects of insulin on the distribution of REM sleep support a similar kinetic interpretation. We know that in normal subjects REM sleep is distributed at the end of sleep. We also know that insulin reduces the occurrence of REM sleep in laboratory animals (Sangiah, S. and Caldwell, D.F., 1988). Thus, high levels of human and beef/pork insulins in the first half of the night reduces the occurrence of REM sleep at this time. This accounts for the normal or slightly prolonged REM onset latencies in these individuals. Peaking insulin levels in the last three and a half hours of sleep, particularly with beef/pork NPH insulin treatment, also correlate with a significantly lower amount of REM sleep. Conversely, while on human N insulin patients spent a longer time in REM sleep in keeping with a reduced insulin effect. Indeed, human R/N insulin treatment produced a normalized REM sleep distribution Interestingly, both human and beef/pork insulin-treated diabetics have selective REMS deprivation if compared to a normal population standard of the same age and gender (human insulin: 18.13±1.94%, beef/pork: 14.5±2.72%, Normative standard: 28.00±5.66%). Thus, even though the effects of chronic hyperinsulinemia are seen
with both insulin species, the brain clearly remains sensitive to hour to hour fluctuations in insulin levels.
4.3 Intermittent Lispro/Human Ultralente Insulin Injection vs. Beef/Pork R/N Insulin Injection

Tables 3.3.1 to 3.3.23 and Figures 3.3.1 to 3.3.11 show relatively few differences between treatment with lispro/human ultralente and beef/pork Iletin R/N insulins. However, there were several interesting findings from this study. First, both insulin treatments caused diabetic subjects to have fragmented sleep. Diabetics spent a disproportionately large percentage of the night in stage-2 and had frequent awakenings, frequent stage shifting and a longer time spent awake after sleep onset. However, in this case the sleep profile on lispro/human ultralente insulin was just as bad, if not worse, than beef/pork Iletin R/N insulin and patients actually had less REM sleep in the second half of the night while on lispro/human ultralente insulin. The MSLT showed the same abnormalities as former studies - short sleep onset latencies and a greater tendency to attain sleep during the day, and there were no differences in daytime cognitive performance.

When this study was designed, we were looking for an insulin species effect. The human-beef/pork insulin comparison had suggested a better sleep profile on human R/N insulin, and it was hypothesized that the kinetic features of lispro/human ultralente insulin would favor an even better outcome. As discussed, lispro insulin is a very short-acting insulin. Its peak concentration is achieved within 45 minutes of administration and it lasts only about four hours. Human ultralente, on the other hand, is a long-acting insulin that starts to have its effect at four to six hours, reaches a peak within eight to 24 hours and persists in the circulation for a full 28 to 36 hours. Thus, when diabetic subjects on lispro/human ultralente insulin inject their evening insulin dose at 5:00 p.m. and are preparing for sleep assessment at 11:00 p.m., we considered that the effects of their lispro insulin would have ended and that the effects of their
human ultralente insulin would be low and constant throughout the night. Also, we expected the time-effect profile of beef/pork Iletin R/N to be the same as Connaught Novo Nordisk beef/pork R/NPH used in the former study. Neither of these assumptions turned out to be correct. Concentrations of monomeric insulin following injection of human ultralente are known to be about 3-fold higher than those obtained following injection of equivalent doses of beef/pork ultralente (Seigler, D.D., et al., 1991). Thus, insulin levels are more likely to be consistently high throughout the night and day on lispro/human ultralente insulin (Figure 4.3.1). Moreover, the time-course of beef/pork Iletin, particularly, Iletin N, is shorter than the Connaught Novo Nordisk formulation. Indeed, there is considerably clinical opinion that Iletin N is more similar to human N insulin in its effect profile (personal communications Drs. R.N. Beck and M.A. Boctor, Division of Endocrinology, Royal University Hospital, Saskatoon).

The finding that the duration of REMS in beef/pork Iletin R/N-treated diabetic patients was greater than that on lispro/human ultralente insulin suggests the nighttime insulin effect was less, particularly in the latter parts of the night. What is more, comparison between beef/pork Iletin R/N and Connaught Novo Nordisk beef/pork R/NPH show a significantly greater duration of total REMS (82.00±8.70 min. vs. 56.40±11.60 min., p = 0.05) and greater duration of REMS in the last three and a half hours of sleep (51.33±6.12 min. vs. 28.75±6.75 min., p = 0.04) on the Iletin formulation. Also seen were significantly fewer wake time after sleep onset (p = 0.003) and fewer REMS periods (p = 0.007). Although this finding were opposite to those anticipated from the original design of the study, once appreciated, they strongly support on insulin effect directly related to the differences in the time-course of the two insulin regimens.
Figure 4.3.1. The estimated time-concentration profile of lispro or regular insulin and NPH or ultralente insulin in patients with Type-I diabetes mellitus while on lispro/human ultralente or beef/pork R/N (Howey, D.C., et al., 1994; Seigler, D.E., et al., 1991). The horizontal bar represents the fluctuation of insulin level related to the meal in healthy, non-diabetic population. The vertical bar represents the sleep period while the patterned part represents the REM sleep time.
4.4 Intermittent Insulin Injection vs. Continuous Subcutaneous Insulin Infusion Administered by an Insulin Pump

Statistical analysis also accepts the last hypothesis that there are differences between the effects of intermittent insulin injection and continuous subcutaneous insulin infusion on sleep and daytime performance in diabetic subjects. Indeed, there are three interesting findings in this study. First, the insulin pump, and in particular the beef/pork insulin pump, normalizes REM sleep in diabetic subjects. Secondly, and in spite of this improvement, diabetic subjects while on the insulin pump, continue to spend a disproportionate amount of time in stage-1 and 2 sleep and to have more frequent stage shifts and awakenings. Indeed, REM sleep was even more fragmented on the pump than on intermittent insulin injection. Finally, diabetic baseline cognitive performance was slightly better while on the insulin pump.

The insulin pump allows diabetic patients to determine their ongoing insulin requirements in relation to meals, exercise and the existing blood sugar levels in a dynamic, interactive, fashion. Insulin pumping also provides diabetics with low basal insulin level during non-meal periods (Figure 4.4.1). Indeed, we intentionally lowered basal insulin infusion rates during the night to reduce the effects of insulin, if any, on sleep. The results show that diabetic subjects had better glucose control on the insulin pump especially during the day of the MSLT and lorazepam studies. However, long-term control, as shown by the HbA1C, was worse than that of intermittent insulin injection. Since HbA1C reflects overall and not just daytime insulin-glycemic control, the higher HbA1C levels on the insulin pump indicate that we were successful in achieving a lessening of insulin effect during the night. Moreover, as shown in Table 3.3.1 diabetic subjects on the insulin pump had a stable blood glucose level at 2:00 am. Thus, the likelihood of undetected nocturnal hypoglycemia is low.
Figure 4.4.1. The estimated time-concentration profile of lispro or beef/pork regular insulin in patients with Type-I diabetes mellitus using an insulin pump (Howey, D.C., et al., 1994). The horizontal bar represents the fluctuation of insulin level related to the meal in healthy, non-diabetic population. The vertical bar represents the sleep period while the patterned part represents the REM sleep time.
Insulin pumping improved several quantitative aspects of the diabetic sleep abnormality. Less time was spent in stage-2 sleep, and more time was spent in SWS, including SWS in the first sleep cycle and SWS in the first 4 hours of sleep, and more time in REMS and REMS at the end of the night. Indeed, the percentages of time spent in these sleep stages compared favorably to normative standards for age and gender. Moreover, and the distribution of SWS and REMS throughout the night were completely normal on the insulin pump. However, the beneficial effects of pumping were clearly limited to pumping with beef/pork insulin. SWS and REMS were improved over their corresponding levels on both intermittent beef/pork R/NPH and intermittent lispro/human ultralente insulins; the differences with lispro generally being larger and significant because sleep was poorest on this insulin regimen. Conversely, these parameters were only minimally affected and, in many cases, worsened by pumping lispro insulin. With all the other evidence in these studies pointing to kinetic differences between insulins, we are inclined to attribute these differences to differences in insulin kinetics, as well. It is possible that lispro insulin, with its greater tendency toward dissociation into the active monomeric form, favors a greater distribution of insulin and a greater insulin effect in the CNS when administered by continuous subcutaneous infusion using an insulin pump. Thus, we believe the beneficial effects of the beef/pork insulin pump resulted from lower night time basal levels of insulin on this regimen.

Interestingly, insulin pumping did not reduce the number of stage shifts and awakenings after sleep onset in diabetic subjects. Indeed, it actually seemed to worsen REM sleep fragmentation as shown by the high number of REMS periods on the pump. This suggests one of two possibilities; namely, 1) diabetic patients are still significantly hyperinsulinemic, even on the pump and that still lower levels of insulin are required to correct this, or 2) other factors besides insulin are at play. We know
that patients receiving exogenous insulin are hyperinsulinemic. Normally, insulin is secreted into the portal circulation where high concentrations inhibit hepatic gluconeogenesis and at the same time are metabolized by the liver, thereby limiting its systemic availability. Much higher levels of exogenous insulin are required to achieve the same level of glycemic control when administered systematically. Thus, despite lowering insulin levels, pumping cannot correct the intrinsic hyperinsulinemia of insulin-treated diabetic patients. There is also evidence in the literature that blood glucose may affect REMS. Lechin et al. (1992; 1993) showed that following a glucose challenge, diabetic patients had more REMS. Oral glucose may affect central cholinergic activity because intestinal glucose stimulates the parasympathetic nervous system. While, it is possible that the higher glucose levels during the night in patients on the insulin pump increased the amount of REM sleep, preliminary data from our insulin clamp studies show quite clearly that it is the insulin and not the glucose.

Daytime sleepiness as measured by the MSLT is about the same while on the insulin pump compared to intermittent insulin injection. Diabetic subjects have slightly, but significantly, longer sleep onset latencies on the beef/pork insulin pump. However, it is obvious that these individuals are still moderately impaired (drowsy) and are apt to sleep at the slightest provocation. Also, it is interesting to observe that even after the apparent quantitative improvements in SWS and REM sleep, and less light sleep and awakening after sleep onset, diabetic patients still have mild daytime sleepiness. It is possible that the frequent stage shifting and awakening, which are not corrected by the pump have a greater influence on daytime sleepiness. It is also possible that sleepiness correlates more with the nature of the insulin therapy. As has been pointed out, hyperinsulinemia persists even with the insulin pump. Moreover, insulin pump therapy resulted in the poorest long-term insulin/glycemic control, and high levels of HbA1c are believed to be related to an increasing level of advanced
glycation end products (AGEs) and increasing production of IL-1 and TNF (Brownlee, M., 1995). These substances are known to effect sleep; IL-1 and TNF increase the sleep tendency, augment SWS amplitudes and inhibit the occurrence of REM sleep (Krueger, J.M., and Toth, L.A., 1994). Therefore, it is possible that mild daytime sleepiness during insulin pump therapy is related to the production of IL-1 and TNF. It is difficult to reconcile the poorer sleep profiles and increased daytime sleepiness with the lower HbA1c in patients on intermittent insulin. However, it shows once again the lack of objective human data on this subject.

Daytime cognitive performance is slightly, but significantly better when diabetics are on the insulin pump (particularly the beef/pork pump) than while on intermittent insulin injection. There are several possible explanations for this including lower insulin levels on the pump, better insulin/glycemic control, and recovery from chronic REM sleep deprivation. We have learned previously that insulin affects the kinetics of several neurotransmitters and their receptors, especially dopamine, noradrenaline and adrenaline (Sauter, A., et al., 1983). In general, the magnitude of the improvements were quite modest. However, it was not the intention of the study to reduce insulin levels during the day or to improve overall glycemic control. The point is, if these can be shown to affect cognitive performance as we believe this experiment suggests, there may be very large gains to be made by controlling these both in diabetic and non-diabetic individuals.
5. Conclusions

The conclusions of our studies are as follows:

1. Patients with insulin-dependent diabetes mellitus have a chronic sleep disorder characterized by a disproportionately large percentage of the night spent in “light” stage-1 and stage-2 sleep with correspondingly low percentages of SWS and REMS, and frequent sleep disruption with many stage shifts, REM periods and awakenings after sleep onset. The problem appears to be an excessive arousal response intrinsic to the sleep abnormality, itself.

2. Diabetic patients are excessively drowsy during the day and readily attain sleep with minimal provocation. This may be related to their disrupted sleep.

3. Young healthy subjects with IDDM perform normally on a variety of tests of cognitive function. Their performance varies throughout the day and this variability is greater than the variability in test scores of non-diabetic individuals.

4. The abnormalities in sleep and cognitive performance in diabetic subjects are directly related to their insulin, with minimal, if any, effect of glucose. Specifically, sleep and performance can be altered by changes in the species or manner of insulin administration.

5. There are differences between the effects of human and beef/pork insulin on sleep, daytime sleepiness and daytime cognitive performance. While on human R/N insulin, diabetic subjects initiate sleep earlier, spend a longer percentage of the night in REM sleep, and have a better overall REM sleep distribution. Also, they are sleepier during the day and are more likely to sleep during the MSLT than on
beef/pork R/NPH insulin treatment. These differences appear to be related to differences in the time-effect profiles of the two insulin preparations, human R/N insulin having a high, short time-effect profile compared to beef/pork R/NPH insulin.

6. There are differences between the effects of lispro/human ultralente and beef/pork R/N insulin on sleep, daytime sleepiness, and daytime cognitive performance. Lispro/human ultralente insulin causes diabetic subjects to spend less time in REM sleep and to have a more disrupted REM sleep pattern in the second half of the night. This appears to be due to the long time-effect profile and high levels of human ultralente insulin.

7. There are similar differences in sleep and day-time performance between the effects of twice daily intermittent subcutaneous insulin injection and continuous subcutaneous insulin infusion using an insulin pump. Insulin pumping increases the amount and distribution of REMS on Overnight Polysomnography. However, the pattern of frequent stage shifts, short frequent REMS periods and frequent awakenings after sleep onset persist. Daytime sleepiness is slightly improved on the insulin pump, although they are still quite drowsy and apt to sleep on the MSLT. Their daytime performance on the DSST, SDMT, and GPBT are slightly, but significantly better while they are on the insulin pump than on intermittent injection. The improvements in sleep and cognitive performance are most apparent on the beef/pork insulin pump, and questionable and in some cases, possibly adverse, on the lispro insulin pump. Again, these differences likely relate to insulin concentrations and distribution into the brain.

8. The rapid, time-dependent, changes in insulin effect in the CNS and their relation to insulin kinetics suggest a dynamic regulation with both short- and long-term abnormalities in patients with IDDM.
6. Recommendations

There are a number of questions that are raised by this study. For example, do patients with IDDM suffer from REM sleep deprivation or is the abnormality an overriding circadian rhythm disorder? Do patients with NIDDM have a corresponding disturbance? How does blood glucose affect sleep, daytime sleepiness and cognitive performance? Are the differences between human insulin and beef/pork and lispro insulins related only to the kinetics differences, or are there additional species-related differences between insulin? Finally, short of reducing insulin, are there other ways to improve the sleep and cognitive impairments in diabetic patients? Unfortunately, like any study that brings new knowledge, or adds significantly to existing knowledge, more questions are raised than are able to be answered. However, this study recommends:

1. Although the sample size used in our studies were sufficient to show the differences between patients with IDDM and non-diabetic controls and between on various insulin regimens, and the patterns of response in respect of these are fairly clear there are a number of limitations inherent in studying small samples. For example, there are several comparisons where a difference would be anticipated but the number of subjects were small and variability large such that the p-value was close, but not at an appropriate level of significance. Obviously, a Type-II error is likely to have occurred. Small sample size also limits the degrees of freedom that can be applied in ANOVA, because of the need to adjust for the variability between subjects. Indeed, if we perform a comparison without consideration of between subject variability, differences will not be detected due
to the high standard deviation. Thus, larger sample sizes are recommended in future studies of sleep and cognitive performance in diabetic populations. This design will allow analyses of the interaction between insulin treatment and the time-effect profile with the appropriate degrees of freedom.

2. Because of the appearance of first-night effect in non-diabetic controls, it is recommended that in future sleep studies control subjects should be studied on the same number of occasions and under the same conditions as diabetic individuals.

3. Extending sleep analysis to include better recording of limb movement, respiratory effort, nasal and oral air flow, and oxygen saturation is recommended in order to be able to explain the cause(s) of disrupted sleep in patients with insulin-dependent diabetes mellitus. Also, Power Spectral Analysis of the various EEG activity in different sleep stages should be obtained.

4. Further studies of the effects of insulin on sleep, daytime sleepiness, and cognitive performance should be extended to include complete 24 hour recording so that the issues of whether diabetics have an isolated sleep disturbance or an overall disturbance in circadian rhythm can be addressed. Measurement of melatonin levels may also be helpful in this regard.

5. It is important to show that the effect of insulin is dose-dependent and to examine the effects of blood sugar in central nervous system function. The insulin/glycemic clamp currently being used is recommended.
6. Our studies have shown that there are differences between insulin regimens in their effects on the CNS. These differences in large measure appear to be related to the kinetics of the evening dose of insulin. It would be premature to recommend one species of insulin or one manner of insulin administration over another at this time. However, our data show that maintaining blood insulin at low levels during non-meal periods improves the sleep and cognitive function of diabetic subjects. Thus, it is recommended that diabetics use the lowest dosage of insulin that is required to maintain their blood sugar within acceptable limits. It is also recommended that diet, exercise and other non-insulin treatments (metformin, α-glycosidase inhibitors, and insulin sensitizers, when available) be considered to facilitate glycemic control. If lifestyle causes a diabetic to use more insulin, avoiding high insulin levels during the night should be considered by giving three injections of short-acting insulin prior to each meal with preferably the last meal injection at 6:00 p.m.. Basal insulin requirements could be managed with a single low dosage of long-acting insulin given in the morning or two injections of intermediate-acting insulin, the second injection given at lunch.

7. Insulin therapy has been shown to increase the life expectancy of patients with IDDM and to reduce the occurrence of diabetic complications. However, administration of exogenous insulin by whatever means is not able to replicate the physiological secretion and effects of endogenous insulin. Insulin therapy causes hyperinsulinemia and it is likely that systemic hyperinsulinemia has adverse biological effects in the human body. We have shown for the first time that patients with IDDM are unable to maintain sleep, have shorter REM onset latencies, have less slow wave sleep and REMS, and suffer from moderate daytime sleepiness and greater REM pressure during the day and, that these
abnormalities are related to their insulin treatment. Other adverse consequences of peripheral insulinization possibly include an accelerated rate of atherosclerosis and ischemic heart diseases. Thus, before the recommendations of the DCCT to employ intensive insulin therapy to control blood sugar in diabetic subjects can be accepted, a careful consideration of the risks and benefits is in order. Longitudinal studies of the quality of life of patients with diabetes mellitus are required and should include sleep, daytime sleepiness and cognitive performance. Response to various treatment interactions should not be limited to biochemical or histopathological outcomes.

8. The prevalence of IDDM is only 5-10% of the total population of diabetic patients in North America. The rest are mostly patients with non insulin-dependent diabetes mellitus. Our study examined the relationship between sleep and insulin insensitivity in patients with IDDM. Meanwhile, insulin insensitivity is a major issue in patients with NIDDM. Therefore, there is a need for further studies of sleep in patients with NIDDM.
REFERENCES


Carskadon, M., To Sleep or Not to Sleep.... *Sleep Medicine Review*, 1993; 1(6): 3.


Feinberg, I., Untreated Type 2 Diabetes as a Cause of Daytime Somnolence [Letter]. *Sleep*, 1993; 16(1): 82.


McCall, A., Gould, J., and Ruderman, N., Abnormal Glucose Metabolism of Brain Microvessels in Diabetes. *Diabetes*, 1982; 31 (suppl. 2), 63A.


Pardridge, W.M., Boado, R.J., Farrell, C.R., Brain-Type Glucose Transporter (GLUT-1) is Selectively Localized to the Blood-Brain Barrier: Studies with Quantitative Western Blotting and In Situ Hybridization. Journal of Biological Chemistry, 1990; 265(29): 18035-18040.


APPENDICES
Consent for Studies in Human Subjects

Title of Research Proposal: Effects of Insulin on Sleep and Response to Lorazepam Sedation in Patients with Insulin-Dependent Diabetes

Principal Investigator: Robert J. Herman, MD

Name of Volunteer: ________________________________ Age: ________________

Address: ________________________________ Phone: ___________________

The following is provided to inform you about the above named research project and your possible participation in it. Please read this form carefully. Feel free to ask any questions regarding the study or the information presented below. To contact Dr. Herman or his research associate call 966-6295 during regular office hours, or 241-1042 in the evenings.

OBJECTIVES OF THE STUDY

The objectives of this study are to examine the effects of different insulins (human versus beef/pork) on the brain. The study consists of 2 parts: 1) a study of the effects of insulin on sleep in the absence of any other drugs, and 2) a study of the effects of insulin on your ability to perform a number of simple tests of memory and coordination before and after administration of a common sedating drug called lorazepam, or Ativan (related to Valium). Thus, we would like to measure your pattern of sleep and extent of impairment of psychomotor performance following lorazepam while on treatment with one type of insulin (human or beef/pork), then switch you to the other insulin type and examine you again. The results of this study will increase our understanding of the effects of insulin in diabetes and its use in patients with this disease.

PROCEDURE

Volunteers will be required to pass a standard medical examination and laboratory screening before being accepted into the study. If you qualify and agree to participate, you must not take any medication (other than insulin) including non-prescription tablets, lotions, or alcohol at least 7 days before, and throughout the study interval.
1. The study begins with a 2 week observation period wherein you will be asked to maintain a stable and adequate sleep habit and to record this in a sleep diary. Twice weekly q.i.d. chemstrip blood sugars will also be measured. Upon satisfactory completion of this, you will be admitted to the Clinical Investigation Unit at Royal University Hospital at approximately 10 pm on a Friday or Saturday evening. EEG electrodes will be attached to your head and you will be given a bed and instructed to sleep. Brain wave recordings will be obtained continuously throughout the night. The following morning you will be awakened at 6 am and asked to participate in a procedure called multiple sleep latency testing. Briefly, this test assesses your level of wakefulness by measuring the time it takes for you to fall asleep. This will be performed on 5 separate occasions at 2 hour intervals throughout the day.

2. One week later, you will return to the Clinical Investigation Unit at approximately 7 am for the second part of the study. EEG leads will again be attached to your head and an intravenous cannula consisting of 0.9% (normal) saline solution will be placed in one arm. Baseline EEG and performance on a number of simple psychometric tests will then be recorded, following which a single dose of 2 mg of lorazepam (Ativan) will be administered by mouth. Blood will be sampled at regular time intervals over the ensuing 12 hours, and at each sampling time, EEG and psychometric testing will be repeated.

3. Upon completion of these studies, you will be switched from your usual insulin to an equivalent dose of an insulin of the alternate type (patients treated initially with beef/pork insulin will be switched to human insulin, and patients on human insulin will be switched to beef/pork insulin). A 2 week observation period will then ensue to allow you to adjust to your new insulin preparation and for completion of another sleep diary and period of blood sugar monitoring. The entire sleep study and lorazepam study will then be repeated.

The study will take approximately 4-6 weeks to complete. The total amount of blood drawn over this period will be approximately 250 mL, or 1/2 of a standard Red Cross donation.

RISKS AND BENEFITS

There are no direct benefits to you for doing the study. The results, however, are intended to increase our understanding of the effects of insulin in diabetes and, thus, may help doctors in treating patients with this disease.

The discomforts, inconveniences, and risks that can be reasonably expected from the study are as follows:
1. Ativan is used as a sedative so that you may feel somewhat drowsy, particularly for the first 2-3 hours after receiving the drug, and to a lesser extent throughout the day. You will be carefully observed while in hospital, and should be free of drug effect by the time you are ready to go home.

2. Changing treatment from animal insulin to human insulin may result in a decrease in the recognition of symptoms of hypoglycemia. Patients should be aware of this possibility and take steps to avoid low blood sugar (don't miss meals and check blood sugars regularly). Changing from human insulin to animal insulins may also cause problems. Sensitivity to non-insulin protein contaminants in animal insulins occasionally occurs, however, this is rare with the new highly purified insulin varieties available today. Animal insulins can also induce the appearance of circulating anti-insulin antibodies, but this is usually mild and should not be a problem. Patients requiring additional assistance with blood sugar monitoring and diabetic control will be referred to the Diabetic Education Center at Royal University Hospital.

4. Blood sampling is a source of minor inconvenience, and may result in slight bruising or soreness.

All data collected during the course of the study will be kept confidential and accessible only to the principal investigator and his assistants. Should the results of this project be published, you will be referred to only by number or letter.

Statement of the Person Agreeing to Participate in this Research Proposal

Consent means that you understand what the study involves and agree to participate. This is not a legal contract and does not absolve the doctors involved in your treatment of the responsibility of your good medical care. You are free to withdraw from the study at any time.

I have read this consent form. The procedure and its risks have been explained to me and all my questions have been answered. I freely and voluntarily agree to participate in this study.

________________________________________  Signature of Volunteer
Date

________________________________________  Signature of Witness
Date

________________________________________  Signature of Study Coordinator
Date
SLEEP DISORDERS CLINIC
Center for Chronobiology
University of Toronto

Name ____________________________

Date Started ____________________________ Day of Week ______________

Instructions:
• Please leave diary near your bedside.
• It is important that you fill out this chart each morning.

• Mark your diary in the following way:

ACTIVITIES
A = each alcoholic drink
B = each caffeinated drink includes coffee, tea, chocolate, cola
P = every time you take a sleeping pill or tranquilizer
M = meals
S = snacks
X = exercise
T = use of toilet during sleep-time
H = noises that disturb your sleep
W = time of wake-up alarm (if any)

SLEEP-TIME (including naps)

• Enter with a “*” event each time you get into bed
• Enter with a “X” event each time you get out of bed
• Enter with a “*” event each time you get up
• Enter with a “X” event each time you go to bed

Example:

<table>
<thead>
<tr>
<th>ACTIVITIES</th>
<th>SLEEP TIME</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>B</td>
</tr>
<tr>
<td>LIGHTS OUT</td>
<td>1230 @pm</td>
</tr>
<tr>
<td>TOTAL SLEEP TIME</td>
<td>5 hrs</td>
</tr>
<tr>
<td>Day</td>
<td>Activities</td>
</tr>
<tr>
<td>-----</td>
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</tr>
<tr>
<td>1</td>
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</tbody>
</table>

**Weekly Sleep Summary**

<table>
<thead>
<tr>
<th>Day</th>
<th>Lights Out</th>
<th>AM/PM</th>
<th>Total Sleep Time</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td></td>
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<td>ACTIVITIES</td>
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<tr>
<td>SLEEP TIME</td>
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<td>LIGHTS OUT _______ am/pm</td>
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<td>TOTAL SLEEP TIME _______ hrs</td>
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<td>LIGHTS OUT _______ am/pm</td>
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<td>TOTAL SLEEP TIME _______ hrs</td>
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<td>LIGHTS OUT _______ am/pm</td>
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<tr>
<td>TOTAL SLEEP TIME _______ hrs</td>
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</tbody>
</table>
INSTRUCTION FOR SLEEP STUDIES

A. The Seven days prior to the study date.

1. Sleep at least 7.5 hours a day.
2. Sleep and wake up at about the same time.
3. Record daily sleep in your Sleep Diary, as instructed on the enclosed pages.
4. Measure blood sugar 4 times a day, twice a week (not on consecutive days) and record glucose level and insulin doses on your Sleep Diary (Diabetics only).
5. Reduce caffeinated beverages prior to the study date: e.g. coffee, tea, cocoa, various COLAs and related beverages
6. No alcohol
7. No medications including non-prescription drugs.

B. On the Dates of the Sleep Study

1. Keep activities as usual
2. Do not sleep or nap during the day
3. Do not drink any coffee, other caffeinated or alcoholic beverages.
4. Meet at the Sleep Disorder Lab at Royal University Hospital at 8:00 PM
5. Wear or bring clothes in which you will be able to sleep comfortably.
6. Bring your insulins (diabetics only) and sleep Diary with you to the hospital
7. The study will finish by 5:30 PM or earlier at the following afternoon.

If you have any question regarding of the study, please call Agus Dahan at 477-3420 (home) or 966-6303 (office), and if you have any problems in controlling blood sugar level please call Dr. Herman at 373-7297 (home) or 966-7960 and 966-6295 (office).
Centre for Sleep and Chronobiology
The Toronto Hospital

Name: ___________________________    Time: __________
Date: _______________    Health Card No.: ___________________________

PRE-SLEEP QUESTIONNAIRE

At what time did you awaken today? ______ a.m.  p.m.

Has today been an unusual day in any way? No ___ Yes ___. If yes, explain:

Did you fall asleep or take a nap today? No ___ Yes ___. If yes, when and for how long:

Did you drink any alcohol today? No ___ Yes ___. If yes, when ______, how much ____________?

Did you, or will you, use any medications (prescription or non-prescription) today? No ___ Yes ___. If yes, specify type and amount:

Have you used any prescription medication in the last 2 weeks? No ___ Yes ___. If yes, specify type and amount:

Please indicate how many cups or glasses of the following that you have consumed today:

__ coffee, __ decaffeinated coffee, __ tea, __ cola, __ chocolate drinks

At what time did you drink your last caffeinated beverage? ____________ a.m.  p.m.
### HOW DO YOU FEEL?

- **CALM**
- **HAPPY**
- **ENERGETIC**
- **RELAXED**

### STANFORD SLEEPINESS SCALE

Please check (✓) the statement which best describes your state of sleepiness. (Choose only one statement)

|   |  
|---|---
| 1 | Feel active and vital; alert; wide awake  
| 2 | Functioning at a high level, but not at peak; able to concentrate  
| 3 | Relaxed; awake; not at full alertness; responsive  
| 4 | A little foggy; not at peak; let down  
| 5 | Fogginess; beginning to lose interest in remaining awake; slowed down  
| 6 | Sleepiness; prefer to be lying down; fighting sleep; woozy  
| 7 | Almost in reverie; sleep onset soon; lost struggle to remain awake  

Put a check mark (✔) in the appropriate column to indicate if you are experiencing any of the following, right now.

<table>
<thead>
<tr>
<th></th>
<th>Not at All</th>
<th>Slightly</th>
<th>Moderately</th>
<th>Intensely</th>
</tr>
</thead>
<tbody>
<tr>
<td>Headache</td>
<td></td>
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<tr>
<td>Unsteadiness</td>
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<td></td>
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<tr>
<td>Faintness</td>
<td></td>
<td></td>
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<tr>
<td>Breathing difficulty</td>
<td></td>
<td></td>
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<tr>
<td>Chest pain</td>
<td></td>
<td></td>
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<tr>
<td>Sweating</td>
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<tr>
<td>Numbness [ specify:</td>
<td></td>
<td></td>
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<tr>
<td>Flushing</td>
<td></td>
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<tr>
<td>Chills</td>
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<tr>
<td>Heart palpitations</td>
<td></td>
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<tr>
<td>Sexual feelings</td>
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<tr>
<td>Hunger</td>
<td></td>
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<tr>
<td>Bloating</td>
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<tr>
<td>Nausea</td>
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<tr>
<td>Gastric fullness</td>
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<tr>
<td>Abdominal pain</td>
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<tr>
<td>Feverishness</td>
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<tr>
<td>Constipation</td>
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<tr>
<td>Diarrhea</td>
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<tr>
<td>Urinary problems</td>
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<tr>
<td>Blurred vision</td>
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<tr>
<td>Irritated eyes</td>
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<tr>
<td>Puffy eyes</td>
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<tr>
<td>Blacking out of sight</td>
<td></td>
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<tr>
<td>Noise in ears</td>
<td></td>
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<tr>
<td>Reduced hearing</td>
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<tr>
<td>Increased taste sensitivity</td>
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<tr>
<td>Increased smell sensitivity</td>
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</tr>
<tr>
<td>Dry mouth</td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>Thirst</td>
<td></td>
<td></td>
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</tr>
</tbody>
</table>
Use the chart below to indicate the severity of any pains, aches, or stiffness that you may be experiencing right now.

On the chart a check (✓) in the row labelled '0' indicates no discomfort. A check (✓) in the row labelled '6' indicates the worst possible discomfort.

FATIGUE SCALE

Please check (✓) the statement which best describes your present state of physical energy or fatigue.

<p>| | |</p>
<table>
<thead>
<tr>
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<th></th>
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<tbody>
<tr>
<td></td>
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</tbody>
</table>

1. Full of energy: enough to tackle my usual physical activities.

2. Energy level is quite high but not at its peak: most physical activities would pose no problem.

3. Energy level is such that one would prefer to be doing very light or sedentary tasks at this point.

4. Energy level is adequate for only routine activities at a leisurely pace.

5. Energy level is such that it would be preferable to rest before doing any routine activity.

6. Energy level is quite low: would strongly prefer to rest rather than do anything else.

7. Totally physically exhausted: unable to undertake the least activity.
Centre for Sleep and Chronobiology
The Toronto Hospital

POST-SLEEP QUESTIONNAIRE

Name: ________________________________

Date: ________________ Time: ________________

Please complete immediately upon the final awakening

How long did it take you to fall asleep last night: ________ minutes

How much sleep do you think you got last night: ________ hours

Please indicate with an X on the line:

____________________________________________________________________

Best Possible Sleep

Worst Possible Sleep

How many times do you think you woke up last night: ________ times.

How did last night differ from your usual night's sleep, taking into account that you slept in a different bed, with electrodes, etc.

Any comments or suggestions:
### Before Going to Bed

<table>
<thead>
<tr>
<th>Feeling</th>
<th>Scale</th>
</tr>
</thead>
<tbody>
<tr>
<td>Asleep quickly</td>
<td>Long time awake</td>
</tr>
<tr>
<td>Felt very physically tense</td>
<td>Felt very physically relaxed</td>
</tr>
<tr>
<td>No worries on my mind</td>
<td>Many worries on my mind</td>
</tr>
<tr>
<td>Many thoughts</td>
<td>No thoughts</td>
</tr>
<tr>
<td>Felt very sleepy</td>
<td>Felt very wide awake</td>
</tr>
<tr>
<td>Felt very exhausted</td>
<td>Not exhausted at all</td>
</tr>
<tr>
<td>Had many physical ailments</td>
<td>Had no physical ailments</td>
</tr>
<tr>
<td>Went to bed in a very bad mood</td>
<td>Went to bed in a very good mood</td>
</tr>
</tbody>
</table>

### During the Night

<table>
<thead>
<tr>
<th>Condition</th>
<th>Scale</th>
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</thead>
<tbody>
<tr>
<td>Frequently awakened</td>
<td>Uninterrupted sleep</td>
</tr>
<tr>
<td>No noises</td>
<td>Very noisy</td>
</tr>
<tr>
<td>Very comfortable room temp.</td>
<td>Extremely hot or cold</td>
</tr>
<tr>
<td>Very uncomfortable bed</td>
<td>Very comfortable bed</td>
</tr>
<tr>
<td>Little or no body movement</td>
<td>Toasted and turned all night</td>
</tr>
<tr>
<td>Awakened and took an extremely long time to go back to sleep</td>
<td>Awakened but immediately went back to sleep</td>
</tr>
<tr>
<td>Lightest sleep possible</td>
<td>Deepest sleep possible</td>
</tr>
</tbody>
</table>
### During the Night (Continued)

<table>
<thead>
<tr>
<th>Adequate amount of sleep</th>
<th>Not enough sleep at all</th>
</tr>
</thead>
<tbody>
<tr>
<td>Many thoughts</td>
<td>No thoughts</td>
</tr>
<tr>
<td>Felt very physically relaxed</td>
<td>Felt very physically tense</td>
</tr>
<tr>
<td>Had many physical ailments</td>
<td>Had no physical ailments</td>
</tr>
<tr>
<td>Extremely pleasant dreams</td>
<td>Extremely unpleasant dreams</td>
</tr>
<tr>
<td>Many dreams</td>
<td>No dreams</td>
</tr>
</tbody>
</table>

### Upon Awakening

<table>
<thead>
<tr>
<th>Woke up long before or after I expected</th>
<th>Woke up exactly when I expected</th>
</tr>
</thead>
<tbody>
<tr>
<td>Woke up extremely tired</td>
<td>Woke up as rested as possible</td>
</tr>
<tr>
<td>Had a very hard time awakening</td>
<td>Woke up as easily as possible</td>
</tr>
<tr>
<td>Woke up in a very good mood</td>
<td>Woke up in a very bad mood</td>
</tr>
<tr>
<td>Remembered extremely unpleasant dreams</td>
<td>Remembered very pleasant dreams</td>
</tr>
<tr>
<td>Woke up feeling as physically poor as possible</td>
<td>Woke up feeling as physically good as possible</td>
</tr>
<tr>
<td>Woke up with no worries on my mind</td>
<td>Woke up with many worries</td>
</tr>
<tr>
<td>Woke up with no thoughts on my mind</td>
<td>Woke up with many thoughts</td>
</tr>
</tbody>
</table>
Put a check mark (✓) in the appropriate column to indicate if you are experiencing any of the following, **right now**.

<table>
<thead>
<tr>
<th>Condition</th>
<th>Not at All</th>
<th>Slightly</th>
<th>Moderately</th>
<th>Intensity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Headache</td>
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<tr>
<td>Unsteadiness</td>
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<tr>
<td>Faintness</td>
<td></td>
<td></td>
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<tr>
<td>Breathing difficulty</td>
<td></td>
<td></td>
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<tr>
<td>Chest pain</td>
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<td></td>
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<tr>
<td>Sweating</td>
<td></td>
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<tr>
<td>Numbness [specify: ___]</td>
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<tr>
<td>Flushing</td>
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<tr>
<td>Chills</td>
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<tr>
<td>Heart palpitations</td>
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<tr>
<td>Sexual feelings</td>
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<tr>
<td>Hunger</td>
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<tr>
<td>Bloating</td>
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<tr>
<td>Nausea</td>
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<tr>
<td>Gastric fullness</td>
<td></td>
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<tr>
<td>Abdominal pain</td>
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<tr>
<td>Feverishness</td>
<td></td>
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<tr>
<td>Constipation</td>
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<tr>
<td>Diarrhea</td>
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<tr>
<td>Urinary problems</td>
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<tr>
<td>Blurred vision</td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>Irritated eyes</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Puffy eyes</td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>Blacking out of sight</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Noise in ears</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Reduced hearing</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Increased taste sensitivity</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Increased smell sensitivity</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dry mouth</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Thirst</td>
<td></td>
<td></td>
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</tr>
</tbody>
</table>
**HOW DO YOU FEEL?**

- Example: CALM | ----------- | IRRITABLE
- CALM
- HAPPY  | SAD
- ENERGETIC | SLUGGISH
- RELAXED  | TENSE

**STANFORD SLEEPINESS SCALE**

Please check (√) the statement which best describes your state of sleepiness. (Choose only one statement)

<p>| | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Feel active and vital; alert; wide awake</td>
</tr>
<tr>
<td>2</td>
<td>Functioning at a high level, but not at peak; able to concentrate</td>
</tr>
<tr>
<td>3</td>
<td>Relaxed; awake; not at full alertness; responsive</td>
</tr>
<tr>
<td>4</td>
<td>A little foggy; not at peak; let down</td>
</tr>
<tr>
<td>5</td>
<td>Fogginess; beginning to lose interest in remaining awake; slowed down</td>
</tr>
<tr>
<td>6</td>
<td>Sleepiness; prefer to be lying down; fighting sleep; woozy</td>
</tr>
<tr>
<td>7</td>
<td>Almost in reverie; sleep onset soon; lost struggle to remain awake</td>
</tr>
</tbody>
</table>
Use the chart below to indicate the severity of any pains, aches, or stiffness that you may be experiencing right now.

On the chart a check (✓) in the row labelled '0' indicates no discomfort.

a check (✓) in the row labelled '6' indicates the worst possible discomfort.

### FATIGUE SCALE

Please check (✓) the statement which best describes your **present** state of physical energy or fatigue.

<p>| | | | | | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Full of energy: enough to tackle my usual physical activities.</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>Energy level is quite high but not at its peak: most physical activities would pose no problem.</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>Energy level is such that one would prefer to be doing very light or sedentary tasks at this point.</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>Energy level is adequate for only routine activities at a leisurely pace.</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>Energy level is such that it would be preferable to rest before doing any routine activity.</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>Energy level is quite low: would strongly prefer to rest rather than do anything else.</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>Totally physically exhausted: unable to undertake the least activity.</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
PRE-MSLT QUESTIONNAIRE

Name: ____________________________ Time: ____________

Date: ____________________ Blood sugar: ____________________

HOW DO YOU FEEL

CALM   ————  IRRITABLE

HAPPY ————  SAD

ENERGETIC ————  SLUGGISH

RELAXED ————  TENSE
**STANFORD SLEEPINESS SCALE**

Please check (✓) the statement which best describes your state of sleepiness. (Choose only one statement)

<table>
<thead>
<tr>
<th></th>
<th>Statement</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Feel active and vital; alert; wide awake</td>
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<td>2</td>
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<tr>
<td>7</td>
<td>Almost in reverie; sleep onset soon; lost struggle to remain awake</td>
</tr>
</tbody>
</table>

**FATIGUE SCALE**

Please check (✓) the statement which best describes your present state of physical energy of fatigue

<table>
<thead>
<tr>
<th></th>
<th>Statement</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Full of energy; enough to tackle my usual physical activities</td>
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<td>2</td>
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<td>Energy level is such that one would prefer to be doing very light or sedentary tasks at this point</td>
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<td>4</td>
<td>Energy level is adequate for only routine activities at a leisurely pace</td>
</tr>
<tr>
<td>5</td>
<td>Energy level is such that it would be preferable to rest before doing any routine activity</td>
</tr>
<tr>
<td>6</td>
<td>Energy level is quite low: would strongly prefer to rest rather than do anything else</td>
</tr>
<tr>
<td>7</td>
<td>Totally physically exhausted: unable to undertake the least activity</td>
</tr>
</tbody>
</table>
POST-MSLT QUESTIONNAIRE

Name:__________________________________________________________

Date:_________________________ Time:___________________________

Please complete immediately upon the final awakening

How long did it take you to fall asleep last time: ____________ minutes

How much sleep do you think you got last time: ____________ minutes

Did you dream? Yes/No

Please indicate with an X on line:

| Best Possible Sleep | Worst Possible Sleep |

How many times do you think you woke up last time: ____________ times.

How did last time differ your usual day's sleep, taking into account that you slept in a different bed, with electrodes, etc.

Any comments or suggestions:
Digit Symbol Substitution Test
### Key

<table>
<thead>
<tr>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
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</table>

### Multiplication Tables

<p>| 2 | 1 | 3 | 7 | 2 | 4 | 8 | 2 | 1 | 3 | 2 | 1 | 4 | 2 | 3 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 5 | 2 | 3 | 1 | 4 | 5 | 6 | 3 | 1 | 4 | 1 | 5 | 4 | 2 | 7 |
| 6 | 3 | 5 | 7 | 2 | 8 | 5 | 4 | 6 | 3 | 7 | 2 | 8 | 1 | 9 |
| 5 | 8 | 4 | 7 | 3 | 6 | 2 | 5 | 1 | 9 | 2 | 8 | 3 | 7 | 4 |
| 6 | 5 | 9 | 4 | 8 | 3 | 7 | 2 | 6 | 1 | 5 | 4 | 6 | 3 | 7 |
| 9 | 2 | 8 | 1 | 7 | 9 | 4 | 6 | 8 | 5 | 9 | 7 | 1 | 8 | 5 |
| 2 | 9 | 4 | 8 | 6 | 3 | 7 | 9 | 8 | 6 | 1 | 4 | 3 | 2 | 7 |
| 9 | 6 | 1 | 5 | 7 | 2 | 6 | 1 | 3 | 9 | 4 | 2 | 7 | 9 | 5 |</p>
<table>
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<td>3</td>
<td>9</td>
<td>4</td>
<td>2</td>
<td>7</td>
<td>9</td>
<td>5</td>
</tr>
</tbody>
</table>
**DSST/SDMT INSTRUCTION**

**MATERIALS**  DSST/SDMT worksheet, printed on the last page of the Record Form

2 pencils without erasers (for use of the subject and the examiner)

Digit Symbol/Symbol Digit Scoring Stencil

**DIRECTIONS**  A smooth drawing surface must be provided. If the table has a rough surface, the Digit Symbol/Symbol Digit worksheet should be placed over a piece of cardboard.

Hand the subject a pencil without eraser. Place the worksheet in front of the subject, point to the Key above the test items, and say,

*Look at these boxes. Notice that each has a number/special mark in the upper part and a special mark/number in the lower part. Each number/mark has its own mark/number.*

Point to 1/first mark and its mark/number, then to 2/second mark and its mark/number.

*Now look down here where the boxes have numbers/marks in the top part but the squares at the bottom are empty.*

Point to the Sample items.

*You are to put each of the empty squares the mark/number that should go there, like this.*

Point to the first several Sample spaces.

*Here is a 2/mark; the 2/mark has this mark/number.*

Point to the first Sample item, then to the mark/number below the 2/mark in the Key.

*So I put it in this square, like this.*

Write in the symbol/number in the first Sample square.

*Here is a 1/another mark; the 1/mark has this mark/number.*

---

Point to the second Sample item, then to the mark/number below the 1/mask in the Key.

So I put it in this square.

Write in the symbol/number.

This number is 3/this is another mark; the 3/mask has this mark/number.

Point to the third Sample item, then to the mark/number below the 3/mask in the Key.

So I put it in this square.

Write in the symbol/number.

After marking the first three Sample items, say,

Now you fill in the squares up to this heavy line.

Point. If the subject makes an error on a sample item, correct the error immediately and review the use of the Key. Continue to help (if necessary) until the seven Sample items have been filled in correctly. Do not proceed with the test until the subject clearly understands the task. When the subject fills in a Sample item correctly, offer encouragement by saying

Yes or Right,

and finally,

Yes, now you know how to do them.

During the Sample exercise, look to see if a left-handed subject blocks or partially blocks the Key when filling in the marks/numbers. If this occurs, fold a separate Record Form in half, exposing only the Digit Symbol/Symbol Digit worksheet, and place it next to the subject’s worksheet on the subject’s right-hand side so that the extra Key is aligned with the one blocked by the subject’s hand. Have the subject use the separate Key to complete the Sample items and to take the actual test.

When the sample exercise has been completed successfully say,

When I tell you to start, you do the rest of them.

Point to the first test item and say,

Begin here and fill in as many squares as you can, one after the other, without skipping any. Keep working until I tell you to stop. Work as quickly as you can without making mistakes.
Sweep across the first row with your finger and say,
   When you finish this line go on to this one.

Point to the first item in row 2.
Say,
   Go ahead,
and begin timing. If the subjects omits an item or starts to do only one type (e.g. only the 1's/one mark), say,
   Do them in order. Don't skip any.

Point to the first item omitted and say,
   Do this one next.

Give no further assistance except (if necessary) to remind the subject to continue until instructed to stop.

At the end of 90 seconds, say,
   Stop.
Timing must be precise on this test.
Symbol Digit Modalities Test
| ( | - | | | + | ) | - | | | | | | |
|---|---|---|---|---|---|---|---|---|
| 4 | 5 | 3 | 7 | 2 | 8 | 9 | 1 | 6 |

### Key

- ( | - | | | + | ) | - | | | | | | |
- ( | - | | | + | ) | - | | | | | | |
- ( | - | | | + | ) | - | | | | | | |
- ( | - | | | + | ) | - | | | | | | |
- ( | - | | | + | ) | - | | | | | | |
- ( | - | | | + | ) | - | | | | | | |
- ( | - | | | + | ) | - | | | | | | |
- ( | - | | | + | ) | - | | | | | | |
- ( | - | | | + | ) | - | | | | | | |
- ( | - | | | + | ) | - | | | | | | |
- ( | - | | | + | ) | - | | | | | | |
- ( | - | | | + | ) | - | | | | | | |
INSTRUCTION FOR THE MFTT

The finger tapper is placed in mid-line with the patient so that the finger tapper is at the edge of the table. The examiner explains the test:

This is a tapping board, a key and a counter.

The examiner points out each and continues.

Place your dominant hand, palm down, on the tapping board with all your fingers extended, and place your index finger on the key. Hold the board with the other hand, so that it does not move while you are tapping. Tap the key with your index finger as quickly as you can, while your hand and arm stay still. Therefore, you can only move your index finger. Make sure that each tap you make is strong enough to be counted by the counter, but not too strong that it reduces your speed.

The examiner demonstrates.

When I say go, begin to tap the key as fast as you can, using only your index finger of your dominant hand. Each test takes 10 seconds to complete. You will do the test three times with brief periods in between. Any questions? Ready, as fast as you can, go.
Name:_____________________________ Time:______
Date:__________ Blood sugar:_________ Score:_____

MANUAL FINGER TAPPING TEST
(10 sec per trial; 3 trials within 3)

  Dominant Hand................
  1. 
  2. 
  3. 
  Average = ................

GROOVED PEGBOARD TEST
(180 sec per trial)

  Dominant hand:.................
  Time of completion .......... 
  Number of errors ............ 
  Number of Pegs .............
INSTRUCTION FOR THE GROOVED PEGBOARD TEST

The Pegboard is placed in mid-line with the patient so that the board is at the edge of the table and the peg tray immediately above the board. The examiner explains the test:

This is a pegboard and these are the pegs.

The examiner points out each and then picks up one of the pegs and continues.

All the pegs are the same. They have a groove, that is, a round side and a square side and so do the holes in the board. What you must do is match the groove of the peg with the groove of the board and put these pegs into the holes like this.

The examiner demonstrates by filling the top row.

When I say go, begin here and put the pegs into the board as fast as you can, using only your (dominant) hand. Fill the top row completely from this side to this side. DO not skip any, fill each row the same way you filled the top row. Any questions? Ready, as fast as you can, go.

---