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The Efficacy of Exogenous Insulin Administered with Total Parenteral Nutrition

Thesis for Master of Science Degree

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ABSTRACT

Anabolic hormones have been proposed as an adjunct to parenteral nutrition to hasten the repletion of the body cell mass. In this study, alternate patients referred for total parenteral nutrition (TPN) received solutions containing 20 to 35 units CZI/L. Diabetics were excluded and studied as a separate group. All patients underwent isotope dilution body composition studies prior to starting TPN and every two weeks.

Control patients did not receive insulin. They doubled their pre-TPN serum insulin with 3-5 days of TPN and maintained this concentration at day 14 and 28. The insulin and diabetic groups increased their serum insulin significantly, $p < 0.05$, compared to controls at the same time interval. Maintenance serum insulin concentration of controls was 59.6 ± 6.6 mcU/ml; the insulin group (99.3 ± 10.4 mcU/ml) and diabetics (96.4 ± 13.7 mcU/ml) demonstrated significantly higher concentrations, $p < 0.05$. Normally nourished patients, by the exchangeable sodium: exchangeable potassium (Na_e/K_e) ratio, maintained normal nutrition whether or not insulin was added. Malnourished control and insulin patients improved equally. No significant effect of insulin was observed. However, by multiple linear regression: Daily change BCM % = $-1.79 + 0.28(Na_e/K_e) + 0.03$(Calories) $+ 0.002$(insulin), $p < 0.01$, insulin ensures maintenance of the body cell mass at lower caloric intakes and increases the repletion rate.

SOMMAIRE

Les hormones anaboliques, telle l'insuline, ajoutées à l'alimentation totale parentérale (ATP) pourraient hâter la restoration de la masse cellulaire déficiente. Dans cette expérience, nous avons ajouté de 20 à 35 U/L d'insuline CZI à la solution parentérale de chaque deuxième patient. Nous avons exclu les patients diabétiques pour en former un troisième groupe. Chaque patient a subi une étude de la composition du corps par dilution d'isotopes avant l'ATP et tous les 15 jours.

Les patients contrôles n'ont pas reçu d'insuline. Après 3 à 5 jours d'ATP ils ont doublé leur concentration d'insuline du départ dans le serum. Cette concentration est restée elevée au 14e et 28e jours. Dans le meme intervalle, la concentration d'insuline dans le serum des groupes insuline et diabétique a augmenté comparée aux contrôles, $p < 0.05$. La concentration d'insuline dans le serum à une infusion constante d'ATP était de 59.6 ± 6.6 mcU/ml chez les controles, 99.3 ± 10.4 mcU/ml chez le groupe insuline ($p < 0.05$) et 96.4 ± 13.7 mcU/ml chez les diabétiques ($p < 0.05$). Les patients considérés bien alimentés selon le critère du rapport sodium échangeable: potassium échangeable (Na_e/K_e), ont conservé leur état normal de nutrition. Les patients des groupes contrôle et insuline en état de nutrition déficiente se sont ameliorés de façon égale. L'insuline n'a pas eu d'effet bénéfique ou néfaste. La régression multiple lineaire: Changement quotidien de la masse cellulaire corporelle % $= -1.79 + 0.28(Na_e/K_e) + 0.03(\text{Calories}) + 0.002(\text{Insuline})$, $p < 0.01$, démontre que l'insuline assure la maintenance de la masse cellulaire corporelle à des infusions moindres en calories et également augmente le taux de restoration du corps déficient en nutrition.

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PREFACE

Many co-workers and collaborators have made this work possible; their aid and support are thankfully acknowledged. Dr. H. Shizgal, who developed the indirect method of body composition that will be referred to below, not only served as my supervisor of research but also guided a good deal of the clinical course of the patients. The staff of the surgical research laboratories, Pauline Crosilla, Debbie Tuitt and Barbara Rhode ensured the accuracy of the technical aspects of the body composition measurements. They prepared the isotope solutions for injection into the patient and counted the activity of various samples. They verified the data with myself before they were entered into the computer data base. I am also appreciative of the work of Pamela Gardiner-Lawson, the parenteral nutrition nurse, who assisted in the management of the patients and who documented the daily infusions of intravenous fluids, intake of oral fluids, calories, biochemistry, microbiology and general progress of the patients.

The insulin data presented here was possible through collaboration with the Polypeptide Laboratory of McGill University under the supervision of Dr. Barry Posner. The insulin radioimmunoassay was carried out as described below.

As resident on the parenteral nutrition service, I reviewed the patients referred for hyperalimentation with Dr. Shizgal and outlined a proposed course of parenteral nutrition appropriate for their condition. After informed consent, patients underwent body composition studies which were performed by the staff of the research labs, supervised by myself. Of these, alternate patients received a parenteral solution which contained insulin; otherwise all patients received the same solution. The same protocol of blood sampling and body composition was followed. I reviewed all charts when the data came to analysis and carried out statistical analysis as described in the text.

Early in the study, I worked with Dr. Armour Forse who preceded me in the research laboratories and was instrumental in developing a great deal of the clinical protocol for administration of exogenous insulin in the parenteral nutrition solution and who did many baseline studies of insulin over time. This allowed us to use a low enough dose of insulin that would be safe yet would ensure adequate blood levels of the hormone.

Lastly, I acknowledge the patience of my wife and family who understood the importance of this work and the time needed to devote to it.

This work is not intended as a review of the copious literature on the subject of insulin. Rather, the nutritional effects of the hormone will be stressed, especially as it is

related to malnutrition and its use in parenteral hyperalimentation. Malnutrition will be discussed in general terms. Body composition, especially using the multiple isotope dilution technique, will be discussed in more detail. Finally, the physiology and endocrinology of several altered states of body composition as it may relate to the patients involved in this study, will be considered.

INTRODUCTION

Malnutrition is not new to science or to humanity. It has been present throughout the ages due to drought, famine, war, poverty, misadventure, plague and disease. Indeed, each of these unfortunate events exists today. Still, many individuals, child and adult, have survived the most extreme forms of malnutrition and many more have weathered milder insults. Yet it is those who have succumbed to its effects by death or morbidity who are the irretrievable loss which might have been prevented. Poor nutrition has subtle but, at times, devastating effects that wisely makes the physician wary, particularly the surgeon, who often must inflict tissue injury in a patient in an already precarious state. Conversely, surgery itself, as well as many medical diseases or malignancy may render the patient malnourished, sometimes in spite of what would otherwise be considered adequate protein and caloric intake. This is the very basis of early and aggressive nutrition in medicine. This field has now become a sophisticated offshoot of the study of medicine. The 'art' of nutrition yields daily to the 'science' as new data come to light. The history of parenteral nutrition will reveal the extent to which it is still somewhat an art.

HISTORY OF INTRAVENOUS HYPERALIMENTATION

Jonathan Rhoads recently presented the important landmarks in the field of parenteral nutrition (1). He notes that in 1913, a protein hydrolysate of casein was administered intravenously to a goat to achieve positive nitrogen balance, by Henriquez and Andersen (2). In 1938, Robscheit-Robbins and Whipple showed that nitrogen balance could be achieved with plasma as the sole protein source (3). It is however an expensive source of protein which must be degraded before native protein is synthesised. Cox et al. described the enzymic hydrolysis of casein in 1939 (4). Elman and Weiner (5) reported the first administration of acid-hydrolysed casein plus tryptophan and cystine to patients, which resulted in improved nitrogen balance, regenerated serum protein and decreased nutritional edema. Prior to this only plasma was used peripherally as a nitrogen source. Stohl et al. (6) gave enzymic hydrolysates of casein to infants as sole source of nitrogen with positive nitrogen balance in both the orally fed and intravenously fed (with 10% glucose) groups. There were febrile reactions with the I. V. mixture. Hartmann et al. (7) in 1942, administered Amigen orally and parenterally with a decreased negative balance. In 1944, Helfrick et al. (8) described feeding an infant a complete diet using 50% glucose, 10% amino acids, 10% fat emulsions, salt, water and vitamins

with successful treatment of marasmus. In 1944, Co Tui (9) gave intravenous Amigen-Dextrose until sufficient calories could be taken by Abbott-Rawson tube. When nitrogen intake was adequate body weight increased. Werner used an amino acid mixture (VUJ, Mead) as a supplement and noted protein sparing in postoperative patients. However, the volumes of fluid infused were high (10). Madden et al. (11) reported that different casein digests, administered either I. V. or per os, would replete the plasma proteins of dogs made protein deficient by plasmapheresis and low protein diets.

Lipid emulsions were introduced as a caloric source in the 1930's by Emmett Holt, Jr. (12). The product was initially unstable and was improved by Stare and Geyer (13). Lipids were eventually removed from the U. S. market due to hyperpyrexic reactions. Research into this product continued in Sweden and Intralipid was reintroduced into the North American market in 1972.

Administering carbohydrate as a caloric source has the drawback that it must be given either in large volumes or as hyperosmolar solutions which could not be infused into peripheral veins. The first was counteracted by potent diuretics. The second could only be solved by developing techniques of catheterisation of the superior vena cava. Parkins, Vars and Rhode (14, 15) first described an apparatus

used in dogs with which access to the superior vena cava was gained via the jugular vein using long intravenous tubing. This was used to deliver a protein hydrolysate (Amigen) and gelatin P-20. Nitrogen balance was improved with this solution but positive balance was not consistently achieved. Subclavian vein puncture used most commonly today was described by Aubaniac (16) in 1952.

The breakthrough in parenteral nutrition occured when Dudrick and Vars fed 12 week old beagles intravenously with hypertonic dextrose, hydrolysed protein, minerals and vitamins by a central vein apparatus. At 72 to 255 days, they were able to show equal growth (measured by serial bony X-ray) and positive weight gain compared to control littermates which were orally fed (17, 18, 19). Wilmore and Dudrick (20) gave hypertonic Dextrose-protein hydrolysate to a newborn with small bowel atresia through a central vein and were able to report positive nitrogen balance, as well as normal growth and development over six weeks of parenteral alimentation. Wilmore et al. published a review of 18 infant patients similarly treated, with positive results (21). Dudrick et al. described, in 1968, an experience with adult patients suffering from chronic intestinal disease, who were supported with intravenous nutrition for 10 to 200 days with generally positive nitrogen balance. No fat was given (22). Fuller et al. were able to administer 20% glucose and 5% fibrin hydrolysates, electrolytes

and vitamins into the superior vena cava in 14 ill infants less than two months old. Their weight gain approximated that of normal infants in spite of septic complications and surgical procedures (23). Again in 1969, Kaplan et al. (24) administered an hyperosmolar hyperalimentation solution by a central vein to seven postsurgical infants with good results in six. Many of the metabolic complications which are well known today were described. In 1969, Dudrick et al. (25) described their entire experience, demonstrating positive nitrogen balance, weight gain, growth and development in infants and improvement of nutritional status in debilitated adults.

Since amino acid and hypertonic dextrose solutions could be easily administered by central vein with positive nitrogen balance, the field of parenteral nutrition has grown immensely. Present research focuses on many areas: trace elements, vitamins and deficiencies, optimum nitrogen to Calorie ratios, optimal caloric source, metabolic needs and responses as measured by oxygen consumption and carbon dioxide production, efficacy of parenteral nutrition as measured by nitrogen balance versus body composition measurements, amino acid and protein turnovers or flux in steady state conditions, as well as other metabolic parameters (including hormones) of malnutrition or injury and of the effects of hyperalimentation.

Malnutrition is classically described as either Kwashiorkor or marasmus. The major characteristic of the former is nutritional edema (and in a child, weight loss greater than 60% of expected); it generally arises from a deficiency of protein in the presence of adequate caloric intake (the African word means "golden boy" due to a red hue in the hair produced by protein deficiency). Marasmus, produced by a deficiency of both proteins and calories, is not usually characterised by edema or the very large weight loss of Kwashiorkor (26). This classification of malnutrition is generally applied to children and intermediate forms are recognised and, at times, it has been applied to adult patients. Blackburn et al. (27, 28) feel that nutritional deficiencies of significance in adult North American patients are associated with combined protein and Calorie deficiencies. Garrow et al. (29) found that in children with extreme malnutrition, the total body protein was decreased to approximately two-thirds that of normal children, with wide variations in the composition of the fat-free body. It is felt that this is due to an imbalance between protein synthesis and protein breakdown and is influenced by the original mass of protein involved in turnover and the protein turnover rate (30). In starvation, the synthesis of protein in muscle promptly falls and there is an increase in protein breakdown to provide the

liver with amino acids for essential protein synthesis and gluconeogenesis (30, 31). Millward (31) found that a protein-free diet does not produce as severe a reduction of the synthesis rate as does starvation, probably due to the effect of circulating carbohydrate and insulin. Again, according to Waterlow et al. (32), muscle mass, or its reduction, is the best indicator available of malnutrition.

In 1912, Benedict studied a volunteer, Mr. L., who underwent a period of starvation lasting 31 days. Benedict published extensive metabolic data accumulated during this time (33a). He noted that the subject lost excessive amounts of nitrogen in the urine in the first few days and that the nitrogen loss per kg of body weight diminished gradually over the period of the experiment. Protein catabolised, calculated as 6.0 X nitrogen, ranged from a maximum of 71.2 gm on the fourth day of fasting to 41.6 gm on the last day. The total loss of nitrogen was 277 gm. Benedict assumed that flesh in the starving individual is 20% protein and calculated the "flesh equivalent" of catabolised protein. This ranged from a maximum 356 gm/day to a minimum 208 gm/day. The total flesh equivalent {cellular mass} lost over 31 days was 8.326 kg (the subject lost 11.96 kg in wieght), an average of 268.6 gm/day. The effect of fasting alone, without other metabolic stress, is thus seen on the cellular protein mass.

Benedict et al. also studied 25 subjects who underwent prolonged reduction in food intake. The group who were subjected to moderate reduction lost an average of 2.1 gm N /day the equivalent of 53 gm of cellular mass. A second group underwent more severe reduction over a short period and lost a mean 3.1 gm nitrogen/day or 77.5 gm cellular mass. Weight loss was more dramatic in this group (33b).

Benedict also noted that the fuel for catabolism in the first few days of starvation was glucose (and amino acids for gluconeogenesis). Eventually, endogenous fat provided about 75% of calories. Fat and amino acids are the only stores available for prolonged use in starvation. Fat metabolism adapts to supervene over glucose so that protein may be conserved. This data has been confirmed by Cahill who postulated that insulin is the controlling hormone as far as fuel mobilisation is concerned. The plasma insulin concentration decreases with fasting and signals increased lipid mobilisation as an endogenous fuel and thus, protein sparing. In the fed state, the plasma insulin concentration is elevated, which is responsible for increased protein anabolism, decreased fat mobilisation, and synthesis and storage of triglyceride (34, 35, 36).

Canon, in 1929, reviewed the current knowledge of the autonomic nervous systems and related stressful stimuli to

sympathetic nervous output as well as the release of "adrenin" (adrenalin) from the adrenal gland (37). The action of "adrenin" is noted to be the same as that of the central nervous system i. e. dilatation of pupils, piloerection, vasoconstriction, cessation of gut activity, restoration of irritability to muscle whether fatigued or denervated, and increased plasma glucose concentration presumably from the liver. He related glycosuria to emotionally stressful situations as well as pain. With little knowledge of insulin and glucagon, he attributed this entirely to "adrenin".

Later, Cuthbertson expanded on the metabolic response to shock and injury. He noted that after bony and non-bony injury, there was a marked output of sulfur, nitrogen and phosphorus, which was disproportionate to the amount of tissue injured and which was maximal on the second to the sixth days. He rationalised that protein might be serving as a source of glucose calories to maintain a level of increased metabolism, similar to the effects of starvation (38). He found that after bony or non-bony injury, the maximum loss of nitrogen might be as high as 23 grams/day and last for several days. Assuming protein = 6.25 X nitrogen and one quarter of the cell is protein, this is the equivalent to 575 grams/day of body cellular mass. At this rate, in ten days, this loss would reach 137 grams of nitrogen which is equivalent to 3.4 kg of cellular mass and deplete the total body nitrogen content by 7.7%. Basal

oxygen consumption usually rose to parallel the nitrogen losses. He selected patients with bony injury and gave them a high protein diet and a high calorie diet or both and found that these failed to eliminate the negative nitrogen balance at the height of catabolism. He also noted that the high nitrogen:sulfur and nitrogen:phosphorus ratios indicated that losses occurred from muscle whether it was directly injured or not. Nitrogen loss may continue to some small extent even beyond six weeks after injury. He notes that "in addition to the severe wasting and autolysis, there appears to be a general increase in katabolism, of proteins in particular, to meet the enhanced metabolism of the repair process." (39)

Moore (46) describes six stimulus-response sequences in surgical patients. Five are summarised here: (A) severe tissue injury, characterised by protein breakdown, oxidation of fat, gluconeogenesis, conservation of ECF, and substrate intolerance. These are mediated by catecholamines primarily, decreased insulin secretion and action, increased glucagon and cortisol as well as the renin-angiotensin-aldosterone mechanism. (B) volume reduction, characterised by sodium retention, aciduria and potassium loss, alkalosis, antidiuresis, minor loss of nitrogen, and fat oxidation. It is mediated by renin-angiotensin and aldosterone, ADH, and secondary increases in ACTH-cortisol and catecholamines. This is usually short-lived. (C) low flow (tissue anaerobiosis) characterised by the same responses as (A)

and (B) but more severe and prolonged, with lactic acidosis, decreased renal function and urine output. It is mediated by an exaggerated catecholamine response, maximal ACTH-cortisol response, increased glucagon with paradoxically low insulin. It may continue with end organ damage. (D) starvation, characterised by gradual proteolysis (mostly muscle), tendency to retain ECF, gluconeogenesis, fat oxidation, and substrate tolerance. There is an aldosterone-like effect, some antidiuresis, gradual increase in glucagon, and decreased insulin. (E) invasive infection characterised by the same as (A) and (B) but more severe and prolonged with markedly increased fat oxidation and oxygen consumption, possibly with an increased cardiac output or low flow state and large losses of protein by catabolism and through exudates. Mediation is by exaggerated catecholamine and corticosteroid, renin-angiotensin-aldosterone, and antidiuretic responses. There is also an insulin intolerance.

BODY COMPOSITION

Nitrogen balance has long been used as a measure of protein metabolism. Positive nitrogen balance represents growth in children (40), repletion in malnourished adults. Nitrogen balance is a measure of the net effect of protein anabolism and catabolism. In the presence of a steady state, protein anabolism is reflected by positive balance, catabolism by negative (41). For accuracy, all nitrogen input to the patient must be scrupulously measured, as well as all the output. Changes are usually small and Vinnars points out that the input tends to be overestimated while the output is underestimated (42). As a result, these errors are systematic and therefore cumulative.

Moore (43) demonstrated that changes in the lean body mass (LBM), reflected by the total body water (TBW) as determined by deuterium or tritium dilution (LBM = TBW/0.73 since 73% of the body weight in the adult is water), is a more accurate representation of positive nitrogen metabolism than is weight change alone. The measurement of body composition has been used in this laboratory to assess a) the nutritional state, b) the effect of I. V. amino acid on protein sparing in postoperative patients, c) caloric requirements in patients receiving TPN, d) the efficacy of TPN solutions (41). In our hands, the Na_e/K_e (total body exchangeable sodium:total body exchangeable

potassium) ratio is a sensitive indicator of malnutrition. The normal ratio in healthy individuals is 0.98 with an upper 95% confidence limit of 1.22. The exchangeable sodium is a reflexion of the extracellular mass, the exchangeable potassium a reflexion of the cellular mass or body cell mass. With malnutrition, the former normally increases, whereas the latter tends to decrease, thus, the ratio increases.

In this study, the Na_e/K_e ratio is used as a definition of malnutrition, values less than 1.22 representing a normal state of nutrition and greater than 1.22 indicating malnutrition. This number represents the upper 95% confidence limit of 25 normal volunteers studied in this laboratory. It has been validated by analysing 500 consecutive body composition studies (44). The ratio was less than 1.22, i. e. a normal nutritional state, in 204 patients. A ratio greater than 1.22, indicating malnutrition, occurred in 296 patients. The total exhangeable potassium for all patients was plotted against TBW using the 95% confidence limits of the 25 normal volunteers. In those patients with a ratio less than 1.22, most of the data points fell within the normal range, whereas if the ratio were greater than 1.22, the data points fell below the 95% confidence limit. The validity of the ratio is thus established.

The body cell mass is the totality of living, oxygen-consuming, energy-exchanging cells in the body. It

includes everything within the cells i. e. nucleus, cytoplasm, cell membrane. It is normally 30-38% of body weight (45). It is derived from the exchangeable potassium as there is a linear relationship between K_e and the body cell mass (43). In contrast to sodium, which has about 1000 mEq which is nonexchangeable in bone, all but 0.5% of the body potassium (including bone) is available for exchange with the isotope, so that K_e is equivalent to total body potassium. Total exchangeable potassium by indirect technique (47) correlates directly with potassium-42 direct isotope dilution. Since the N:K ratio in the cells is constant at 3mEq K/gram N (2.8 to 3.2) and one quarter of the wet cell weight is protein (25.8%) and assuming protein is 6.25 X cell nitrogen, then BCM may be determined from the exchangeable potassium as follows: BCM = 8.33 X K_e. This has been verified by a cadaver study by Moore (45). All energy exchanges, synthesis, and mitosis occur within the cellular compartment. Therefore the nutritional state can best be evaluated by measuring the body cell mass.

The lean body mass, which is equivalent to the fat free mass, is body weight minus body fat. This is best measured by estimating the total body water, as LBM = TBW / 0.73. The lean body mass is 75-80% of body weight. Lean body mass minus body cell mass is the extracellular mass (ECM). This includes structural extracellular proteins (hair and dermis, cartilage, tendon, skeleton and fascia), fluids (plasma, extracellular

water, joint fluid, lymph, pericardial, pleural and peritoneal fluid, third space, connective tissue structures of adipose tissue, cerebrospinal fluid and the extracellular supporting system of the CNS and peripheral nerves) as well as extracellular proteins in solution (albumin, immunoglobulins, extracellular enzymes including those in the gastrointestinal tract). It also includes urine in the collecting ducts, ureters and kidneys, and feces in the gastrointestinal tract (45). Note that no energy exchange takes place in the extracellular mass. Nutritional edema of malnutrition occurs in this compartment.

Body composition may be measured by various isotope dilutions. Total body water may be measured by dilution of either deuterium or tritium. Either Na22 or Na24 is used to measure the exchangeable sodium in the body. Exchangeable potassium may be measured by a direct technique (43, 45, 54, 55) using dilution of K42 or K43. These two isotopes have a half-life of 12.5 hours and 22.4 hours respectively and therefore there must be a constant fresh supply, they must be used immediately and there can be no delays in counting. K40 is an isotope which exists as 0.012% of naturally occurring potassium. This can be counted directly by a whole body counter using gamma-ray spectrometry (56, 57, 58). The facilities required for this counting are expensive and calibration is a major problem. Potassium may also be measured by an indirect technique, as in this laboratory. This technique is based on

the fact that the cations sodium and potassium are distributed in water throughout the body at a constant value, R, in all tissues but bone (45, 47, 59, 60). This R value can be determined by assaying whole blood sodium, potassium and water content. The exchangeable potassium is the same function of R, exchangeable sodium and total body water as is the whole blood potassium a function of R, whole blood sodium and water content. The calculations are found below, in the method section.

Isotope dilution body composition determinations are static measurements of the body compartments. Error in this method is random (due to errors in counting of isotope) but is not cummulative, as is the error of metabolic balance.

HYPOTHESIS

Cahill (36) attributes the over-all control of fuel to the hormone insulin. Insulin is an anabolic hormone. In the post prandial state, high levels of insulin serve to maintain serum glucose within narrow limits, store glucose as glycogen for emergency use, store excess calories as fat. It also promotes protein synthesis. Excess calories, even in the form of protein, are converted to fat, with elimination of nitrogen. In the fasting state, low serum insulin concentrations cause proteolysis to serve the need for gluconeogenesis and mobilisation of fat stores. The effect due to insulin of increasing amino acid uptake by cells, decreasing amino acid release and increasing protein synthesis as well as fat deposition would be welcome in the clinical situation in which malnourished patients are being refed by hyperalimentation.

Insulin secretion is stimulated moderately by low dose carbohydrate, maximally by large doses, moderately by amino acids but the largest response occurs in the presence of both carbohydrate and protein. Little release occurs in diabetes and prolonged fast (36). Total parenteral nutrition, then, with hypertonic dextrose and high concentration of amino acids, would be expected to stimulate endogenous insulin release. This would be partially responsible for the repletion of the body cell mass that we do observe in malnourished patients. Would the addition

of exogenous insulin result in a further increase in the repletion rate of the body cell mass?

Starvation, injury, sepsis, shock all are associated with significant loss of body cell mass. Benedict (33a) and Cuthbertson (39) have emphasized the magnitude of the initial losses of nitrogenous mass after fasting or injury, which may reach 8% of the total body nitrogen (Cuthbertson) (or up to one fifth the body cell mass) at 10 days. The rate of repletion of this loss, in spite of an adequate caloric intake is considerably slower than the rate at which it was lost. A method to increase this rate of repletion would be welcome when dealing with malnourished patients who are ill and often develop further compications of their disease.

Since the abnormal stimulus-response sequences in starvation and disease are mediated by hormones, hormone manipulation has been attempted to restore early positive nitrogen balance. Growth hormone has been used along with oral intake in five burn patients with limitied success (48). Anabolic steroids (methandienone), administered with high Calorie and casein hydrolysate intravenous feedings, were ineffective in countering the negative nitrogen balance 5 days postoperatively. However, these were normally nourished patients (49). Hinton, Littlejohn, Lloyd and Allison, in 1971, reasoned that the nitrogen losses noted after injury might not

be related wholly to the catabolic action of catecholamines and cortisol (50), but also to decreased activity of insulin. They treated 28 burn patients, who were in negative nitrogen balance, with a glucose and insulin regimen intravenously and a group of nine patients who received the regimen intermittently since their admission, whenever urea or potassium excretion were elevated. Urea excretion was used as a rough estimate of nitrogen balance. Insulin was given in high doses (200 to 600 units daily) with 50% dextrose to maintain normoglycemia. This significantly reduced the negative nitrogen (urea) balance. There was a decrease in potassium excretion as well (51). However, this study failed to establish whether the improved nitrogen balance resulted from the increased carbohydrate or because of exogenous insulin. Subsequently, Woolfson, Heatley and Allison (52) used three-day isocaloric crossover studies with constant amino acid infusion (9.4 g nitrogen daily). The three regimens consisted of (A) 30% sorbitol, 5% glucose (1400 kcal) plus Intralipid (1000 kcal), (B) 30% glucose (2400 kcal) with insulin given only for glucose above 15 mmol/L., (C) same as B except insulin was used to maintain glucose between 4 and 8 mmol/L. Again, urea production was used to approximate nitrogen metabolism. Sorbitol was used to provide calories without stimulating insulin release. Differences occurred only in catabolic patients. The glucose plus insulin group showed a marked difference in the inhibition of protein catabolism

compared to glucose alone. The glucose group had a slightly more protein-sparing effect than the sorbitol-lipid group. These changes were only apparent when the crossover changed the rate of urea production. They concluded that insulin has an important protein-sparing effect in catabolic patients. Serum insulin concentrations were significantly higher in those patients receiving insulin. The change in urea production rate (Δ Pu) was related both to the initial rate of production (Pu) and to the change in serum insulin (Δ ins):

$\Delta Pu = 4.32 - 0.311(Pu) - 0.012(\Delta ins)$.

There was a significant correlation between change in insulin and change in urea prodiction: Greater increases in insulin produced greater reduction in urea production. In a recent review of these studies, Allison demonstrated that the serum insulin concentration of the Intralipid and sorbitol group were different from those receiving glucose alone. The concentration was even higher, $p < 0.005$ in those patients receiving glucose and insulin compared to those receiving glucose alone. In the pooled data of all studies, change in plasma insulin correlates with a fall in the urea production rate and the relation is a straight line.

We therfore set out to test the hypothesis that (a) serum insulin levels in starved and stressed patients could be increased above that level which is known to be stimulated by TPN, by administration of exogenous insulin (this should be well

above pre-TPN levels) and (b) malnourished patients receiving exogenous insulin could replete their body cell mass at a faster rate than controls who did not receive insulin. With this in mind, we added low-dose insulin in the TPN solution for alternate patients and studied body composition at two-week intervals. Serum insulin levels were measured prior to TPN, when the patients achieved maintenance infusion rates of TPN and with each subsequent body composition test, every 2 weeks. Patients were then divided into normally-nourished and malnourished groups on the basis of their body composition. Diabetics, as well and patients who required an insulin infusion were excluded and thus formed a third group (diabetic).

METHODS

SELECTION OF PATIENTS

All patients referred to the hyperalimentation team at the Royal Victoria Hospital were candidates for the study. Only those patients who agreed to participate were included. Informed consent was obtained from all the patients. Alternate patients receiving total parenteral nutrition received either no exogenous insulin in the TPN solution (control group) or 20-35 units/litre crystalline zinc insulin (CZI) in the solution (insulin group). Patients who were known to be diabetics at the time of consultation and those patients who were found subsequently to require additional insulin by continuous infusion for control of hyperglycemia were excluded and considered as a separate group (diabetic group). Insulin was sometimes added to the solution itself to control hyperglycemia for the latter group. Certain patients in all three groups at times received subcutaneous CZI on a sliding scale for >1% glycosuria (approximates serum glucose of 200 mg/dl).

TPN SOLUTION

All patients received a solution of 2.75% crystalline L-amino acids (Travasol, Baxter) with 25% Dextrose.

Electrolytes were adjusted for the patient's clinical condition; multivitamins were given five days/week, vitamin B12 30 mcg, vitamin K 10 mg, folic acid 6 mg once weekly, zinc 4 mg five days/week and copper 1 mg, iodine 0.12 mg, manganese 0.2 mg and chromium 0.002 mg three days/week. The solution was administered through a central vein by a silastic or polyethylene catheter. The ideal infusion rate was suggested to the attending service by the TPN consultant and was never less than 50 kcal/kg/day (usually 60 kcal/kg/day). Solutions were gradually increased to maintenance usually over three days, to prevent hyperosmolar, nonketotic dehydration. Whenever the solution was discontinued, 10% dextrose was administered to prevent hypoglycemia. This regimen was supplemented by 500 ml of Intralipid twice weekly to prevent essential fatty acid deficiency. All solutions were prepared and distributed through the hospital pharmacy.

In the early period of this study, CZI was added to the solution for the insulin group in the amount of 35 units/ L. Later, this was changed to 20 units/L due to biochemical hypoglycemia in several patients.

MONITORING

All patients were monitored daily for glucose, creatinine, blood urea nitrogen, and electrolytes until maintenance TPN

infusion rates were achieved. Thereafter, depending on their condition, twice weekly they had complete blood count, glucose, electrolytes, renal function, liver function, calcium phosphate on a standard SMAC (multianalysis counter). Metabolic and septic complications were investigated appropriately.

All intake and output for each patient was kept on an hourly basis on "shock sheets". The total intake was calculated for the period between 2 studies, the TPN period, and converted into a mean daily caloric intake. This was further subdivided into protein, carbohydrate and fat Calories/Kg/day.

Fractional urines were tested every six hours for glucose and acetone. Excessive glycosuria was treated by an appropriate sliding scale for subcutaneous insulin.

INSULIN SAMPLING

All patients had a pre-TPN blood sample drawn and the serum was stored by immediate freezing for later insulin determination. Subsequent serum insulin determinations were obtained when the patients were on maintenance TPN rates, usually 3-5 days after initiation of TPN. Thereafter, levels were drawn along with subsequent body composition studies, performed at 2 week intervals. The majority of specimens were drawn between 9 a. m. and 11 a. m.

INSULIN RADIOIMMUNOASSAY

Serum specimens were thawed and analysed in the Polypeptide Laboratory at McGill University under the supervision of Dr. Barry Posner and according to the method of Morgan and Lazarow (53). The basis of this method is that insulin, bound to antibodies in a soluble form in serum, can be precipitated by an antibody against gamma globulins.

The method is used with insulin standards each time serum samples are analysed. Insulin is incubated with anti-insulin antibodies from insulin-immunised guinea pigs (AIS-GP). This forms a soluble antigen-antibody complex. This complex is precipitated by an antibody against guinea pig serum from rabbits immunised with guinea pig serum (AGPS-R). I-125-labeled insulin is used as a tracer. The amount of radioisotope counted in the precipitate is a reflexion of the total insulin in the solution (labeled and unlabeled). The assay is based on the difference in the percent of tracer insulin precipitated (Δ% ppt) when varying concentrations of insulin are present (53).

All incubations are carried out at 4 degrees Celsius. On day 1 of the assay, 0.5 ml 1% bovine serum albumen (BSA) in phosphate buffered saline is mixed with 0.05 ml of insulin standard or assay sample of serum, along with 0.1 ml of Guinea pig antiserum to insulin (AIS-GP), 1:40,000. This incubates for

24 hours. On the second day this mixture is incubated with 0.1 ml of I-125-insulin (30,000 cpm specific activity, 140 mcCi/mcg). On day 3, 0.1 ml of rabbit anti-Guinea pig serum (AGPS-R) 1:200 and 0.1 ml of normal Guinea pig serum (NGPS) 1:14 are added. A precipitate appears and on the fourth day it is centrifuged at 3000 rpm for 30 minutes and decanted. Only the precipitate is counted. The samples are counted in a Wallace LKB 1280 Ultrogamma counter with 40% efficiency. The percent precipitate is:

$$\% \text{ I-125-insulin bound to AIS-GP} = \frac{\text{cpm in precipitate}}{\text{total cpm}}$$

A logarithmic curve of standard concentrations is constructed and the assay samples are read from this.

BODY COMPOSITION STUDIES

Body composition tests were performed after informed consent was obtained from patients. A multiple isotope dilution technique was employed (47).

BASELINE SAMPLES: Prior to injection of isotopes, a 10 ml heparinized blood sample was drawn for baseline beta and gamma radiation. These were prepared as described below.

PREPARATION OF ISOTOPES FOR INJECTION: Sodium-22 (New England Nuclear) arrives as a 1 ml (1 mCi) solution in a 10 ml vial. It is not pyrogen-free. It is made pyrogen-free by repeatedly washing the 10 ml vial with bacteriostatic (benzyl alcohol) saline, passing the contents through a sterile 0.2 micron filter (Nalgene, Sybron). The final dilution of the 1 ml of Na22 is to 200 ml of bacteriostatic (benzyl alcohol) saline. Thus, 2 ml of this solution is approximately 10 mcCi. The solution is then steam-autoclaved for 40 min. at 12 psi. (82.7 kilopascals) and 115 degrees Celsius.

Tritium (New England Nuclear) arrives as 25 mCi in a 10 ml vial. The vial is washed with sterile distilled water, filtered in the same fashion, but to a dilution of 25 ml. Five ml are placed in each of five bottles (5 mCi each) and diluted up to 100 ml with bacteriostatic (benzyl alcohol) saline. Ten ml of this solution will contain approximately 500 mcCi of tritium. The solution is autoclaved in the same fashion.

PREPARATION OF STANDARDS FOR GAMMA AND LIQUID SCINTILLATION COUNTING: As each injection bottle contains a slightly different amount of radioactivity, a standard must be prepared for each bottle to be compared with the patients' concentrations.

One ml of the radioactive solution is withdrawn in a one ml disposable plastic syringe and weighed. The contents of the syringe are injected into a 100 ml volumetric flask and the syringe reweighed. The flask is filled to 100 ml with distilled water and agitated. Two ml of this diluted standard is pipetted into a new, clean 7 ml red-stoppered tube (glass, without additives, Hemovac) containing 2 ml of 10% (w/v) trichloroacetic acid (TCA, Fischer) and agitated. One ml of the resulting solution is transferred into each of three glass liquid scintillation vials (New England Nuclear) containing 10 ml of Aquasol (New England Nuclear). The vials are capped and labeled with the isotope, bottle of origin and date of preparation. This is done in triplicate i. e. 9 vials, and repeated each time the counter is run.

For the gamma counter standards, 3 ml of the diluted standard are pipetted into each of 3 plastic gamma counter vials (New England Nuclear). This procedure is done in duplicate as only sodium-22 is being counted. These are counted directly in the counter.

INJECTION OF ISOTOPES: Ten ml of tritium are drawn up in a sterile plastic 10 ml syringe, containing approximately 500 mcCi. In a 3 ml sterile plastic syringe, are drawn up 2 ml of Na-22, containing approximately 8 mcCi. These are the concentrations for the first study only. The second uses 10.4 ml tritium, containing 520 mcCi and 2.2 ml Na-22 containing 8.8 mcCi. These are increased slightly at each further study, in order to overcome any background counts left by the previous study(ies). The syringes, with their needles and caps, are weighed. They will be weighed again after the isotope has been injected, with the same needle and cap, to determine the exact volume injected.

A free-flowing intravenous is established and, after the time 0 blood has been drawn for background, the two isotope solutions are injected by bolus, as close to the entry site as possible, and the line flushed with a minimum of ten ml of sterile saline or the prevailing solution if the I. V. is to remain indwelling. Any break in technique is noted on the patient's isotope chart. The nurses and patient are then instructed to collect all urine and any other body fluid for 24 hours following the injection. A notation is made in the patient's ward chart of the amount of isotopes injected.

FURTHER SAMPLING FOR ISOTOPE COUNTING: Four hours after injection, 20 ml (depending on the patient's hematocrit) of

blood are drawn in a heparinised tube. 24 hours after injection, 30 ml of blood are drawn in a heparinised tube as well as 10 ml of clotted blood for serum electrolytes (done by our lab on the flame photometer and checked with the SMAC in biochemistry). At 24 hours, the urine and drainage are measured and a representative aliquot taken of each. If some of either has been discarded, the time of this loss during the 24 hour period is noted. If the loss occured during the early part i. e. before the isotopes have had time to equilibrate, the sample will still be representative. If the sample occured late, the patient could be asked to void, or an aliquot of drainage taken at the end of the period and would be representative of equilibrated losses. At any rate, it is imperative that the quantity of external fluid losses be measured.

PREPARATION OF SAMPLES FOR COUNTING: For the gamma counter (Beckman 8000), no special preparation is necessary. For the beta (liquid scintillation) counter (Beckman LS 8000), samples must be clear and virtually colorless. Plasma is mixed with 10% TCA to deproteinate. Urine is decolorised with charcoal and filtered. Drainage is deproteinated using 10% TCA but usually cannot be filtered due to its consistency. Quenching is corrected by means of counting with an external standard and a microprocessor in the counter.

Time 0 (background) samples: Heparinised blood is centrifuged. Three ml of plasma is placed in each of 2 gamma counting vials i. e. these are counted in duplicate. The beta samples are counted in triplicate. Four ml of plasma are deproteinated with 4 ml of 10% TCA, mixed and centrifuged. One ml of the supernatant is pipetted into each of 3 vials. Ten ml of Aquasol (liquid scintillation counter cocktail) is added and the solution mixed.

4 hour samples: Prepared in the same fashion as the 0 hour.

24 hour samples: Plasma beta and gamma samples as for the 0 hour. Three ml each of urine and drainage are used directly for gamma counting. The beta urine sample is prepared by mixing an aliquot with charcoal, filtering it, then mixing 2 ml of the filtrate with 2 ml of 10% TCA. If a protein precipitates, it is centrifuged. One ml samples are counted in duplicate, after adding 10 ml Aquasol to each vial. The beta drainage samples (if there is more than one drainage site, they are counted separately) are prepared by mixing 2 ml of drainage with 2 ml of 10% TCA. This is centrifuged and the supernatant is treated exactly as the plasma supernatant, except in duplicate.

Plasma water is obtained by using a TS (total solid) Meter (American Optical). Two drops of plasma are placed on the meter and the protein concentration is read. A standard conversion table is used to obtain the concentration of water.

Triplicate one ml samples of whole blood are dessicated; the wet weight minus the dry weight is the whole blood water. Whole blood electrolytes are determined on the same tube of blood using a flame photometer.

RADIOISOTOPE COUNTING: The counting windows of the beta and the gamma counters are established from the spectrum of each isotope. The meV "windows" are adjusted to maximise the counts of each isotope while minimising the spillover from the other isotope. Sodium-22 activity is determined in both the beta and gamma counters as this isotope emits both beta particles and gamma rays. Tritium only gives rise to a beta particle and therefore can only be detected in the beta counter.

The crossover of one isotope into the window of another can be estimated by measuring the standards in each window. There is no crossover of tritium into the sodium window so no correction is necessary. The fractional crossover for sodium in the tritium window is:

$$\frac{\text{Na-22 standard in the tritium window}}{\text{Na-22 standard in the Na-22 window}}$$

This will be used in calculations below.

The gamma standards are counted in duplicate and the beta standards in triplicate. Each of the patient samples are counted in duplicate in the gamma counter and in triplicate in the beta counter. The gamma samples are counted for ten minutes

each and the beta samples for twenty. The mean counts are used in each instance.

TOTAL BODY WATER: TBW is calculated at the 4 hour and 24 hour interval. Tritium equilibrates very quickly in the body and will be representative of the TBW at 4 hours in most patients.

Correction for isotope crossover:

$$\text{H3 cts/min} = \frac{\text{cts/min H3 win} - \text{Na22 std in H3 win}}{\text{Na22 std in Na22 win}} \text{ (Na22 cts/min)}$$

Counts injected:

$$\text{Cts injected} = \frac{\text{wt sol. injected} \times \text{cts/min H3 std} \times 100}{\text{wt of injection solution diluted in 100 ml}}$$

where H3 is tritium, cts/min is counts per minute, Na22 is sodium-22, std is the standard, win is an isotope window.

At 4 hours:

$$\text{TBW} = \frac{\text{counts injected}}{\text{H3 cts/min at 4 hr} - \text{H3 cts/min at 0 hr}} \times \text{plasma water}$$

At 24 hours:

$$\text{TBW} = \frac{\text{(counts injected - counts excreted)}}{\text{H3 cts/min at 24 hr} - \text{H3 cts/min at 0 hr}} \text{(plasma water)}$$

The mean of the 4 and 24 hour TBW is used unless a gross error occurs in one.

TOTAL EXCHANGEABLE SODIUM: Na_e is calculated from the 24 hour plasma specific activity using both the beta and gamma counts.

Counts injected:

$$\text{Counts injected} = \frac{(\text{wt sol. injected}) \times (\text{cts/min Na22 std})}{\text{wt of solution diluted in 100 ml flask}} \times 100$$

At 24 hours:

$$Na_e = \frac{(\text{cts injected} - \text{cts excreted})}{(\text{Na22 cts/min at 24 hr} - \text{Na22 cts/min at 0 hr})} \times \text{plasma Na}$$

Na_e is determined using both the beta and gamma specific activity and the mean is used unless there is a gross error. The plasma sodium concentration is determined by flame photometry.

TOTAL EXCHANGEABLE POTASSIUM: K_e is determined indirectly (47). This determination is based on the premise that

$$R = \frac{[Na] + [K]}{\text{water content}}$$

where R = a constant,

[Na] = sodium concentration

[K] = potassium concentration

for all tissues in the body except bone. Algebraic summation of all the subunits results in:

$$R = \frac{Na_e + K_e}{TBW}$$

Thus $K_e = R(TBW) - Na_e$.

The value of R is obtained by measuring sodium and potassium concentration and the water content, by dessication, in a sample of whole blood. K_e is subsequently calculated. The accuracy of this indirect measurement has been experimentally validated (47).

SUMMARY OF PARAMETERS MEASURED:

Na_e = total exchangeable sodium

TBW = total body water

weight

SUMMARY OF PARAMETERS CALCULATED:

Fat = weight - TBW

K_e = total exchangeable potassium

BCM = body cell mass = K_e x 0.00833

LBM = lean body mass = TBW / 0.73

ECM = extracellular mass = LBM - BCM

Exchangeable sodium and potassium are often expressed as a function of TBW to correct for body size.

STATISTICAL METHODS

Early in the study, serum insulin data was analysed in a timed sequence. Patients had insulin levels drawn prior to TPN (day 0), when maintenance TPN was achieved (usually day 3 to 5), and at day 14 and 28, when the second and third composition studies were done. These concentrations were compared within each of the groups in a time sequence. The insulin and diabetic groups were also compared to controls. Analysis of variance was used to detect statistical significance and a T statistic and Scheffe's test were applied to detect within which groups the significance lay. This analysis of variance was carried out as above at time 0, day 3 to 5, day 14 and day 28.

Later in the study, pre-TPN serum glucose concentrations were compared between groups, by analysis of variance. The same analysis was used to compare maintenance TPN serum glucose concentration (drawn the same day as the serum insulin) between groups. T-test was used to detect significant change within groups between pre-TPN and maintenance concentrations.

Body composition studies done serially were broken down into TPN periods defined as the number of days between two consecutive body composition studies, usually 14 days. In addition to body composition data for each study i. e. at the beginning and end of a TPN period, a number of data are

calculated for the period itself. This includes the actual number of days, total calories for the period expressed as kilocalories/kg (body weight)/day, carbohydrate, lipid and protein Calories/kg/day, the daily change in K_e (delta K_e/day) and the daily change in BCM expressed as a percentage of initial BCM, to correct for body size (delta BCM/BCM %).

The compositional changes from the initial study of the TPN period and the final were analysed by paired Student's T-tests. Caloric data, delta K_e/day, percent daily change in BCM and serum insulin concentration were compared by analysis of variance (T-statistics and Scheffe's test) between groups.

The number of TPN periods in each group is considered inadequate to compare initial compositional data between groups, especially the normally nourished. It had been planned to have 100 patients in each of the control and insulin groups but this goal could not be achieved in the twenty months of this study.

RESULTS

Results are always expressed as means with the standard error of the mean.

PATIENTS:

Patients are subdivided into controls, who did not receive exogenous insulin, and an insulin group which received 20 to 35 units of CZI in each litre of TPN solution. Patients considered diabetic or who required a continuous CZI drip to control hyperglycemia were considered separately, as they would not be expected to respond to insulin in the same fashion as the other two groups. We wished to determine whether the adminsitration of exogenous insulin would increase serum insulin concentration and, if so, would that group improve their state of nutrition more than the controls.

A further subdivision was based on nutritional status as determined by the Na_e/K_e ratio. Patients were divided into normally nourished, having a ratio less than 1.22, and malnourished, greater than 1.22, subgroups. The normally nourished patient would be expected to conserve his body cell mass, given adequate nutrition and presuming there were not excessive demands resulting from his disease. The malnourished individual, however, would be expected, under the same

conditions, to increase or replete his BCM and improve his nutritional state.

There were 74 patients included in this study (Table I). There were 29 control patients who did not receive insulin. In this group, 93 body composition determinations were performed to evaluate 62 periods of TPN. The patient was considered normally nourished on the basis of an $Na_e/K_e < 1.22$, at the beginning of the TPN period, in ten of these 62 periods. These ten periods (20 studies) had a mean duration of 15.0 ± 0.5 days. In the remaining 52 TPN periods (80 studies) the patients were initially malnourished on the basis of the Na_e/K_e ratio. These periods were of a mean 15.3 ± 0.5 days.

31 patients received insulin with their TPN. In these patients 90 body composition studies were performed to evaluate 55 periods of TPN. The patients were initially malnourished in 40 of these periods (70 studies) of a duration of 14.3 ± 0.4 days and normally nourished in 15 periods (30 studies) lasting 15.0 ± 1.1 days.

14 patients were considered to be diabetic or required a continuous CZI infusion to control hyperglycemia. 25 TPN periods were evaluated by 38 body composition studies. In four of these periods (8 studies) of a mean duration of 15.0 ± 0.4 days, the initial Na_e/K_e ratio was less than 1.22. The ratio indicated malnutrition in 21 TPN periods (36 body composition studies). These 21 periods were of 14.0 ± 0.1 days duration.

There was no statistical difference between groups as to the duration of the TPN period. The minimum continuous duration of TPN was 12 days, the maximum, 130 days (9 TPN periods).

Tables II, III and IV indicate the major diagnoses of each patient. It is of note that one patient is included in two groups; early in his illness, he required a CZI drip to control hyperglycemia due to sepsis; later, he was restarted on TPN and being normoglycemic, was randomised to the insulin group. Virtually all patients included were severely ill patients, a large number of which underwent a surgical procedure either immediately before or during the study period. Five days were allowed for body fluids to return to equilibrium before perfoming a body composition determination.

The regimen of low dose CZI was generally well tolerated. Occasionally, a patient would require additional CZI for hyperglycemia , usually by the subcutaneous route. The regimen originally consisted of 35 units CZI/litre but because of two cases of asymptomatic, biochemical hypoglycemia of < 40 mg/dl, this was changed to 20 units/litre and the serum insulin levels followed in the same fashion.

SERUM INSULIN:

The serum insulin concentrations were studied over time to determine first, the rise of serum insulin concentration that

occurs with TPN alone, as the control group would demonstrate, and secondly, we wished to determine how much the insulin concentration would rise further with addition of low dose insulin. The patients were therfore subdivided into control, insulin and diabetic groups.

Table V shows the serum insulin concentration of 46 patients, illustrated in graph I. The pre-TPN concentration was low and equal in all three groups. The control patients doubled their insulin concentration by the time maintenance TPN was achieved (17.3 $\pm$ 3.1 to 36.4 $\pm$ 5.8) and at the time of the second and third body composition studies, which were performed at two week intervals, maintained this level. In these patients, the changes in the plasma insulin concentration was not significantly different from pre-TPN concentrations.

Both the insulin group and the diabetics behaved in a similar fashion but of greater magnitude. The insulin concentration rose from 16.8 $\pm$ 2.9 to 76.7 $\pm$ 7.4 mcU/ml ($p < 0.05$) in the insulin group and from 18.6 $\pm$ 8.0 to 87.9 $\pm$ 15.8 mcU/ml ($p < 0.05$) in the diabetics, at day 3-5. Both groups maintained these levels at the subsequent two studies. All maintenance concentrations were statistically significantly ($p < 0.05$) different from the pre-TPN levels. At day 3 to 5 and day 14, the serum insulin concentrations of the insulin and diabetic groups were significantly higher ($p < 0.05$) than controls by

Scheffe's test and T-statistics (analysis of variance). Statistical significance was not achieved at 4 weeks presumably because of the large variance and/or the diminishing sample size, as several patients did not undergo a third body composition study at 28 days.

The above demonstrates well that the addition of exogenous insulin does indeed result in a statistically significant elevation of the serum insulin above control levels which are elevated due to TPN.

SERUM GLUCOSE CONCENTRATION:

Serum glucose was analysed to determine whether there was a difference between groups that might account for an altered response to insulin. Certainly, the serum glucose of the diabetic is expected to be elevated. The concentrations of the control and insulin groups should be normal and not statistically different from each other prior to TPN. An elevation is expected at maintenance TPN due to the high dextrose concentration. This may not show statistical significance due, to the elevated serum insulin concentrations that have been observed.

Table VI indicates the serum glucose of the three groups subdivided into their state of nutrition. The pre-TPN concentration of the control and insulin groups, whether

normally or malnourished, were not statistically different by analysis of variance. Both groups of diabetics had statistically significantly higher pre-TPN serum glucose concentrations than the control and insulin groups ($p < 0.05$).

Note that the n value of the pre-TPN glucose concentration subgroups represents the number of patients, whereas in the maintenance subgroups it represents TPN periods, since several patients with repeat studies were included. The maintenance glucose concentration was higher than pre-TPN concentrations in all groups but was not statistically significantly different in any group. The diabetics again had statistically significantly ($p < 0.05$) higher blood glucose at maintenance than the other four groups.

These data justify the exclusion of diabetics at least on the basis of blood glucose. Even with continuous CZI, they are not well controlled. The serum glucose of the other two groups rises but not significantly, indicating the effect of a stimulated rise in serum insulin. The fact that the glucose in the insulin group did not remain at pre-TPN concentrations or fall below indicates our dosage was low enough so as not to affect glucose concentration, yet high enough to increase serum insulin.

MAINTENANCE INSULIN:

In general, maintenance insulin was defined as the serum concentration at the second study of a TPN period. If this sample was not available, the concentration from the first study or day 5 may be used as justified by the data in the time study.

Since the dosage of insulin was changed in the middle of the study from 35 U/L to 20 U/L, in order to avoid hypoglycemia, we needed to prove that the lower dose could still provide adequate serum concentrations of insulin. If this assumption is indeed valid, then the combined data of the two dosage regimens should still indicate that the insulin group have a higher serum insulin concentration at maintenance than the control group.

First, the insulin group was subdivided for analysis into those who received 35 U/L and those who received 20 U/L. The 35 U/L dosage was given through 27 TPN periods, the latter dosage through 28 periods.

In the 35 U/L subgroup, 8 normally nourished had a mean serum insulin concentration of 100.1 $\pm$ 9.5 mcU/ml, 19 malnourished had a mean concentration of 88.5 $\pm$ 8.7 mcU/ml, the combined 27 had a mean of 91.9 $\pm$ 6.7 mcU/ml. As expected, there was no statistical difference between normally nourished and malnourished patients.

In the 20 U/L subgroup, 7 normally nourished had a mean serum insulin concentration of 92.9 ± 19.5 mcU/ml, 21 malnourished had a mean concentration of 110.8 ± 25.2 mcU/ml, the combined group had a mean of 106.3 ± 19.4 mcU/ml. Again, there was no difference between the normally nourished and the malnourished. More important, there was no statistical difference between any of the 35 U/L subgroup and any of those receiving 20 U/L. This was unexpected but probably indicates that both of these doses are low and in spite of binding to the solution bag and tubing, deterioration with time, the delivery to the patient is the same.

Table VII lists the maintenance serum insulin concentration for all patients in all groups of this study. The data indicate that the insulin and diabetic groups have statistically significantly higher maintenance serum insulin than the controls. The normally nourished groups are small. The controls (77.7 ± 15.4 mcU/ml insulin) do not differ significantly from the insulin group (96.7 ± 10.1 mcU/ml) and the diabetics (120.0 ± 43.6 mcU/ml), probably due to sample size. The malnourished groups are larger. The controls (56.1 ± 7.2 mcU/ml) have a significantly ($p < 0.05$) lower maintenance concentration than the insulin group (100.2 ± 13.8 mcU/ml) and the diabetics (91.9 ± 14.4 mcU/ml). The combined groups show the same difference ($p < 0.05$) compared to the control group (control 59.6 ± 6.6 mcU/ml, insulin 99.3 ± 10.4 mcU/ml and diabetic 96.4 ± 13.7 mcU/ml).

Thus, one may conclude that the addition of low dose insulin to the TPN solution does give rise to increased serum concentrations of insulin, as does CZI by infusion in the case of diabetic patients. The 20 U/L dose of CZI is safe and as effective as the 35 U/L dose.

BODY COMPOSITION DATA:

Tables VIII and IX show the body composition data for the normally nourished and the malnourished patients respectively. "Pre" represents the means of the initial studies of a TPN period, "post", the final studies. Initial and final study parameters are analysed within a group by paired T-test. These changes are illustrated by means of histograms I and II.

The population of normally nourished patients is small: 10 controls, 15 insulin and 4 diabetics. There was a positive weight gain in both the control (51.4 $\pm$ 2.7 to 54.0 $\pm$ 3.2 kg, $p < 0.05$) and insulin groups (65.9 $\pm$ 2.3 to 69.5 $\pm$ 2.9 kg, $p < 0.01$). The diabetics did not change significantly. Fat stayed the same or increased slightly in all groups. The positive weight gain was reflected mostly by an increase in the lean body mass in the controls (46.1 $\pm$ 2.0 to 48.3 $\pm$ 2.8 kg) and insulin group (49.6 $\pm$ 2.2 to 53.2 $\pm$ 3.0 kg). Likewise, the slight weight loss by the diabetics is reflected by a decreased LBM (47.8 $\pm$ 2.1 to 42.2 $\pm$ 1.4 kg). In the control and insulin groups, the body cell mass remained virtually the same, thus

this increase is due to expansion of the extracellular mass (26.1 ± 1.1 to 28.3 ± 2.1 kg in the controls, 27.9 ± 1.2 to 31.1 ± 1.8 kg, $p < 0.05$ in the insulin group). The diabetics lost in both BCM and ECM, but not significantly so. Na_e and K_e are expressed as a function of TBW to minimise differences in body size. There was no statistically significant change in either parameter by any group but the trend is interesting to note, especially in the insulin group. The Na_e/TBW increases which explains the increase in ECM (mostly represented by sodium). The K_e/TBW decreases (71.8 ± 1.0 to 67.9 ± 1.9 mEq/L) indicating that the changes in potassium are small and that the increase in TBW has overshadowed the increased K_e that was seen in the BCM change. The nutritional status as indicated by the Na_e/K_e ratio did not change significantly in any group (controls 1.1 ± 0.04 to 1.17 ± 0.07, insulin 1.08 ± 0.03 to 1.21 ± 0.06 and diabetics 1.09 ± 0.09 to 1.21 ± 0.08) and still remained within the normal range.

Normally nourished patients usually maintain their body cell mass and nutritional state provided they remain stable clinically and are provided with adequate Calories. All three groups maintained their normal state of nutrition, the control and insulin groups maintained body cell mass. Thus, insulin has no added advantage, no detrimental effects in patients with normal nutritional status. This is exactly as expected.

The malnourished patients comprised by far the larger group: 52 TPN periods included in the malnourished control group, 40 in the insulin group and 21 in the diabetic. There was no significant change in weight although insulin and diabetic group patients gained slightly (insulin 66.1 ± 2.1 to 67.0 ± 2.3 kg, diabetics 71.3 ± 4.1 to 73.1 ± 3.7 kg). As in the normally nourished, fat remained the same or increased slightly. Lean body mass remained the same in the controls (44.7 ± 1.4 to 44.2 ± 1.3 kg) and insulin group (49.8 ± 1.7 to 49.5 ± 1.7 kg) but increased in the diabetics (54.8 ± 2.9 to 57.2 ± 2.8 kg) though not significantly. As in the normally nourished, this is broken down into changes in the extracellular mass and body cell mass. The largest change in ECM occured as an increase in the diabetic group (37.7 ± 2.4 to 39.6 ± 2.7 kg), still not significant. The extracellular mass remained stable (slight decrease) in the insulin group (32.5 ± 1.3 to 31.7 ± 1.4 kg) as well as those who did not receive insulin (30.1 ± 1.1 to 29.1 ± 1.1 kg). Body cell mass improved slightly in all groups but changes were minimal (control, 14.7 ± 0.6 to 15.2 ± 0.6 kg, insulin, 17.3 ± 0.6 to 17.8 ± 0.6 kg and diabetic, 17.2 ± 0.8 to 17.6 ± 1.0 kg). The magnitude of the change in Na_e expressed as a function of TBW is somewhat larger than the miniscule change in ECM. The control group decreased from 93.6 ± 1.5 to 91.9 ± 1.7 mEq/L and the insulin group from 92.1 ± 1.4 to 89.0 ± 1.7 mEq/L ($p < 0.05$). In contrast, the diabetics increased from

94.4 ± 2.1 to 98.5 ± 3.0 mEq/L, corresponding to their increase in LBM and ECM. K_e/TBW also improved in the control (54.3 ± 1.4 to 56.8 ± 1.6 mEq/L, $p < 0.05$) and insulin group (57.7 ± 1.1 to 60.0 ± 1.6 mEq/L). The diabetic group maintained their K_e/TBW (52.5 ± 1.7 to 52.1 ± 3.1 mEq/L), as they did their BCM. The change in the Na_e/K_e ratios summarises the other changes that have occured. The control and insulin groups decreased their Na_e and increased in K_e, thus, the ratios have improved (controls, 1.83 ± 0.09 to 1.75 ± 0.10 and insulin, 1.64 ± 0.06 to 1.57 ± 0.08). The diabetics maintained K_e and BCM, while expanding their ECM and Na_e. Their ratios worsened from 1.86 ± 0.10 to 2.11 ± 0.19 as a result.

These data are more difficult to interpret as they are somewhat unexpected. The general improvement of the control group was predictable since malnourished patients tend to replete their BCM and decrease an expanded ECM, thus improving their ratio, when provided with adequate calories and protein. However, the insulin group underwent changes of approximately the same magnitude, improving as well. Exogenous insulin has not been detrimental but the expected improvement over the control group has not been demonstrated. Sample size, although not ideal, should be adequate to detect this change.

CALORIC DATA:

There was no difference in total caloric intake between any of the normally nourished groups although the controls (61.4 ± 2.2 kcal/kg/day) received the highest amount of Calories of all six groups (Tables X, XI). The normally nourished insulin group received 55.1 ± 2.4 kcal/kg/day and the diabetic, 46.2 ± 6.8 kcal/kg/day. There was no difference either between malnourished groups as far as total Calories. The malnourished controls received 50.9 ± 2.2 kcal/kg/day, the insulin group, 46.8 ± 1.8 kcal/kg/day and the diabetics, 47.9 ± 3.1 kcal/kg/day. By analysis of variance and T-statistics the normal controls appeared to have statistically significantly higher total caloric intake than all malnourished groups ($p < 0.05$) but this difference was not apparent when Scheffe's test was applied. The same T-statistics applied to analysis of variance showed that the normal insulin group received significantly higher ($p < 0.05$) total intake than the malnourished insulin group. This was not so when Scheffe's test was applied.

Fat Calories were no different between any of the groups. Patients received from 1.1 ± 0.1 kcal/kg/day (normal diabetics) to 2.1 ± 0.4 kcal/kg/day (normal controls).

Carbohydrate Calories showed exactly the same differences between groups as did the total Calories. Again, this was not significant by analysis of variance to which Scheffe's test is

applied. The normally nourished controls received 52.7 ± 1.9 kcal/kg/day in the form of carbohydrate, the insulin group, 47.5 ± 2.2 kcal/kg/day and the diabetics 40.5 ± 6.1 kcal/kg/day. The malnourished groups appear to receive somewhat less: controls, 43.9 ± 1.9, insulin, 40.4 ± 1.6 and the diabetics 41.8 ± 2.7.

Protein calories are distributed in a similar manner. There is however a difference between the normally nourished groups now. The controls received 6.6 ± 0.3 kcal/kg/day, the insulin group, 5.9 ± 0.2 and the diabetics 4.6 ± 0.7. The diabetics differed from the controls by T-statistics applied to analysis of variance, $p < 0.05$. The significance was lost when Scheffe's test was applied. There were no differences between the malnourished groups: Controls absorbed 5.4 ± 0.2 kcal/kg/day as protein Calories, the insulin group, 5.0 ± 0.2 and the diabetics, 5.0 ± 0.3. The normally nourished controls showed significantly higher protein Calories by analysis of variance, T-statistics, $p < 0.05$, (but not by Scheffe's) compared to all malnourished groups. The normally nourished insluin group received more protein than the malnourished insulin and diabetic groups, $p < 0.05$ by the same analysis. None of these differences were significant when Scheffe's test was used with analysis of variance.

When a difference is demonstrated by T-statistics but not by Scheffe's test, a more rigid test of significance, the

difference is questionable. With the exception of the normally nourished controls, all groups received approximately the same amount of Calories.

Delta K_e/day is defined as the mean daily change in exchangeable potassium during the TPN period. There was no difference in the mean delta K_e/day between any of the groups. This was probably because the changes in K_e are small and daily changes even smaller. Where the population size is small, the differences are notable but not significant: normally nourished controls, -0.6 $\pm$5.4 mEq/day, insulin, 4.9 $\pm$ 5.7 mEq/day and diabetics, -28.1 $\pm$ 14.1 mEq/day. In the malnourished groups the differences are small: controls, 3.8 $\pm$ 2.8 mEq/day, insulin, 4.7 $\pm$ 3.2 and diabetic, 3.7 $\pm$ 6.4. The actual daily change in body cell mass corrected for body size (the initial body cell mass) is expressed as the percent daily delta BCM for the malnourished patients: controls, 0.31% $\pm$ 0.16, insulin, 0.25% $\pm$ 0.15 and diabetics, 0.21% $\pm$ 0.27. There is no statistical difference. Since the changes are small and the population heterogeneous and few in number, many more TPN periods would have to be included to obtain statistical significance. The delta Ke/day data and that of the percent daily change in BCM will be used below in the formation of a multiple linear regression equation.

MULTIPLE LINEAR REGRESSION

Multiple linear regression is a statistical method whereby one may express one dependent variable such as delta K_e/day or % delta BCM/day as a function of more than one independent variable. Independent variables which would correlate with such changes in nutritional parameters are: the status of nutrition (Na_e/K_e), the amount and type of Calories, the serum insulin concentration. Multiple linear regression will fit all the variables into a theoretical straight line even though it would require more than two dimensions to draw such a line. The equation is in the form of

$$y = a + bx + cw + dz$$

where x, w and z are independent variables. Tables XII and XIII express the delta K_e/day and the percent delta BCM/day respectively as a function of the Na_e/K_e ratio (state of nutrition), total Calories, and serum insulin. This was carried out only for the malnourished patients as the normally nourished tend to maintain their body cell mass, not increase above their maximum. Subdividing the Calories into the carbohydrate, lipid and protein portions did not add significance and these are not included.

Graphs II, III, and IV illustrate the regression lines of each group separately and graph V is a summary of the three.

These lines were obtained by keeping the state of nutrition constant at 1.5 Na_e/K_e. The concentration of insulin used was the mean of the group in question. The Calories were then varied to obtain the line as illustrated. One can appreciate from the equations, that the intercepts are similar in all three groups. The multiple correlation coefficients, which indicate how well the data points fit the line, are similar. The regressions are not significant. One can appreciate by the graphs, that the slope of all three lines are similar. Thus, when the nutritional state, the least significant of the three independent variables, is constant, the three groups behave in a similar fashion, which will vary according to the insulin concentration. Whereas the malnourished controls require 46 kcal/kg/day to maintain their K_e, the insulin group and diabetic group require 38 and 39 kcal/kg/day, respectively. At a TPN rate supplying 60 kcal/kg/day, the patients receiving no insulin will increase their K_e by 6 mEq/day, whereas the insulin group will increase it at a rate of 12.5 mEq/day, the diabetics by 10 mEq/day. The beneficial effects of insulin are clearly demonstrated. It is of note, however, that in the diabetic group, the serum insulin concentration has a negative correlation with delta K_e, though not statistically significant.

Graphs VI, VII, VIII and IX illustrate the multiple linear regressions of the three groups with percent delta BCM/day as dependent variable. One will note by the equations in Table

XIII that the intercepts are similar in each group. The total calories are statistically significant in the control group, $p < 0.01$ and in the insulin group, $p < 0.05$. The regressions are not significant but the correlation coefficients are similar, especially for the control and insulin groups. The graphs were again constructed using an $Na_e/K_e = 1.5$ and using the maintenance serum insulin concentration of the group in question. One can appreciate that the slopes are similar in the control insulin groups. The malnourished control group, under these conditions, required 44 kcal/kg/day to maintain their BCM. The insulin group required less, 38 kcal/kg/day and the diabetics, 33 kcal/kg/day, although the repletion rate of the diabetic group at higher caloric intakes is slower, as indicated by the slope of the line.

Assuming a theoretical BCM of 20 kg and a rate of 60 kcal/kg/day, the equations would indicate that the controls would gain 99 gm of BCM daily, the diabetics would gain 106 gm/day and the insulin group would gain 131 gm daily. Again, the beneficial effect of insulin is demonstated: less Calories are required to maintain the body cell mass and at a given caloric intake, the repletion rate is faster.

This is borne out when the control and insulin groups are combined to form a regression. This can be done since they behave in a similar fashion, except that the insulin group

correlates negatively for Na_e/K_e, but this is far from statistically significant. The resulting regression becomes statistically significant and the significance of the serum insulin and Calories variables are increased. This regression appears on graph X. These lines are remarkably close to the individual regression line of each group. The same beneficial effect of insulin is illustrated.

To be complete, the regression for the combined data of the three groups is included (graph XI). The regression is significant, but one is not justified in combining the diabetics with the other two groups. The slope of the regression of this group is totally dissimilar to the others, there is a negative correlation between Na_e/K_e and percent daily delta BCM, as well as between insulin and percent daily delta BCM. In the graph, it appears as if the diabetics do as well as the insulin group because the mean maintenance serum insulin concentrations of these groups are almost identical.

The delta K_e/day regression and the percent daily delta BCM regression are dissimilar, in the case of the diabetic group, because the latter regression corrects for body size, an important correction in a group numbering only 21 TPN periods.

Table XIV illustrates the changes that would occur when one of the variables is changed. When the Na_e/K_e is made worse, from 1.5 to 2.0, the increases in BCM are small. For example,

at a serum insulin concentration of 100 mcU/ml and 50 kcal/kg/day the daily increase in body cell mass is from 66 gm to 94 gm. When the state of nutrition and caloric intake are kept constant, variation in the serum insulin produces small changes. The increase is from 46 gm/day to 66 gm/day and 86 gm/day when the serum insulin is 50 mcU/ml, 100 mcU/ml and 150 mcU/ml respectively, at a TPN rate supplying 50 kcal/kg/day. Varying the caloric intake produces the greatest change. The daily increase in body cell mass increases by 60 gm/day with each successive 10 kcal/kg/day. Of course, the greatest increase would occur when the patient is very malnourished, has a high serum insulin concentration and receives the maximum Calories tolerated.

DISCUSSION

We have succeeded in adding exogenous insulin to our parenteral nutrition solution in doses which are low enough to be safe and without risk of hypoglycemia yet which ensure elevated plasma levels of the hormone. It has been demonstrated above, that 20 units of CZI in a litre of solution and 35 U/L render equal plasma concentrations of insulin. There were several cases of hypoglycemia with the higher dose, although asymptomatic. It is interesting to note that two dosage regimens, one almost half the second, would result in identical plasma concentrations. However, these are dilute concentrations of insulin and surely all of the initial dose does not reach the patient's bloodstream. Care is taken that the CZI is added to the TPN solution and refrigerated but there is still some degradation of insulin when the solution is hung at the bedside. Insulin is known to bind to the plastic of the solution administration bags and tubing. Even continuous infusions of insulin are variable in the delivery of the hormone to the patient depending on the concentration and the type of infusion set (61). However, in spite of binding of insulin to plastic, it has been demonstrated by double isotope tagging, that constant insulin delivery to the patient can be assured when adminsitered in a TPN solution (65). Albumen is not necessary to add stability to the solution. It is of importance to verify

the serum concentrations of the hormone and to correlate body composition data with these rather than with the dose administered.

The population of patients studied was very heterogenous, and severely ill. The majority were malnourished. Although the total number of patients was less than what was set out as an ideal at the beginning of this study, the numbers of the control and insulin malnourished groups are sufficient to study the questions posed in the hypothesis. At the onset of this study, the diabetics were excluded on the basis of supposed insulin resistence, although the nutritional effects of insulin are not necessarily always related to the glucose effect. The glucose data, both prior to TPN and at maintenance, supports the exclusion of diabetics, at least on the basis of glucose intolerance. As a group, the diabetics received the lowest amount of calories, probably because of poor control of serum glucose. Several of the patients within this group were hyperglycemic on the basis of sepsis rather than true diabetes alone. The fluid and caloric demands of such a patient would be even higher than the non-septic patient or true diabetic. Presumably, these demands were not met. The small number of patients in the diabetic group, as well as their heterogeneity, make it difficult to draw any fast conclusions from their data, with the exception of the serum glucose and insulin data which were statistically different from controls.

The release of insulin in response to a glucose infusion is known to be rapid and the levels sustained (36, 62, 63). TPN solutions are sustained for long periods in the patients studied, a minimum of two weeks. The study of serum insulin concentration on days of TPN, in a subpopulation of patients, indicated that, in fact, the controls had reached maintenance serum insulin concentration by the time their parenteral solution was being infused at maintenance rates. This concentration of insulin was double the pre-TPN concentration and was maintained in the subsequent two studies.

The patients receiving insulin, either as part of the protocol, the insulin group, or for hyperglycemia, also demonstrated sustained levels of insulin but significantly higher than the controls. The maintenance insulin concentration that was used to correlate with body composition data, was that obtained at the time of the second body compostion study of a TPN period. This concentration was always higher in the insulin and diabetic group than the control, $p < 0.05$. Thus, the addition of CZI to the TPN solution resulted in increased serum concentration above and beyond the increase observed in controls, which is due to the solution itself. Exogenous insulin does not, apparently, turn off endogenous production of insulin.

Does this increased serum insulin concentration have a

positive effect on the body's nutritional status? The relative insulin resistance after shock, surgery, sepsis or starvation has been described (35, 46, 65, 66). This may be due to low concentrations of insulin as occurs in fasting or thermal burns. It is related especially to a relative overabundance of catabolic hormones, in different combinations as described above. Insulin is the prime anabolic hormone of the body. In high concentrations, insulin will stimulate protein synthesis, glycogen synthesis and triglyceride synthesis and storage. Allison et al. (51, 52) have felt that the decreased activity of insulin could be counteracted by delivering large doses of insulin to the patient. They succeeded in demonstrating improved nitrogen balance, as estimated by decreased urea production, when insulin was added to their crossover hyperalimentation solutions. There was a significant correlation between urea production and serum insulin. These changes occurred in catabolic patients.

There are similarities between the results presented here and those of Allison's group, even though the studies were different. Allison's patients receiving glucose alone had serum insulin concentrations (46.9 $\pm$ 39 mU/L) similar to our control group maintenance insulin concentration. The insulin group in our study achieved statistically significantly higher serum insulin concentration than controls (99.3 $\pm$ 10.4 mcU/ml). Allison's insulin group achieved levels of 223 $\pm$ 219 mU/L which

may be accounted for by radioimmunoassay techniques and especially the higher dose of insulin administered. Note that in Allison's work, changes occurred in catabolic patients. In our study, the malnourished patients improved in body composition.

The errors in nitrogen balance have been discussed. The method of urea excretion is an approximation of nitrogen balance. Body composition, as performed in this laboratory, has proved a sensitive tool in measuring response to manipulation of nutrition (41, 67, 68, 69, 70, 71). The indirect method of body composition has proved valuable in evaluating changes in body cell mass and extracellular mass in protein-sparing experiments in which weight change alone was not significant. In previous studies, such as that comparing fat emulsion and amino acids to dextrose and amino acids, it was necessary to determine the patients' initial nutritional status in order to determine their response to therapy as a group. This same principle was used in our study. The sensitivity of the Na_e/K_e ratio has been validated (44) by studying 500 consecutive body composition studies. These were compared to normal, healthy volunteers. When total body exchangeable potassium was plotted against TBW, the data points of the patients with a normal ratio < 1.22 fell mostly within the 95% confidence limits of the normal volunteers, whereas those with a ratio > 1.22 fell below this limit. Therefore, in our estimation, this method serves better

to determine chagnes in the body cell mass which may be brought about by insulin than would nitrogen balance.

Patients in a normal state of nutrition, do not tend to increase their body cell mass when given excess calories. Rather, they tend to convert the excess to fat and conserve their body cell mass. In a normally nourished adult, the body cell mass does not usually increase unless stimulated by exercise. The patients in our study with an Na_e/K_e less than 1.22, normally nourished, would not be expected then to increase their body cell mass, whether they received insulin or not. In fact, both the normally nourished control and insulin groups, maintained their normal state of nutrition, both gaining in weight, extracellular mass, without significant change in body cell mass. The performance of the normally nourished diabetics was generally poor. Lack of significance was due to population size. Exogenous insulin has shown no positive or negative effects on the nutrition of these normally nourished patients, nor is significant benefit expected.

The malnourished control and insulin groups both improved. At first glance, it appears that insulin has no added advantage. The control group and insulin group both increased their body cell mass by approximately the same magnitude. Neither group showed statistically significant improvement. The control group did improve the K_e/TBW, $p < 0.05$ but the insulin group improved,

as well, though this improvement was not of statistical significance. Both groups decreased the Na_e/TBW, this time the change of statistical significance is demonstrated by the insulin group. This resulted in improvement by both groups but no demonstrable effect of insulin alone is noted; nor was there any detrimental effect.

The positive effect of insulin can be demonstrated by the multiple linear regression data. In both the delta K_e/day and percent delta BCM/day multiple linear regression data, the effect of high serum insulin concentration is clear. The insulin group requires less Calories to maintain their body cell mass. When the amount of calories is increased above this minimum, the insulin group responds with faster repletion of the K_e or body cell mass. The statistical significance of the combined control and insulin multiple linear regressions adds credence to this view.

It is interesting to note that the variable that was most often statistically significant in these equations was the total Calories/kg/day. All patients received more than the 34.7 kcal/kg/day calculated as a minimum rate, below which body cell mass does not improve (67) {recalculated from (70)}. According to the regression and under fixed conditions, the insulin group required approximately this rate of caloric intake to maintain their body cell mass, the control group, slightly more. The

Na_e/K_e ratio would be of more significance if normally nourished patients had been included in the regression. This was not done as it was not the purpose of this manipulation to determine the effect of malnutrition on the repletion rate, rather the effect of insulin. Malnourished patients only were included since it was those patients who, as a group, increased their body cell mass with nutritional therapy.

The changes in BCM and total exchangeable potassium, within a TPN period, are small. As emphasized above, the rate of repletion lags far behind the rate at which the body cell mass was lost. This is confirmed by the body composition data of the malnourished patients and illustrated in the examples of multiple linear regression. A gain of 50 grams/day of body cell mass is small if one considers that the patient may have lost over 300 gm/day before parenteral nutrition was instituted.

Naturally, the pre-TPN losses are not measured by our laboratory, only those changes occuring with TPN. Since the changes are small, and the population so heterogeneous, both in body size and in pathology, it will require much larger numbers than included in this study to demonstrate a significant difference in the insulin group.

CONCLUSIONS

This study of 74 patients receiving total parenteral nutrition demonstrates that:

1. Prior to administration of TPN all patients had a low serum insulin concentration.

2. By the time maintenance TPN was achieved, usually at 3 to 5 days, patients who did not receive insulin doubled their pre-TPN serum insulin concentration and these concentrations were maintained at two subsequent body composition studies (day 14 and day 28). However, these concentrations were not statistically significantly different from pre-TPN concentrations.

3. Patients receiving exogenous insulin, the insulin and diabetic groups, increased their serum insulin concentration by the time maintenance TPN was achieved, to a level which was statistically significantly higher ($p < 0.05$) than control concentrations. These concentrations remained elevated through the two subsequent body composition studies.

4. Maintenance serum insulin concentration was statistically significantly higher ($p < 0.05$) in those patients receiving insulin, compared to controls.

5. The serum glucose concentration of the diabetic group was statistically significantly elevated over that of control and insulin groups, both prior to TPN and at maintenance TPN. The poor control of the diabetic group justifies its exclusion.

6. The serum glucose concentration of all groups increased with TPN but was not statistically significantly different from pre-TPN.

7. The two dosage regimens used, 35 Units CZI/L and 20 U/L, both rendered approximately equal concentrations of insulin in the serum. This is probably because both are low dilutions of the hormone, but enough reaches the bloodstream of the patients to make a significant difference in concentration over controls. There were no cases of hypoglycemia with the 20 U/L dose.

8. Patients who were normally nourished maintained their normal state of nutrition, particularly body cell mass. As expected, insulin had no added advantage or disadvantage in this population.

9. In the malnourished patient subgroup, the larger group, both the control and insulin groups improved their nutritional status, including body cell mass. These changes failed to reach statistical significance probably due to sample size. Exogenous insulin has failed to bring about a significant benefit in the

number of patients considered, but has shown no detrimental effect.

10. Multiple linear regression expressing delta K_e/day and percent daily change in BCM as a function of Na_e/K_e (nutritional status), total Calories/day and serum insulin concentration at maintenance show that Calories is a significant variable. The regression resulting from combining the control and insulin group is significant and shows that high concentrations of insulin allow maintenance of the body cell mass at lower caloric intakes and also increases the rate of repletion of the malnourished body cell mass.

11. Multiple linear regression also indicates that the diabetic group behaves differently from the other two. No definite conclusions can be drawn about the effect of insulin on this group.

TABLE I

DIVISION OF 74 PATIENTS

	CONTROL	INSULIN	DIABETIC
Number of patients	29	31	14
Number of studies	93	90	38
Number of TPN periods	62	55	25
Initial Na_e/K_e less than 1.22			
Number of studies	20	30	8
TPN periods	10	15	4
* Mean duration of TPN period (days)	15.0 ± 0.5	15.0 ± 1.1	15.0 ± 0.4
Initial Na_e/K_e greater than 1.22			
Number of studies	80	70	36
TPN periods	52	40	21
* Mean duration of TPN period (days)	15.3 ± 0.5	14.3 ± 0.4	14.0 ± 0.1

* There was no statistically significant difference in the mean duration of the TPN period between groups by analysis of variance.

TABLE II

MAJOR DIAGNOSES OF THE CONTROL GROUP

1. gastrointestinal bleed/ sepsis
2. small bowel obstruction/ carcinoma colon
3. peptic ulcer resection/ duodenal stump leak
4. carcinoma esophagus/ obstruction
5. resection of esophageal webs/ leakage
6. carcinoma esophagus/ subphrenic abscess
7. pancreatitis/ pseudocyst/ abscess
8. fecal peritonitis/ sepsis
9. fractured hip/ esophageal stricture
10. gastric carcinoma resection/ anastomotic leakage
11. carcinoma mouth
12. sepsis post colon resection/ prolonged ileus
13. carcinoma esophagus
14. carcinoma esophagus
15. severe infected sacral ulcer due to pilonidal abscess
16. intraabdominal abscess
17. infarcted bowel/short bowel syndrome
18. upper gastrointestinal bleed/ sepsis
19. odontoid fracture/ dysphagia
20. perforated bladder/ sepsis
21. aorto-coronary bypass/ mitral valve replacement/ sepsis
22. leukemia
23. small bowel obstruction
24. diverticulitis
25. leukemia
26. appendiceal abscess
27. carcinoma esophagus
28. duodenal ulcer
29. gastric dysfunction

TABLE III

MAJOR DIAGNOSES OF THE INSULIN GROUP

1. Crohn's disease
2. pancreatitis
3. carcinoma rectum/ anastomotic leakage
4. radiation enteritis/ nonfuctioning bowel
5. small bowel fistula
6. small bowel fistula
7. trauma/ sepsis/ pancreatitis
8. mitral valve replacement/ sepsis
9. pancreatic ascites/ pseudocyst
10. carcinoma pancreas/ subphrenic abscess
11. upper gastrointestinal bleed/ duodenal leakage
12. carcinoma of gastroesophageal junction/ anastomotic leakage
13. gastrointestinal bleed/ abscess
14. gastrointestinal bleed/ sepsis
15. upper gastrointestinal bleed
16. bowel obstruction/ peritonitis
17. carcinoma stomach
18. radiation enteritis/ fistula.
19. carcinoma neck
20. common bile duct stone removal/ respiratory failure
21. carcinoma stomach/ obstruction
22. perforated, strangulated hernia/ sepsis
23. splenectomy/ gastric fistula
24. esophageal perforation
25. carcinoma esophagus/gastric leakage
26. femoral fracture/ duodenal bleed
27. esophageal obstruction
28. pancreatitis/ abscess/ perforated bowel
29. pancreatitis
30. carcinoma pancreas/obstruction postop
31. perforated bowel/ sepsis/ renal failure

TABLE IV

MAJOR DIAGNOSES OF THE DIABETIC GROUP

1. Mallory-Weiss tear/sepsis
2. small bowel infarct resection/ respiratory failure
3. perforated strangulated hernia/ sepsis
4. duodenal stump leakage/ sepsis
5. fistula/ abscess/ renal failure
6. burns/ sepsis
7. carcinoma pancreas/ anastomotic leakage
8. carcinoma esophagus
9. small bowel obstruction
10. aorto-femoral bypass/ fistula to infected graft
11. carcinoma lung/ bronchopleural fistula
12. renal failure/ aortic aneurysm resection/ infarcted bowel
13. cerebral hemorrhage/ mesenteric infarction
14. abdominal aortic aneurysm resection/ gangrene leg

TABLE V

SERUM INSULIN CONCENTRATION (mcU/ml)

	CONTROL n = 15	INSULIN n = 24	DIABETIC n = 7
Pre TPN	17.3 ± 3.1	16.8 ± 2.9	18.6 ± 8.0
Day 3-5	36.4 ± 5.8	76.7 ± 7.4* #	87.9 ± 15.8* #
Day 14	33.6 ± 6.0	82.8 ± 15.6* #	101.0 ± 13.1* #
Day 28	34.2 ± 7.5	100.9 ± 21.7*	97.8 ± 31.4*

* significantly higher ($p<0.05$) than pre TPN serum insulin concentration.

\# significantly higher ($p<0.05$) than Control serum insulin concentration at the same time sequence.

TABLE VI

SERUM GLUCOSE CONCENTRATION (mg/dl)

NORMALLY NOURISHED

	CONTROL	INSULIN	DIABETIC
Pre TPN	102.3 ± 8.8 n = 4	106.0 ± 12.3 n = 7	260.3 ± 15.9* n = 3
Maintenance	134.8 ± 14.5 n = 6	145.0 ± 15.8 n = 12	232.3 ± 44.1* n = 6

MALNOURISHED

	CONTROL	INSULIN	DIABETIC
Pre TPN	119.6 ± 8.4 n = 9	124.8 ± 8.4 n = 18	217.8 ± 45.5* n = 10
Maintenance	156.3 ± 15.5 n = 22	141.5 ± 10.4 n = 35	312.7 ± 31.4* n = 18

* statistically higher ($p<0.05$) than the concentrations of the Control and Insulin groups.

In the pre TPN subgroup, n represents the number of patients whose glucose concentration was recorded. In the maintenance subgroup, the n is higher since these same patients were included with subsequent body composition studies.

TABLE VII

MAINTENANCE SERUM INSULIN CONCENTRATION (mcU/ml)

	CONTROL	INSULIN	DIABETIC
Normally nourished	77.7 ± 15.4 n = 10	96.7 ± 10.1 n = 15	120.0 ± 43.6 n = 4
Malnourished	56.1 ± 7.2 n = 52	100.2 ± 13.8 * n = 40	91.9 ± 14.4 * n = 21
Combined	59.6 ± 6.6 n = 62	99.3 ± 10.4 * n = 55	96.4 ± 13.7 * n = 25

* statistically significantly higher ($p < 0.05$) than control concentrations by analysis of variance.

TABLE VIII

BODY COMPOSITION DATA OF NORMALLY NOURISHED PATIENTS

	CONTROL		INSULIN		DIABETIC	
	PRE	POST	PRE	POST	PRE	POST
Weight (Kg)	51.4 ± 2.7	54.0 ± 3.2*	65.9 ± 2.3	69.5 ± 2.9#	59.9 ± 3.5	55.3 ± 3.8
Fat (Kg)	5.3 ± 1.2	5.7 ± 1.2	16.3 ± 1.7	16.3 ± 1.7	12.2 ± 2.9	13.1 ± 2.7
LBM (Kg)	46.1 ± 2.0	48.3 ± 2.8	49.6 ± 2.2	53.2 ± 3.0*	47.8 ± 2.1	42.2 ± 1.4
ECM (Kg)	26.1 ± 1.1	28.3 ± 2.1	27.9 ± 1.2	31.1 ± 1.8*	26.9 ± 1.0	24.8 ± 0.6
BCM (Kg)	20.1 ± 1.0	19.9 ± 1.1	21.7 ± 1.1	22.1 ± 1.5	20.9 ± 1.8	17.5 ± 1.3
Na_e/TBW (mEq/L)	78.3 ± 1.6	78.5 ± 1.9	77.2 ± 1.1	80.9 ± 2.3	76.9 ± 3.1	81.3 ± 1.9
K_e/TBW (mEq/L)	71.4 ± 1.2	68.6 ± 2.5	71.8 ± 1.0	67.9 ± 1.9	71.5 ± 3.7	67.8 ± 3.2
Na_e/K_e	1.10 ±0.04	1.17 ±0.07	1.08 ±0.03	1.21 ±0.06	1.09 ±0.09	1.21 ±0.08
n	10		15		4	

* $p < 0.05$ # $p < 0.01$

LBM= lean body mass, ECM = extracellular mass, BCM = body cell mass, TBW = total body water, Na_e = total body exchangeable sodium, K_e = total body exchangeable potassium.

Pre = initial body composition study of a TPN period, Post = second study of period.

TABLE IX

BODY COMPOSITION DATA OF MALNOURISHED PATIENTS

	CONTROL		INSULIN		DIABETIC	
	PRE	POST	PRE	POST	PRE	POST
Weight (Kg)	56.7 ± 2.0	56.7 ± 1.9	66.1 ± 2.1	67.0 ± 2.3	71.3 ± 4.1	73.1 ± 3.7
Fat (Kg)	11.9 ± 1.3	12.5 ± 1.2	16.3 ± 1.2	17.5 ± 1.1	16.5 ± 2.0	16.0 ± 2.0
LBM (Kg)	44.7 ± 1.4	44.2 ± 1.3	49.8 ± 1.7	49.5 ± 1.7	54.8 ± 2.9	57.2 ± 2.8
ECM (Kg)	30.1 ± 1.1	29.1 ± 1.1	32.5 ± 1.3	31.7 ± 1.4	37.7 ± 2.4	39.6 ± 2.7
BCM (Kg)	14.7 ± 0.6	15.2 ± 0.6	17.3 ± 0.6	17.8 ± 0.6	17.2 ± 0.8	17.6 ± 1.0
Na_e/TBW (mEq/L)	93.6 ± 1.5	91.9 ± 1.7	92.1 ± 1.4	89.0 ± 1.7*	94.4 ± 2.1	98.5 ± 3.0
K_e/TBW (mEq/L)	54.3 ± 1.4	56.8 ± 1.6*	57.7 ± 1.1	60.0 ± 1.6	52.5 ± 1.7	52.1 ± 3.1
Na_e/K_e	1.83 ±0.09	1.75 ±0.10	1.64 ±0.06	1.57 ±0.08	1.86 ±0.10	2.11 ±0.19
n	52		40		21	

* $p < 0.05$

LBM = lean body mass, ECM = extracellular mass, BCM = body cell mass, TBW = total body water, Na_e = total body exchangeable sodium, K_e = total body exchangeable potassium.

Pre = initial body composition of a TPN period, Post = second study of a period.

TABLE X

CALORIC DATA OF NORMALLY NOURISHED PATIENTS

	CONTROL	INSULIN	DIABETIC
Carbohydrate (Cal/Kg/day)	52.7 ± 1.9	47.5 ± 2.2	40.5 ± 6.1
Fat (Cal/Kg/day)	2.1 ± 0.4	1.7 ± 0.3	1.1 ± 0.1
Protein (Cal/Kg/day)	6.6 ± 0.3	5.9 ± 0.2	4.6 ± 0.7*
Total Calories/Kg/day	61.4 ± 2.2	55.1 ± 2.4	46.2 ± 6.8
Delta K_e/day (mEq/day)	-0.6 ± 5.4	4.9 ± 5.7	-28.1 ± 14.1

* significantly less ($p < 0.05$) than controls.

TABLE XI

CALORIC DATA OF MALNOURISHED PATIENTS

	CONTROL	INSULIN	DIABETIC
Carbohydrate (Kcal/Kg/day)	43.9 ± 1.9	40.4 ± 1.6	41.8 ± 2.7
Fat (Kcal/Kg/day)	1.6 ± 0.2	1.4 ± 0.2	1.1 ± 0.2
Protein (Kcal/Kg/day)	5.4 ± 0.2	5.0 ± 0.2	5.0 ± 0.3
Total Calories/Kg/day	50.9 ± 2.2	46.8 ± 1.8	47.9 ± 3.1
Delta K_e/day (mEq/day)	3.8 ± 2.8	4.7 ± 3.2	3.7 ± 6.4
$\frac{\text{Delta BCM/day}}{\text{BCM}}$ %	0.31 ± 0.16	0.25 ± 0.15	0.21 ± 0.27

TABLE XII

MULTIPLE LINEAR REGRESSION: DELTA K_e / DAY

CONTROL: Multiple correlation coefficient: 0.33. Regression not significant.

ΔK_e / day = $-29.89 + 4.94(Na_e/K_e) + 0.45(\text{Calories}) + 0.03(\text{Insulin})$

$p < 0.3$ $p < 0.02^*$ $p < 0.6$

INSULIN: Multiple correlation coefficient: 0.35. Regression not significant.

ΔK_e / day = $-14.80 - 5.20(Na_e/K_e) + 0.53(\text{Calories}) + 0.03(\text{Insulin})$

$p < 0.6$ $p < 0.1$ $p < 0.4$

DIABETIC: Multiple correlation coefficient: 0.35. Regression not significant.

ΔK_e / day = $- 9.43 - 1.58(Na_e/K_e) + 0.48(\text{Calories}) - 0.07(\text{Insulin})$

$p < 0.9$ $p < 0.5$ $p < 0.6$

CONTROL + INSULIN: MCC: 0.31. Regression significant, $p < 0.05$.*

ΔK_e / day = $-24.61 + 2.29(Na_e/K_e) + 0.46(\text{Calories}) + 0.03(\text{Insulin})$

$p < 0.6$ $p < 0.01^*$ $p < 0.4$

All patients included were malnourished.

TABLE XIII

MULTIPLE LINEAR REGRESSION: $\frac{\text{DELTA BCM / DAY}}{\text{BCM}}$ %

CONTROL: Multiple correlation coefficient: 0.38. Regression not significant.

$$\% \frac{\Delta \text{ BCM / day}}{\text{BCM}} = -2.07 + 0.42(Na_e/K_e) + 0.03(\text{Calories}) + 0.0024(\text{Insulin})$$
$$p < 0.2 \qquad p < 0.01^* \qquad p < 0.5$$

INSULIN: Multiple correlation coefficient: 0.39. Regression not significant.

$$\% \frac{\Delta \text{ BCM / day}}{\text{BCM}} = -1.15 - 0.13(Na_e/K_e) + 0.03(\text{Calories}) + 0.0020(\text{Insulin})$$
$$p < 0.8 \qquad p < 0.05^* \qquad p < 0.3$$

DAIBETIC: Multiple correlation coefficient: 0.29. Regression not significant.

$$\% \frac{\Delta \text{ BCM / day}}{\text{BCM}} = -0.31 - 0.11(Na_e/K_e) + 0.02(\text{Calories}) - 0.0021(\text{Insulin})$$
$$p < 0.9 \qquad p < 0.5 \qquad p < 0.7$$

CONTROL + INSULIN: MCC: 0.36. Regression significant, $p < 0.01^*$

$$\% \frac{\Delta \text{ BCM / day}}{\text{BCM}} = -1.79 + 0.28(Na_e/K_e) + 0.03(\text{Calories}) + 0.0020(\text{Insulin})$$
$$p < 0.2 \qquad p < 0.001^* \qquad p < 0.2$$

CONTROL + INSULIN + DIABETIC: MCC: 0.34. Regression significant, $p < 0.01$. *

$$\% \frac{\Delta \text{ BCM / day}}{\text{BCM}} = -1.63 + 0.23(Na_e/K_e) + 0.03(\text{Calories}) + 0.0014(\text{Insulin})$$
$$p < 0.3 \qquad p < 0.001^* \qquad p < 0.4$$

ALL PATIENTS INCLUDED WERE MALNOURISHED.

TABLE XIV

COMBINED CONTROL AND INSULIN MULTIPLE LINEAR REGRESSION:

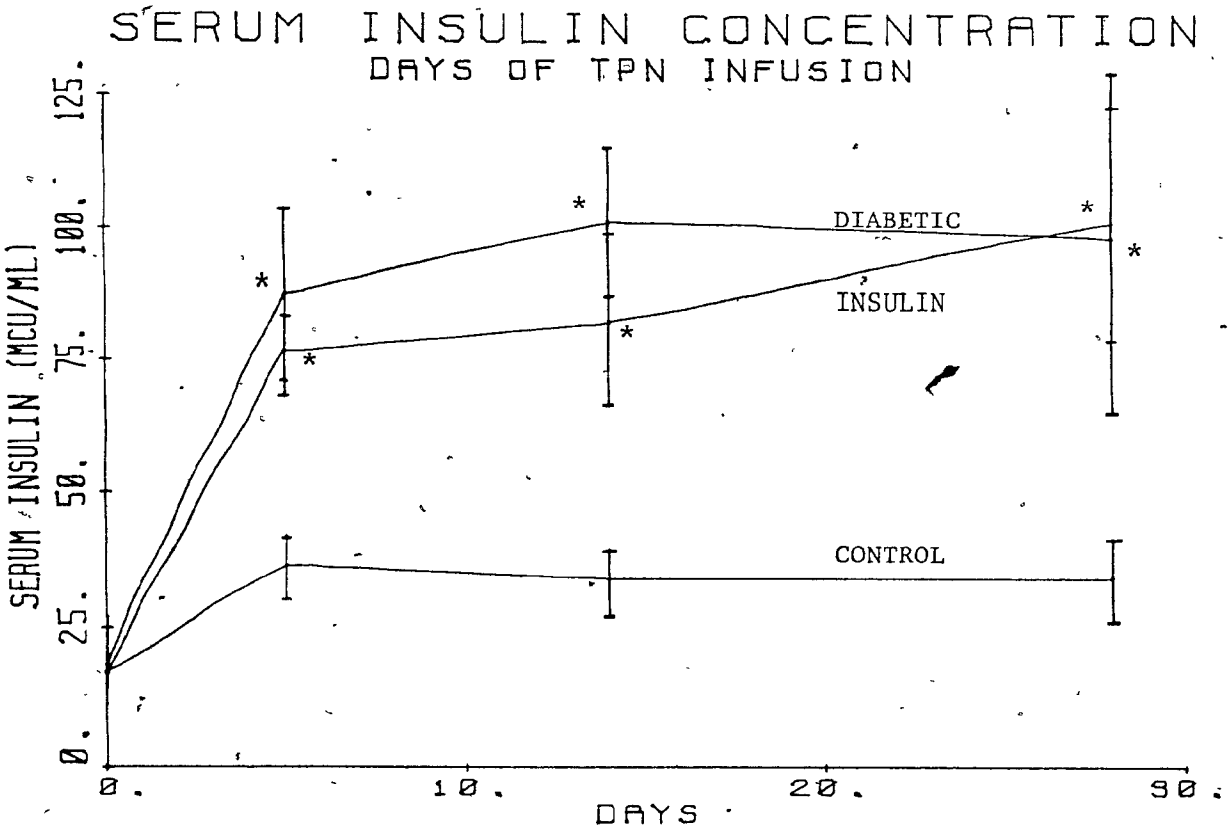
variations of Na_e/K_e, Calories/Kg/day, serum insulin (mcU/ml)

in an hypothetical malnourished BCM = 20 Kg:

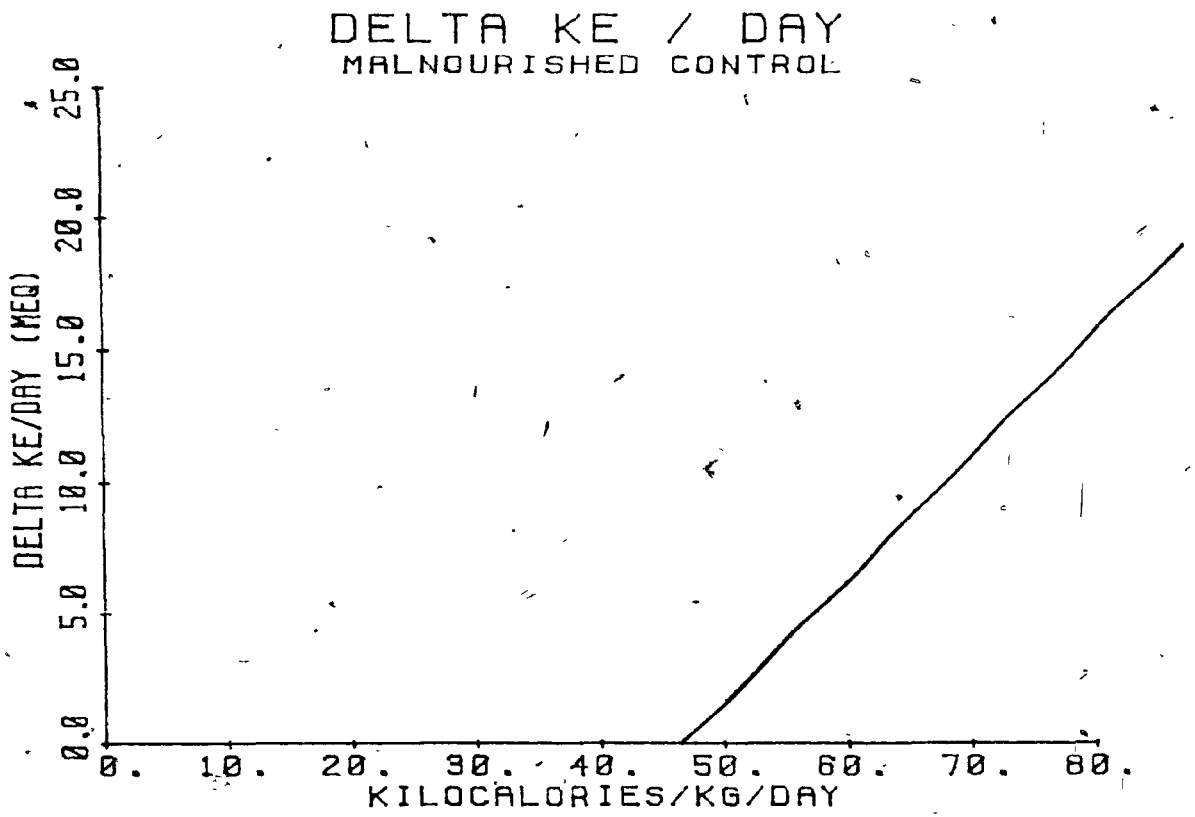
	Na_e/K_e = 1.5			Na_e/K_e = 2.0		
Serum insulin →	50	100	150	50	100	150
Kcal/Kg/day ↓						
40	-14 gm	+6	26	+14	34	54
50	46	66	86	74	94	114
60	106	126	146	134	154	174

These results represent the theoretical daily increase in body cell mass when the above variables are substituted in the multiple linear regression:

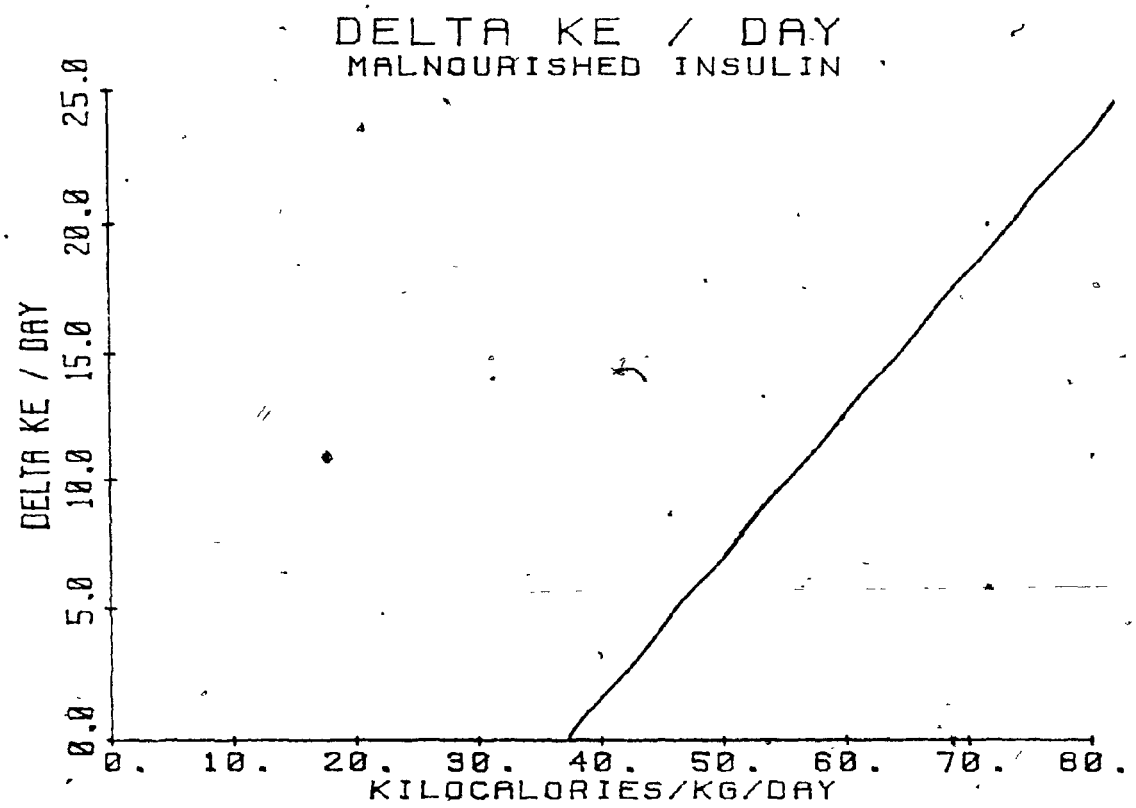
$$\% \frac{\Delta BCM / day}{BCM} = -1.79 + 0.28(Na_e/K_e) + 0.03(Calories) + 0.002(Insulin).$$


SERUM INSULIN CONCENTRATION
DAYS OF TPN INFUSION
SERUM INSULIN (MCU/ML)
0.
25.
50.
75.
100.
125.
DIABETIC
INSULIN
CONTROL
0.
10.
20.
30.
DAYS

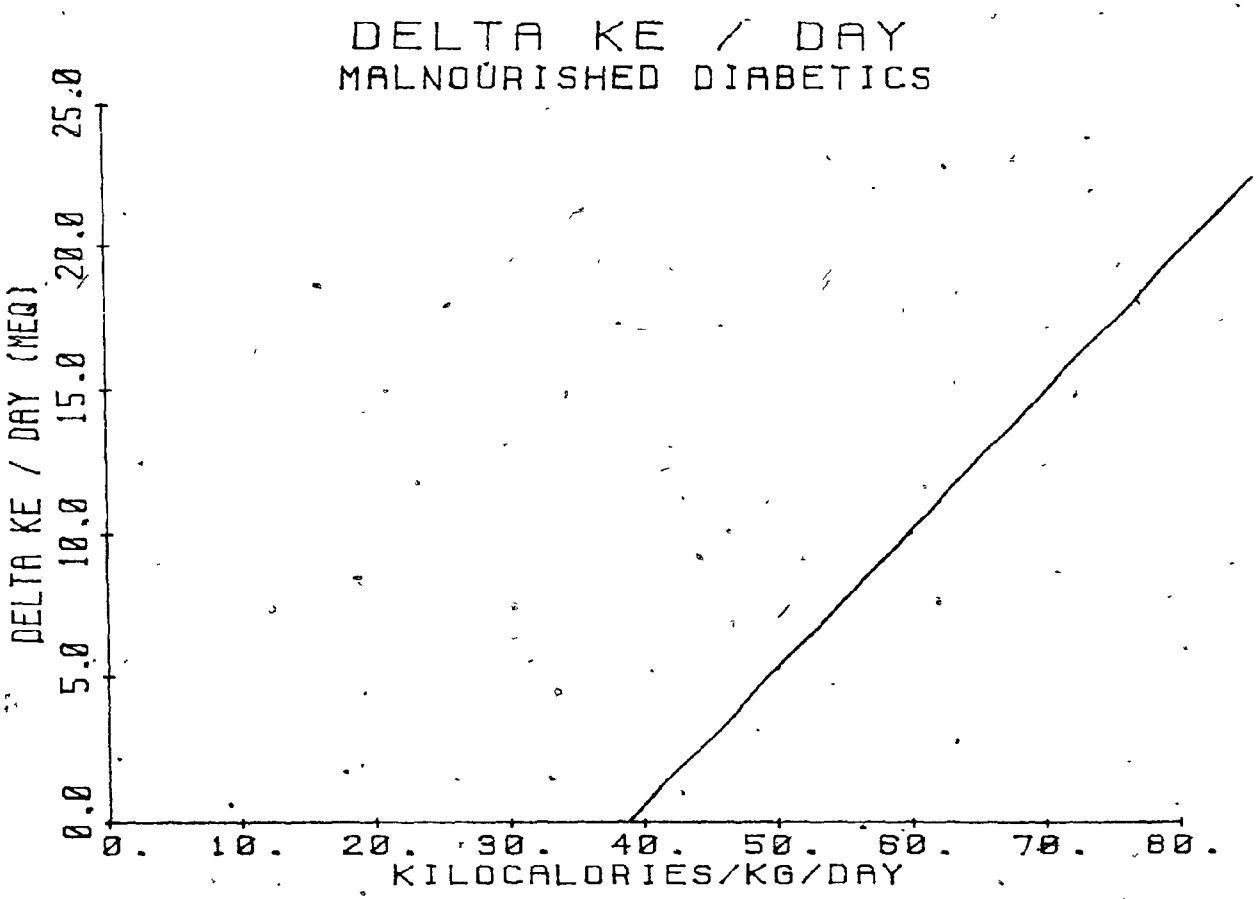
GRAPH I


DELTA KE / DAY
MALNOURISHED CONTROL
DELTA KE/DAY (MEQ)
0.0
5.0
10.0
15.0
20.0
25.0
0.
10.
20.
30.
40.
50.
60.
70.
80.
KILOCALORIES/KG/DAY

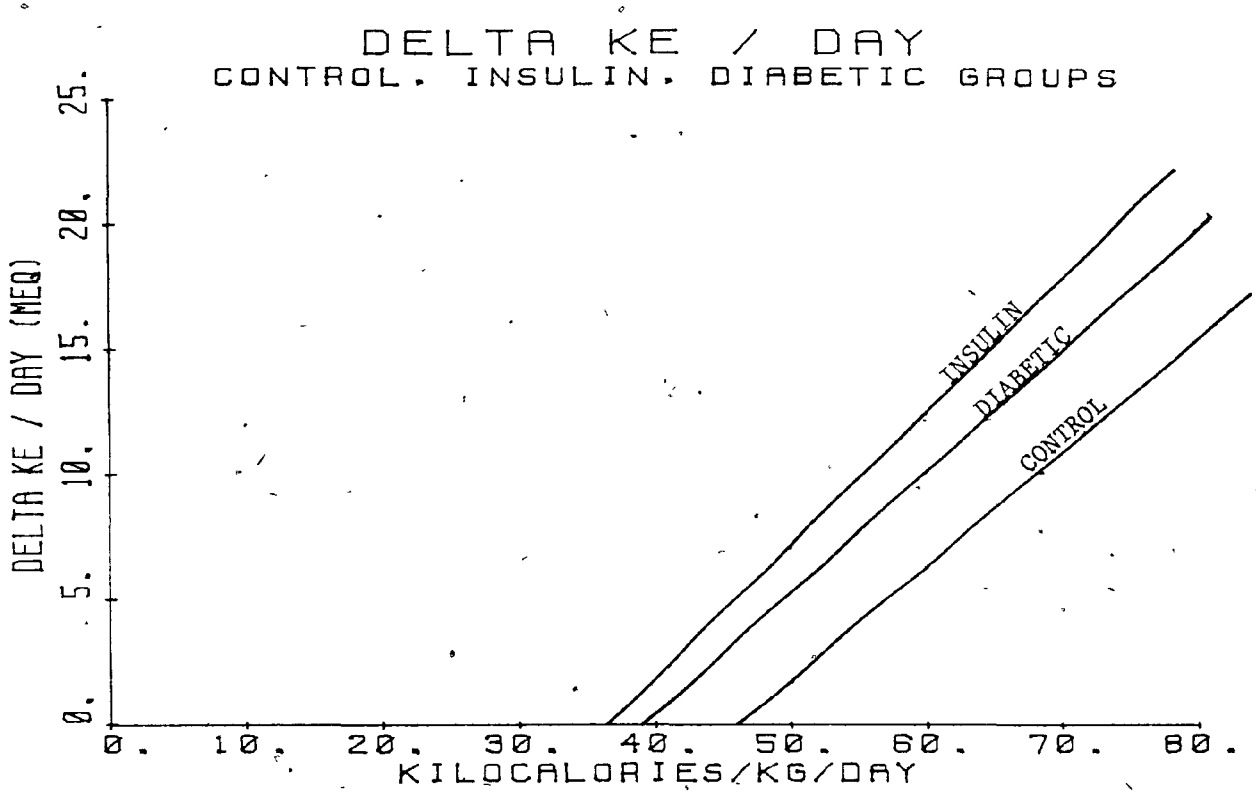
GRAPH II


DELTA KE / DAY
MALNOURISHED INSULIN
DELTA KE / DAY
0.0
5.0
10.0
15.0
20.0
25.0
0.
10.
20.
30.
40.
50.
60.
70.
80.
KILOCALORIES/KG/DAY

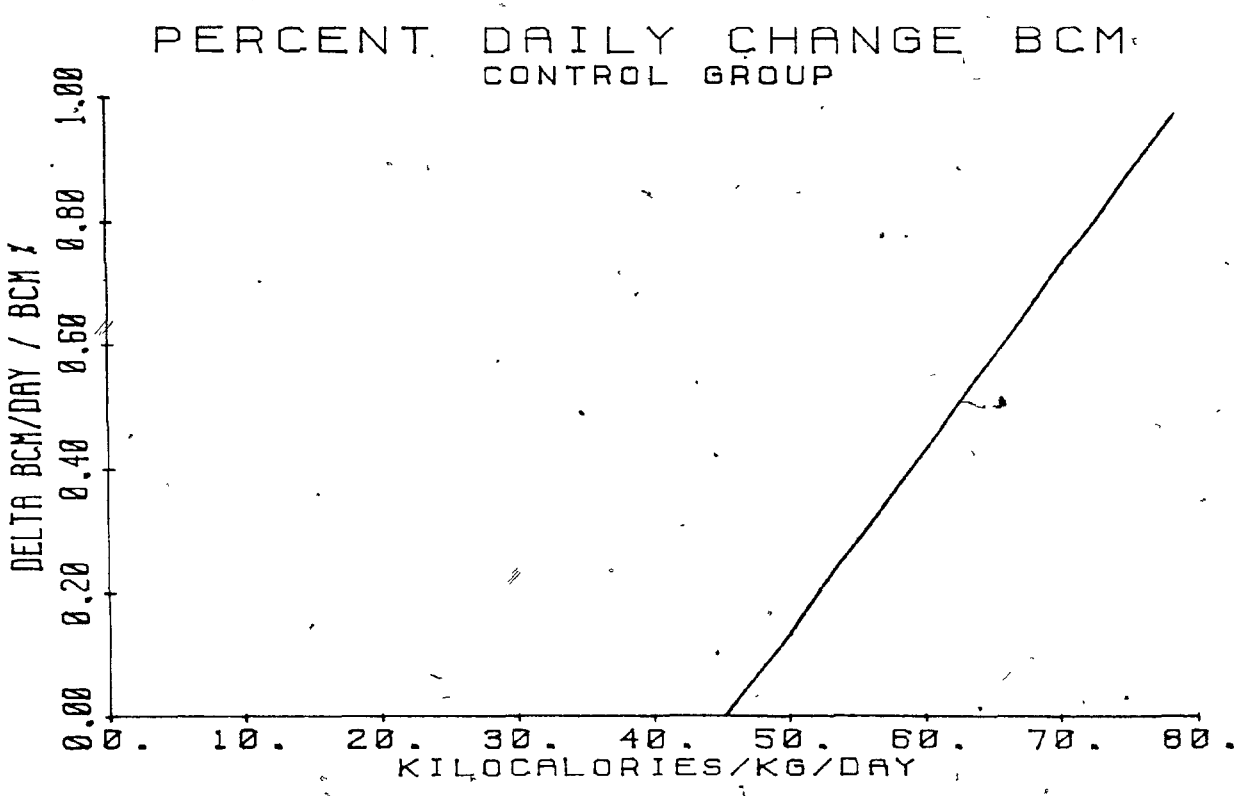
GRAPH III


DELTA KE / DAY
MALNOURISHED DIABETICS
DELTA KE / DAY (MEQ)
0.0
5.0
10.0
15.0
20.0
25.0
0.
10.
20.
30.
40.
50.
60.
70.
80.
KILOCALORIES/KG/DAY

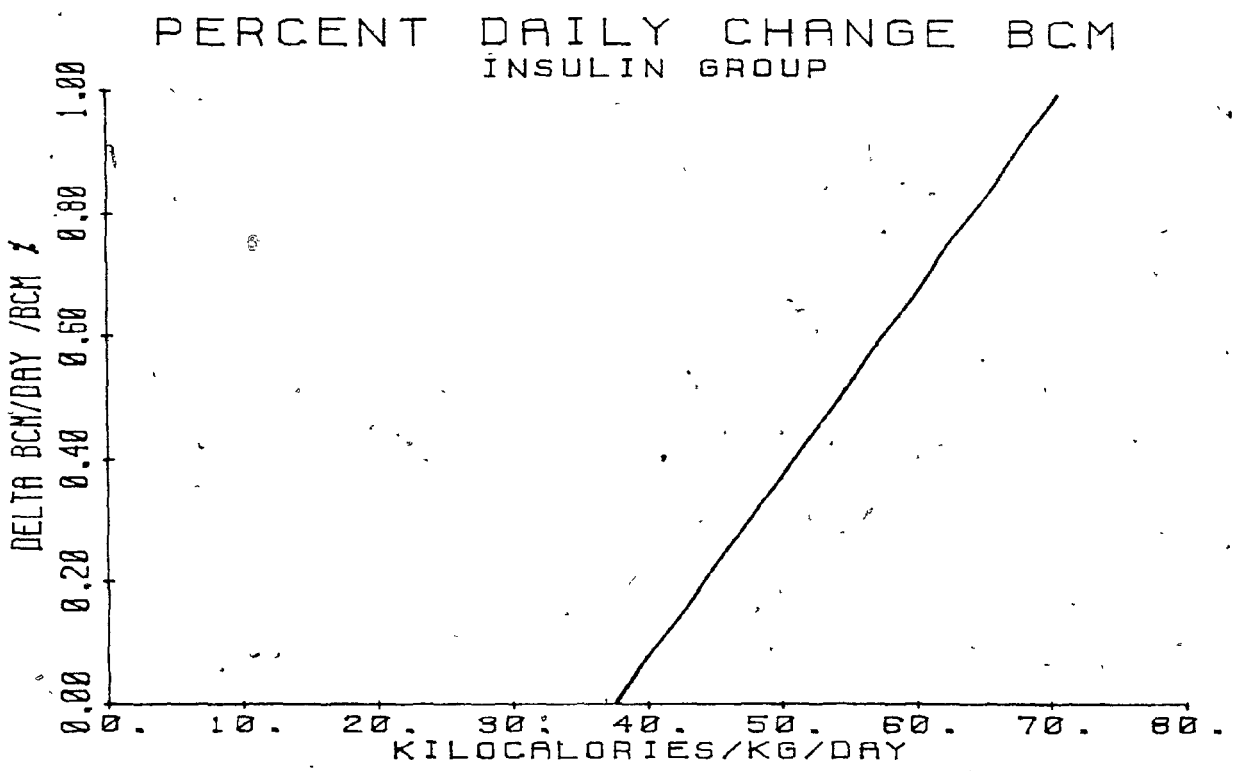
GRAPH IV


DELTA KE / DAY
CONTROL. INSULIN. DIABETIC GROUPS
DELTA KE / DAY (MEQ)
0.
5.
10.
15.
20.
25.
0.
10.
20.
30.
40.
50.
60.
70.
80.
KILOCALORIES/KG/DAY
INSULIN
DIABETIC
CONTROL

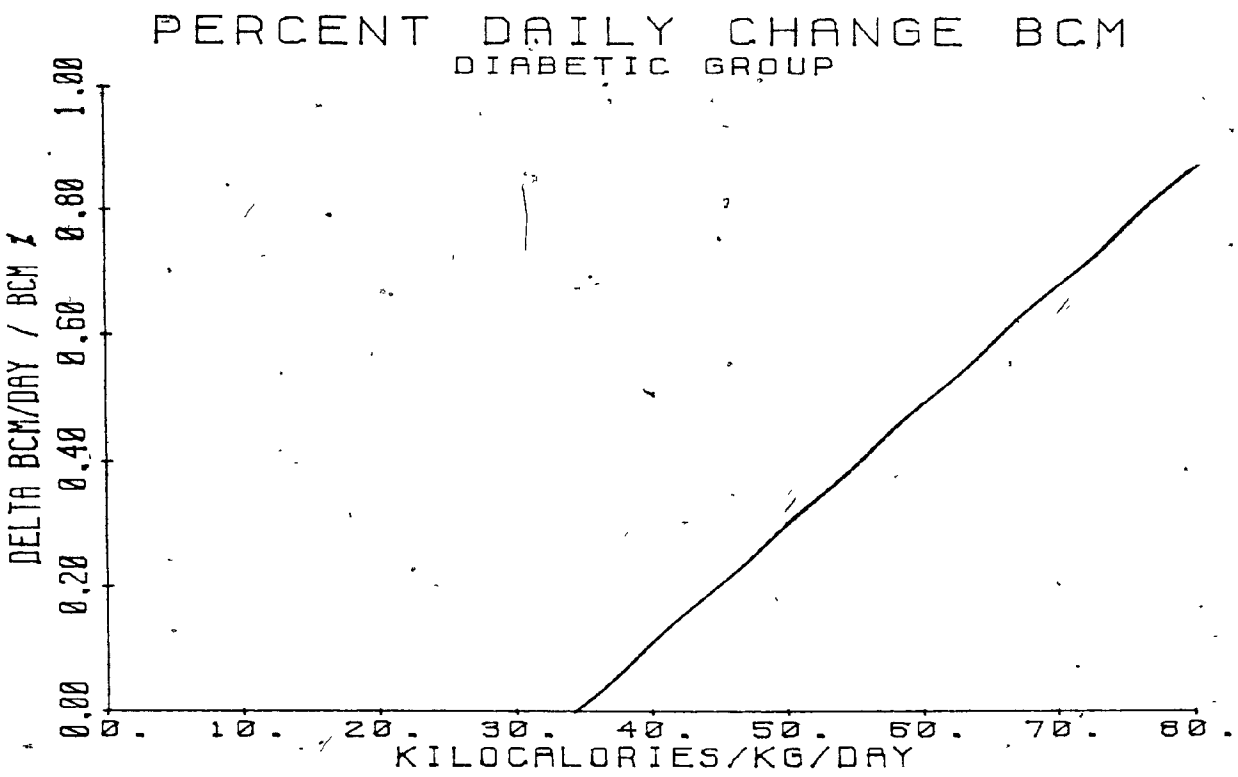
GRAPH V


PERCENT DAILY CHANGE BCM
CONTROL GROUP
DELTA BCM/DAY / BCM %
0.00
0.20
0.40
0.60
0.80
1.00
0.
10.
20.
30.
40.
50.
60.
70.
80.
KILOCALORIES/KG/DAY

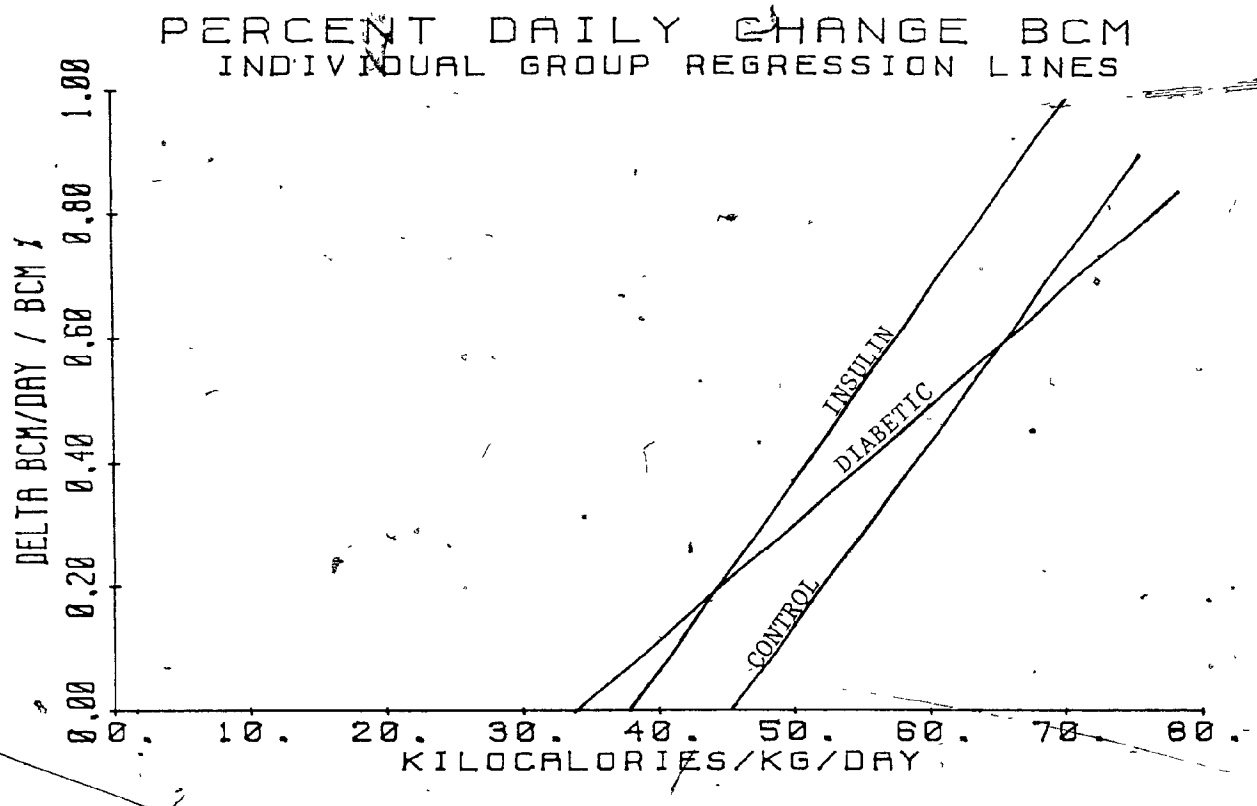
GRAPH VI


PERCENT DAILY CHANGE BCM
INSULIN GROUP
DELTA BCM/DAY /BCM %
0.00
0.20
0.40
0.60
0.80
1.00
0.
10.
20.
30.
40.
50.
60.
70.
80.
KILOCALORIES/KG/DAY

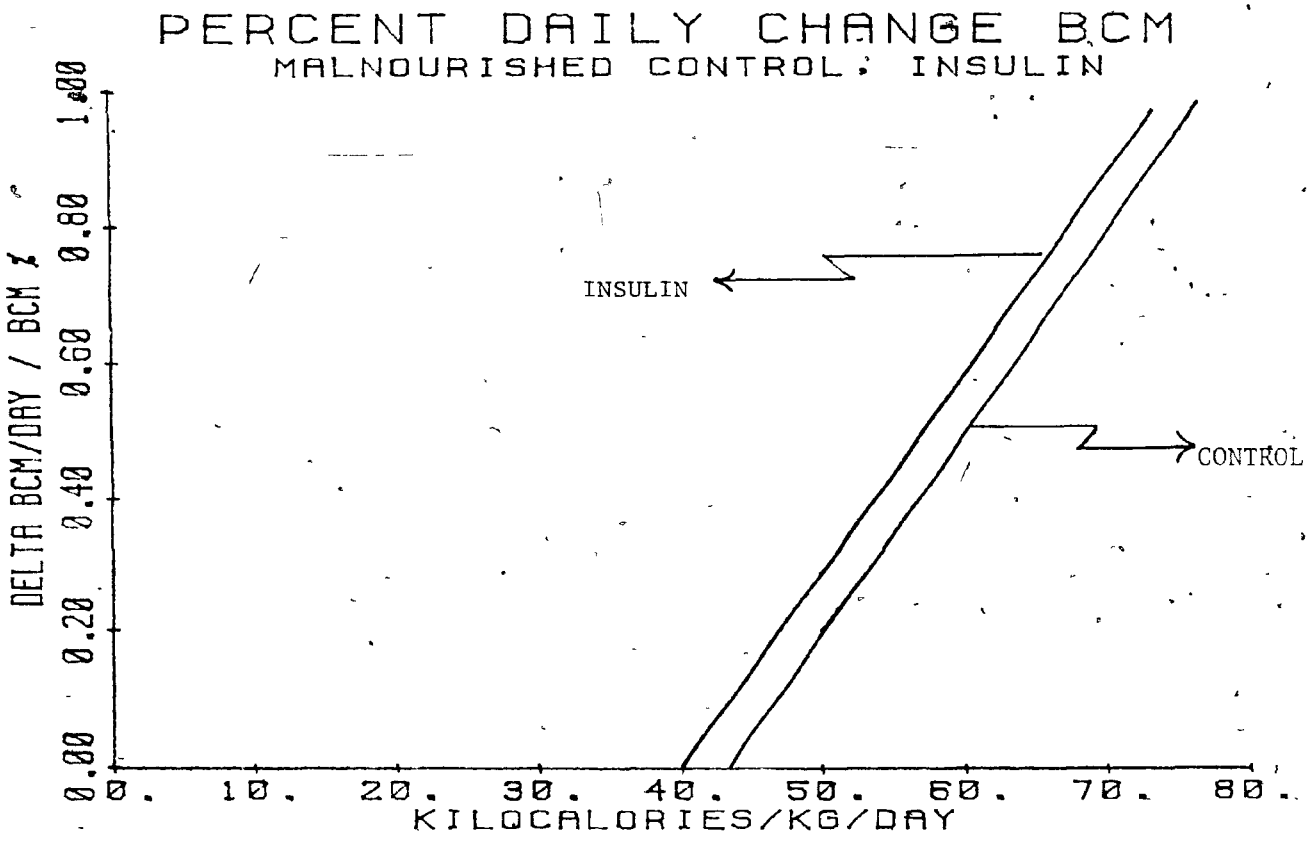
GRAPH VII


PERCENT DAILY CHANGE BCM
DIABETIC GROUP
DELTA BCM/DAY / BCM %
0.00
0.20
0.40
0.60
0.80
1.00
0.
10.
20.
30.
40.
50.
60.
70.
80.
KILOCALORIES/KG/DAY

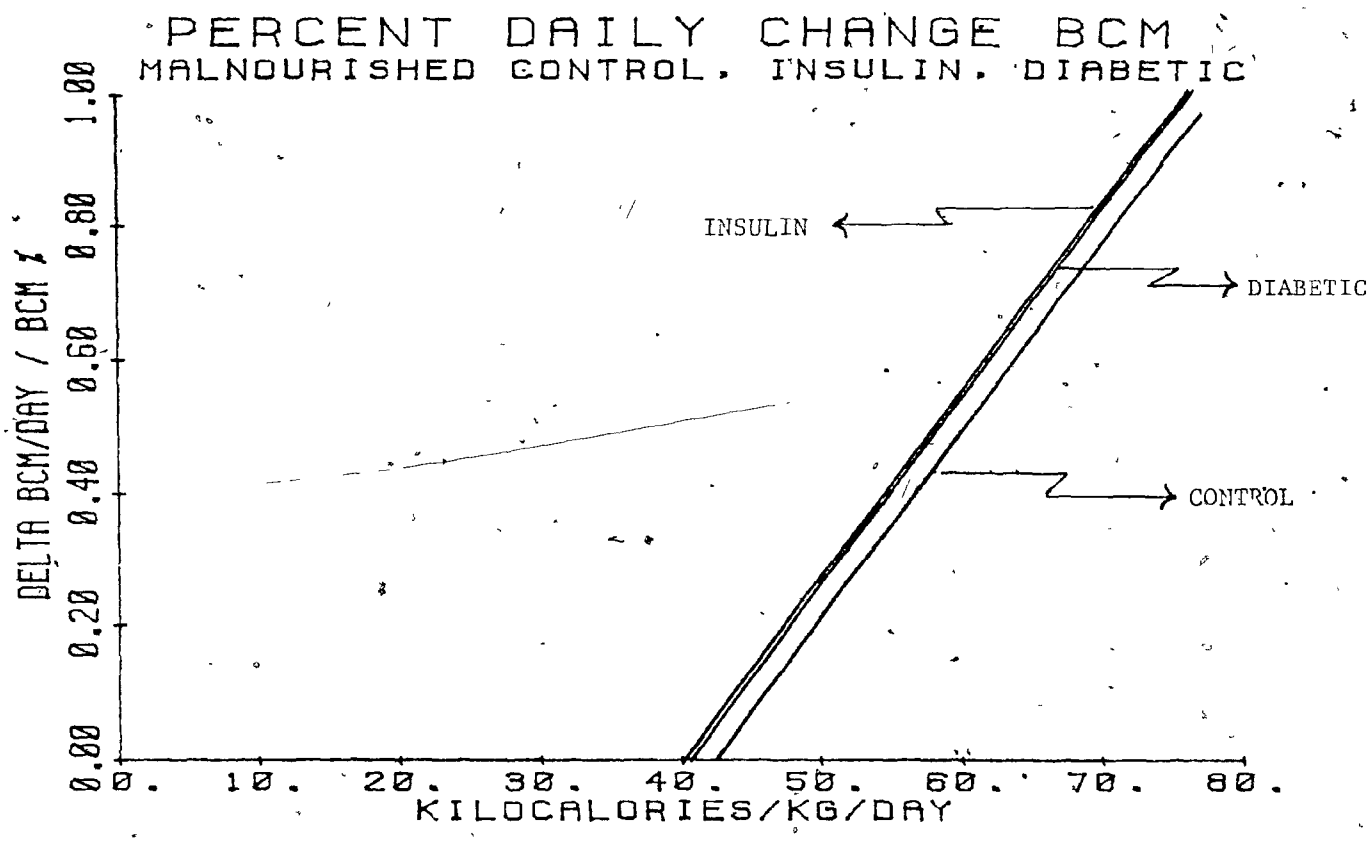
GRAPH VIII


PERCENT DAILY CHANGE BCM
INDIVIDUAL GROUP REGRESSION LINES
DELTA BCM/DAY / BCM %
0.00
0.20
0.40
0.60
0.80
1.00
0.
10.
20.
30.
40.
50.
60.
70.
80.
KILOCALORIES/KG/DAY
INSULIN
DIABETIC
CONTROL

GRAPH IX


PERCENT DAILY CHANGE BCM
MALNOURISHED CONTROL: INSULIN
DELTA BCM/DAY / BCM %
0.00
0.20
0.40
0.60
0.80
1.00
INSULIN
CONTROL
0.
10.
20.
30.
40.
50.
60.
70.
80.
KILOCALORIES/KG/DAY

GRAPH X

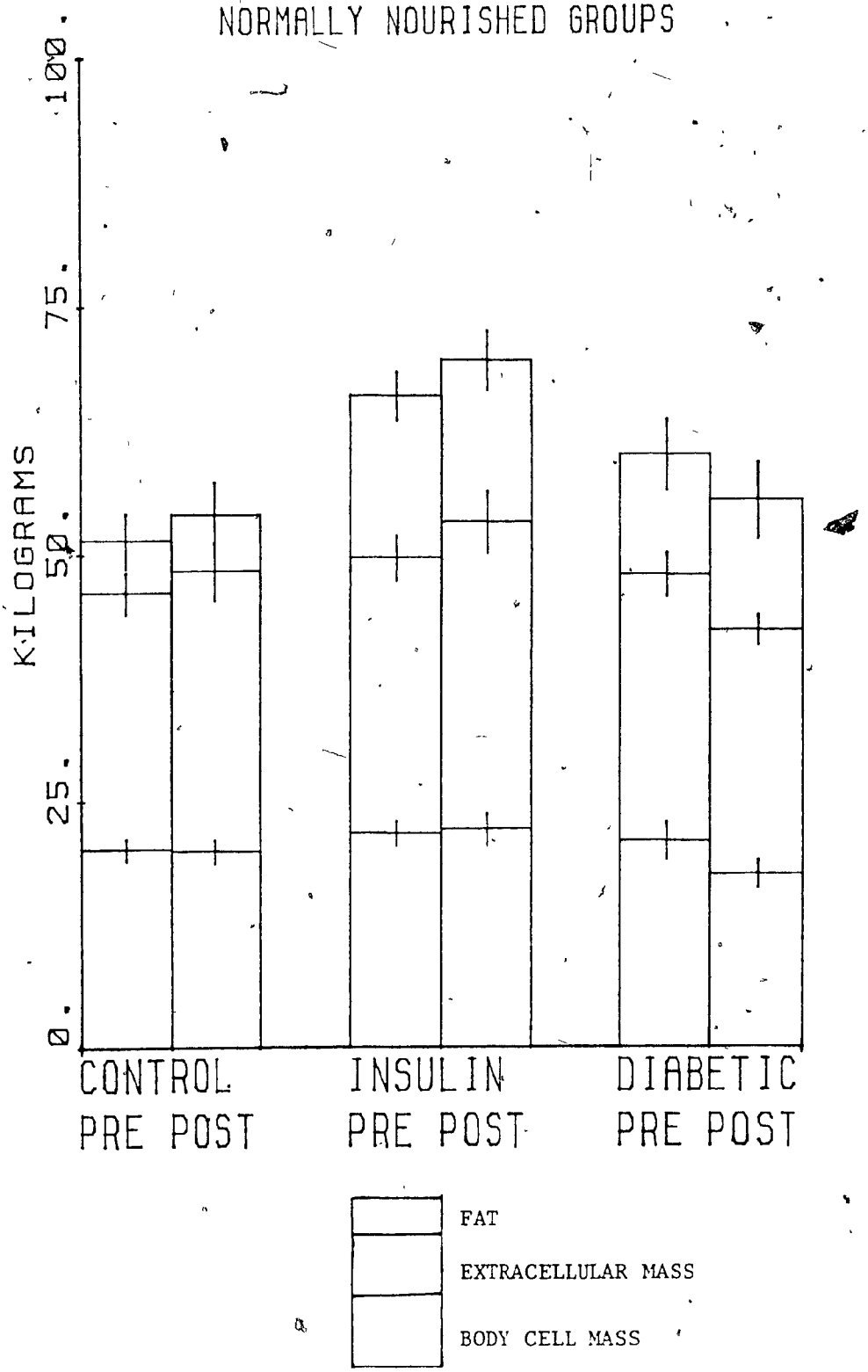

PERCENT DAILY CHANGE BCM
MALNOURISHED CONTROL. INSULIN. DIABETIC
DELTA BCM/DAY / BCM %
0.00
0.20
0.40
0.60
0.80
1.00
0. 10. 20. 30. 40. 50. 60. 70. 80.
KILOCALORIES/KG/DAY
INSULIN
DIABETIC
CONTROL

GRAPH XI

HISTOGRAM I

BODY COMPOSITION

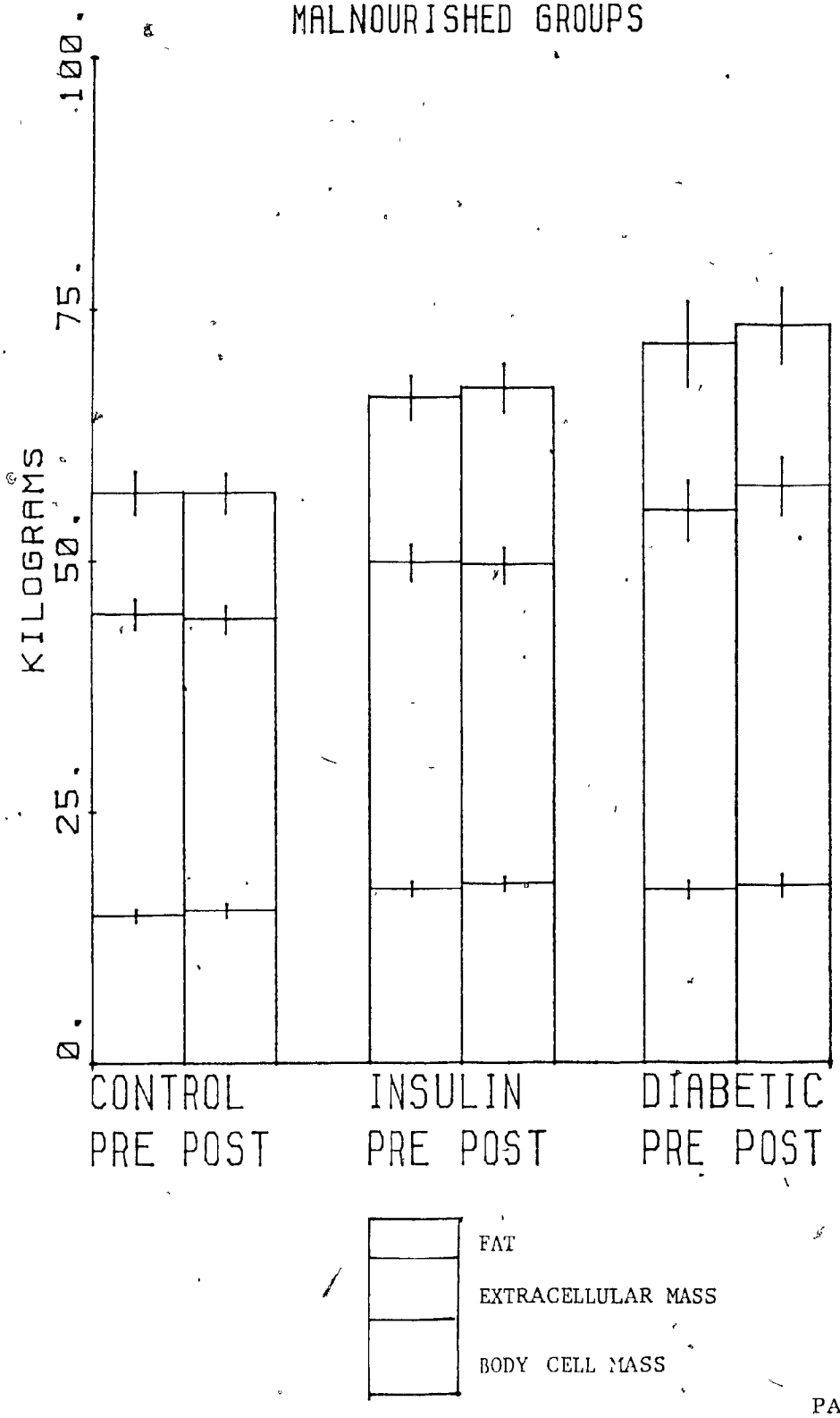
NORMALLY NOURISHED GROUPS



HISTOGRAM II

BODY COMPOSITION

MALNOURISHED GROUPS



BIBLIOGRAPHY

1. Rhoads, J. E.: The History of Parenteral Nutrition. in The Manual of Surgical Nutrition. The American College of Surgeons Committee on Pre and Postoperative Care. ed. Ballinger, W. F., Collins, J. A., Brucker, W. R., Dudrick, S. J., Zeppa, R. W. B. Saunders, Philedelphia, 1975

2. Henriquez, V., Andersen, A. C.: Über parenterale Ernährung durch intravenöse Injektion. Ztchr. f. physiol. chem. 88:357, 1913 (reference only)

3. Daft, F. S., Robscheit-Robbins, F. S., Whipple, G. H.: Plasma protein given by vein and its influence upon body metabolism. J. Biol. Chem. 123: 87, 1938

4. Cox, W. M. Jr., Mueller, A. J.: Nitrogen retention on casein digests. Proc. Soc. Exp. Biol. & Med. 42:658, 1939

5. Elman, R., Weiner, D. O.: Intravenous alimentation: with special reference to protein (amino acid) metabolism. J. A. M. A. 112:796, 1939

6. Stohl, A. T., Butler, A. M., Blackfan, K. C., MacLachlan, E.: Nitrogen metabolism during the oral and parenteral administration of the amino acids of hydrolysed casein. J. Pediatr. 15:469, 1939

7. Hartmann, A. F., Meaker, C. S., Perley, A. M., McGinnis, H. G.: Studies of amino acid administration. J. Pediatr. 20:308, 1942

8. Helfirck, F. W., Abelson, N. M.: Intravenous feeding of a complete diet in a child. J. Pediatr. 25:400, 1944

9. Co Tui, _., Wright, A. M., Carrabba, V., Barcham, I., Vinci, V. J., Mulholland, J. H.: Studies on surgical convalescence: I. Sources of nitrogen loss postgastrectomy and effect of high amino-acid and high caloric intake on convalescence. Ann. Surg. 120:99, 1944

10. Werner, S. C.: Use of a mixture of pure amino acids in surgical nutrition. Ann. Surg. 126:169, 1947

11. Madden, S. C., Kattus, A. A., Carter, J. R., Miller, L. L., Whipple, G. H.: Plasma protein production as influenced by parenteral protein digests; Very high protein feeding and RBC catabolism. J. Exp. Med. 82:181, 1945

12. Holt, L. E. Jr., Tidwell, H. C., Scott, T. F. M.: Intravenous administration of fat; a practical therapeutic procedure. J. Pediatr. 6: 151, 1935

13. Geyer, R. P., Mann, G. V., Stare, F. J.: Parenteral nutrition; improved techniques for preparation of fat emulsions for intravenous nutrition. J. Lab. & Clin. Med. 33:153, 1948

Parenteral nutrition: fat emulsions for intravenous nutrition; turbidometric determination of infused fat emulsions. J. Lab. & Clin. Med. 33:175, 1948

et al.: Parenteral nutrition; studies on soybean phosphatides as emulsifiers for intravenous fat emulsions. J. Lab. & Clin. Med. 33:163, 1948

14. Rhode, C. M., Parkins, W., Tourtellotte, D., Vars, H. M.: Method for continuous intravenous administration of nutritive solutions suitable for prolonged metabolic studies in dogs. Am. J. Physiology. 159:400, 1949

15. Rhode, C. M., Parkins, W. M., Vars, H. M.: Nitrogen balances of dogs infused with 50 percent glucose and protein preparations. Am. J. Physiology 159:415, 1949

16. Aubaniac, R.: L'injection intraveneuse sous-claviaire: avantages et technique. Presse Med. 60:1456, 1952

17. Dudrick, S. J., Vars, H. M., Rawnsley, H. M., Rhoads, J. E.: Total intravenous feeding and growth in puppies. (Abstract) Fed. Proc. 25:481, 1966

18. Dudrick, S. J., Vars, H. M., Rhoads, J. E.: Growth of puppies receiving all nutritional requirements by vein. Fortschritte der Parenteralen Ernahung 2:16, 1967 (reference only)

19. Dudrick, S. J., Wilmore, D. W., Vars, H. M.: Long-term total parenteral nutrition with growth in puppies and positive nitrogen balance in patients. Surg. Forum. 18:356, 1967

20. Wilmore, D. W., Dudrick, S. J.: Growth and development of an infant receiving all nutrients exclusively by vein. J. A. M. A. 203:860, 1968

21. Wilmore, D. W., Diller, B. G., Bishop, H. C.: Total parenteral nutrition in infants with catastrophic gastrointestinal anomalies. J. Pediatr. Surg. 4:181, 1969

22. Dudrick, S. J., Wilmore, D. W., Vars, H. M.: Long-term total parenteral nutrition with growth, development and positive nitrogen balance. Surgery 64: 134, 1968

23. Fuller, R. M., Eraklis, A. J., Rubin, V. G.: Long-term total parenteral nutrition in infants. N. E. J. M. 281:589, 1969

24. Kaplan, M. S., Mares, A., Quintana, P., : High caloric glucose-nitrogen infusions. Arch. Surg. 99:567, 1969

25. Dudrick, S. J., Wilmore, D. W., Vars, H. M.: Can intravenous feeding as sole means of nutrition support growth in a child and restore weight loss in adults? Affirmative answer. Ann. Surg. 169:974, 1969

26. _____, : Classification of infantile malnutrition. Lancet 2:302, 1970

27. Bistrian, B. R., Blackburn, G. L., Serimshaw, N. S., Flatt, J. P.: Cellular immunity in semi-starved states in hospitalized adults. Am. J. Clin. Nutr. 28:1147, 1975

28. Blackburn, G. L., Bistrian, B. R.: Nutritional care of the injured and/or septic patient. Symposium on Response to Infection and Injury II. in Surg. Clinics N. Am. 1195, 1976

29. Garrow, J. S., Fletcher, K., Halliday, D.: Body composition in severe infantile malnutrition. J. Clin. Invest. 44:417, 1965

30. James, W. P. T.: Protein synthesis and breakdown and amino acid catabolism in protein-calorie malnutrition. Proc. Nutr. Soc. 31:225, 1972

31. Millward, D. J.: Protein turnover in skeletal muscle. II. The effect of starvation and a protein-free diet on the synthesis and catabolism of skeletal muscle proteins in comparison to liver. Clinical Sci. 39:591, 1970

32. Waterlow, J. C., Neale, R. J., Rowe, L., Palin, I.: Effects of diet and infection on creatine turnover in the rat. Am. J. Clin. Nutr. 25:371, 1972

33a. Benedict, F. G.: A study of prolonged fasting. Carnegie Institute of Washington Publication No. 203. Washington, D. C. 1915

33b. Benedict, F. G., Miles, W. R., Roth, P., Monmouth Smith, H.: Human vitality and efficiency under prolonged restricted diet. Carnegie Institute of Washington Publication No. 280. Washington, D. C., 1919

34. Cahill, G. F. Jr., Herrara, M. G., Morgan, A. P., Soeldner, J. S., Steinke, J., Levy, P. L., Reichard, G. A. Jr., Kipnis, D. M.: Hormone-fuel interrelationships during fasting. J. Clin. Invest. 45:1751, 1966

35. Cahill, G. F. Jr.: Starvation in man. N. E. J. M. 282:668,1970

36. Cahill, G. F. Jr.: Physiology of insulin in man. Diabetes 20:785, 1971

37. Canon, W. B.: Bodily Changes in Pain, Hunger, Fear and Rage. 2nd ed. Appleton and Co. New York, 1929

38. Cuthbertson, D. P.: The disturbance of metabolism produced by bony and non-bony injury with notes on certain abnormal conditions of bone. Biochem. J. 24:1244, 1930

39. Cuthbertson, D. P.: Post-shock metabolic response. Lancet I:6189 Apr. 11, 1942

40. Graystone, J., Cheek, D. B.: Nutrition and Restricted Nutrition in Fetal and Postnatal Cellular Growth: Hormones and Nutrition ed. Cheek, D. B. New York, John Wiley and Sons, 1975

41. Shizgal, H. M.: The use of body composition mesurement to assess the efficacy of parenteral nutrition. Ch. 35 Advances in Parenteral Nutrition. MTP. Lancaster, Eng. 1978

42. Vinnars, E.: Effect of intravenous amino acid administration on nitrogen retention. Scan. J. Clin. Lab. Invest. 27(suppl)117:1, 1971

43. Moore, F. D., Olsen, K. H., McMurrey, J. D., Parker, H. V., Ball, M. R., Boyden, C. M.: The Body Cell Mass and Its Supporting Environment. W. B. Saunders, Philedelphia, 1963

44. Forse, R. A., Shizgal, H. M.: The Na_e/K_e ratio; a predictor of malnutrition. Surg. Forum 31:89, 1980

45. Moore, F. D.: Energy and maintenance of the body cell mass. J. P. E. N. 4:228, 1980

46. Moore, F. D.: La maladie post-opératoire: is there order in variety: the six stimulus-response sequences. Surg. Clin. N. Am. 56(4):803, 1976

47. Shizgal, H. M., Spanier, A. H., Humes, J., Wood, D.: Indirect measurement of total exchangeable potassium. Am. J. Physiol. 233:F253, 1977

48. Liljedahl, S.-O., Gemzell, C.-A., Plantin, L.-O., Birke, G.: Effect of human growth hormone in patients with severe burns. Acta Chir. Scand. 122:1, 1961

49. Johnston, I. D. A., Marino, J. D., Stevens, J. Z.: The effect of intravenous feeding on the balances of nitrogen, sodium and potassium after operation. Brit. J. Surg. 53:885, 1966

50. Birke, G., Dunér, H., Liljedahl, S.-O., Pernow, B., Plantin, L.-O., Troell, L.: Histamine, catecholamines and adrenocortical steroids in burns. Acta Chir. Scand. 114:87, 1957

51. Hinton, P., Littlejohn, S., Lloyd, J., Allison, S. P.: Insulin and glucose to reduce catabolic response to injury in burned patients. Lancet I:767, 1971

52. Woolfson, A. M. J., Heatley, R. V., Allison, S. P.: Insulin to inhibit protein catabolism after injury. N.E.J.M. 300:14 1979

53. Morgan, C. R., Lazarow, A.: Immunoassay of insulin: Two antibody system. Plasma insulin levels of normal, subdiabetic and diabetic rats. Diabetes 12:115, 1963

54. Corsa, L. Jr., Olney, J. M., Steenburg, R. W.: The measurement of exchangeable potassium in man by isotope dilution. J. Clin. Invest. 29:1280 1950

55. James, A. H., Brooks, L., Edelman, I. S.: Body sodium and potassium I. The simultaneous measurement of exchangeable sodium and potassium in man by isotope dilution. Metabolism 3:313 1954

56. Cohn, S. H., Dombrowski, C. S.: Absolute measurement of whole body potassium by gamma ray spectrometry. J. Nucl. Med. 11:239 1970

57. Woodward, K. T., Randolph, C. L. Jr., Van Hoeck, R.: The Walter Reed whole body counting facility, Washington, D. C. Walter Reed Inst. Med. Res. 11:11, 1960 (reference only)

58. Tyson, I., Genna, S., Jones, R. L., Bikerman, V., Burrows, B.A.: Body potassium measurements with a total body counter. J. Nucl. Med. 11:225, 1970

59. Edelman, I. S., Leibman, J., O'Meara, M. P., Birkenfeld, L. W.: Interrelations between serum sodium concentration, serum osmolarity and total exchangeable sodium, total exchangeable potassium and total body water. J. Clin. Invest. 37:1236, 1958

60. Olesen, K. H.: Inter-relations between total exchangeable sodium, potassium, body water and serum sodium and potassium concentrations in hyponatremic and normonatremic heart disease. Circulation 25:895, 1967

61. Whalen, F. J., LeCain, W. K., Latiolais, C. J.: Availability of insulin from continuous low-dose insulin infusions. Am. J. Hosp. Pharm. 36:330, 1979

62. Zierler, K. L.: Insulin, ions and membrane potentials. Ch. 22 Handbook of Physiology. Section 7. Endocrinology I: Endocrine Pancreas. American Physiological Society. Washington, D. C. 1972

63. Randle, P. J., Hales, C. N.: Insulin release mechanisms. Ch. 13. Handbook of Physiology. Section 7. Endocrinology I: Endocrine Pancreas. American Physiological Society. Washington, D. C. 1972

64. Allison, S. P.: Effect of insulin on metabolic response to injury. J. P. E. N. 4:175, 1980

65. Moore, F. D.: Metabolic Care of the Surgical Patient. W. B. Saunders Philedelphia, 1959

66. Alberti, K. G. M. M., Batstone, G. F., Foster, K. J., Johnston, D. G.: Relative role of various hormones in mediating the metabolic response to injury. J. P. E. N. 4:141, 1980

67. Shizgal, H. M.: The effect of nutritional support on body composition. Ch. 16 in Practical Nutritional Support. Pitman Medical Publishing, London, England, 1980

68. Forse, R. A., Shizgal, H. M.: The efficacy of total parenteral nutrition with lipid. Ann. Roy. Coll. Phys. & Surg. Can. 13:127, 1980

69. Forse, R. A., Shizgal, H. M.: Protein and calorie requirements with total parenteral nutrition. Ann. Surg. 192:562, 1980

70. Spanier, A. H., Shizgal, H. M.: Caloric requirements of the critically ill patient receiving intravenous hyperalimentation. Am. J. Surg. 133:99, 1977

71. Shizgal, H. M., Milne, C. A., Spanier, A. H.: The effect of protein-sparing intraveously administered fluids on postoperative body composition. Surgery 85:496, 1979