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THE EFFECT OF GLYCEMIC CONTROL ON PROTEIN METABOLISM IN OBESE SUBJECTS WITH TYPE II DIABETES MELLITUS

by

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THE EFFECT OF GLYCEMIC CONTROL ON PROTEIN METABOLISM IN OBESE SUBJECTS WITH TYPE II DIABETES MELLITUS

Karin Styhler

ABSTRACT

We questioned whether improved glycemic control achieved by oral hypoglycemia agent (gliclazide) would correct the altered protein metabolism during an iso-energetic (ISO) and a iow energetic (50% of ISO) diets. Seven diabetic (DM) and 7 matched obese control (OB) subjects were given ISO for 14 (DM) or 7 (OB) days, followed by 28 days of the low energetic diet with constant 1.5 g protein/kg BMI_{25}/d. Gliclazide (+ metformin in 4 DM) was given during days 8-14 of ISO and the low energety diet to DM. With ISO and gliclazide, fasting plasma glucose decreased and plasma insulin and nitrogen retention increased while 3-methylhistidine excretion and resting metabolic rate decreased to levels no longer different from OB. With moderate energy restriction, weight decreased in all subjects and glycemia normalized in DM. Nitrogen equilibrium was maintained and 3-methylhistidine excretion did not change. The altered protein metabolism observed during hyperglycemia can be improved with oral hypoglycemic agent therapy ± the low energy diet. Moderate energy restriction with oral hypoglycemic agent therapy achieves diabetes control, nitrogen equilibrium, and a modest decrease in resting metabolic rate.

L'EFFET DE LA CONTROLE GLYCÉMIQUE SUR LA MÉTABOLISME DES PROTÉINES CHEZ LES SUJETS OBESE AVEC LA DIABÉTE TYPE II

Karin Styhler

RÉSUMÉ

Nous avons vérifié si l'amélioration de la glycémie par un agent hypoglycémiant (gliclazide) corrige le métabolisme altéré des protéines durant des régimes isoénergétique (ISO) et hypoénergétique (50% ISO). Sept sujets diabétiques (DM) et 7 sujets témoins obèses (OB) ont reçu une formule ISO pendant 14 (DM) ou 7 (OB) jours, suivie pendant 28 jours d'une formule hypoénergétique, avec un apport protéique de 1.5 g/kg IMC_{25}/jour. Gliclazide (+ metformin chez 4 DM) a été donné durant les jours 8-14 d'ISO et durant le régime hypoénergétique aux sujets DM. Avec ISO + médicament, la glycémie baisse, l'insuline plasmatique monte associée à une retention accrue d'azote et une excrétion de la 3-méthylhistidine et un métabolisme de base diminuée à des valeurs semblables au groupe témoin. Avec le régime hypoénergétique, le poids chute, la glycémie se normalise chez DM, le bilan d'azote est à l'équilibre et la 3-méthylhistidine ne change pas. Le métabolisme altéré des protéines durant l'hyperglycémie peut être amélioré par l'agent hypoglycémiant ± un régime hypoénergétique. Une restriction énergétique modérée combinée à la prise d'un agent hypoglycémiant améliore le contrôle du diabète, maintient un bilan d'azote à l'équilibre et induit une réduction modeste du métabolisme de base.

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This thesis is lovingly dedicated to the memory and brilliant work of Christine Gagnon, close friend and confidante, she will be sadly missed, but never forgotten.

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LIST OF ABBREVIATIONS

%BF	-	Percentage of Body Fat
BIA	-	Bio-electrical Impedance Analysis
BMI	-	Body Mass Index
BW	-	Body Weight
CBG	-	Capillary Blood Glucose
CHO	-	Carbohydrate
CIU	-	Clinical Investigation Unit
DM	-	Type II Diabetes Mellitus Study Group
FFA	-	Fasting Plasma Free Fatty Acids
FPG	-	Fasting Plasma Glucose
FPI	-	Fasting Plasma Insulin
IRI	-	Immunoreactive Insulin
ISO	-	Iso-energetic diet
ISO+OHA	-	Iso-energetic diet with Oral Hypoglycemic Agent
K	-	Potassium
LBM	-	Lean Body Mass
LED	-	Low Energy Diet
3-MeHis	-	Urinary 3-Methylhistidine
N	-	Nitrogen
Na	-	Sodium
OB	-	Obese Control Group
3-OH	-	3-beta-hydroxybutyrate
OHA	-	Oral Hypoglycemic Agent
PRO	-	Protein
RMR	-	Resting Metabolic Rate
RQ	-	Respiratory Quotient
RVH	-	Royal Victoria Hospital

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1.0 INTRODUCTION

The hallmark of diabetes mellitus is hyperglycemia. This chronic metabolic condition is not characterized by aberrations solely in carbohydrate metabolism, but in lipid and protein metabolism as well. The association between diabetes and abnormal protein metabolism was noted as early as 600 BC in the Sanscrit literature; severe muscle wasting was one consequence of this *"honey urine disease"* (Frank, 1957). In 200 AD Aretaeus observed the emaciation that is seen in a state of complete insulin deficiency, and termed this condition of *"melting down of flesh to sweet urine"* as *diabetes (to flow through)*, with reference to the polyuria (Reed, 1954). In 1675 Thomas Willis refined the nomenclature of this condition into what is known today as *diabetes mellitus (sweetened with honey)*.

In the 19th century Carl Voit conducted a series of nitrogen balance experiments in which he observed that whereas healthy subjects were in a state of nitrogen equilibrium, poorly controlled diabetic subjects were not (Sönksen, 1992). These diabetic subjects were in a state of negative nitrogen balance; that is the body, due to the lack of insulin, was not using the readily available glucose present in the blood, but searching for glucogenic precursors, namely amino acids. One such source of amino acids was skeletal muscle and visceral proteins. The breakdown of these tissues resulted in the increased excretion of urinary nitrogen.

With Banting and Best's discovery of insulin in 1922, this previously observed negative nitrogen balance was reversed to a state of positive nitrogen balance and/or equilibrium (Sokhey & Allan, 1924; Atchley et al., 1933). It was this

reversal of the negative nitrogen balance that led early scientists to deem insulin as having an anabolic nature, leading to the popular belief that the nitrogen imbalance was due to a decrease in protein synthesis, rather than an increase in protein breakdown. The role of insulin on protein homeostasis is now reasonably well documented for type I diabetes (Jefferson, 1980; Nair et al., 1983; Umpleby et al., 1986; Tessari et al., 1986; Nair et al., 1987; Pacy et al., 1989; Luzi et al., 1990; Nair, 1992), however its role in type II diabetes is less understood.

It has been reported that protein metabolism is accelerated in moderately hyperglycemic obese type II diabetic subjects when compared to a matched obese control group during an iso-energetic diet with ample protein intake (Gougeon et al., 1994). These alterations in protein metabolism observed in this group of obese diabetic subjects could be corrected with exogenous insulin therapy sufficient to achieve euglycemia (personal communication by R.Gougeon). By contrast, restoration of euglycemia with a very-low-energy diet (VLED) could not completely achieve nitrogen equilibrium in a type II diabetic group (Gougeon et al., 1994). However, the VLED did allow for nitrogen equilibrium to be established by 3 weeks in an obese control group (Gougeon et al., 1995). This suggests that there are alterations in the adaptive mechanisms for protein sparing during a VLED in type II diabetes (Gougeon et al., 1994; Gougeon et al., 1995).

The experimental strategy which led us to the present study was therefore very clear. It had been observed that protein metabolism was abnormal in poorly controlled type II diabetic subjects consuming a weight-maintaining diet, yet could be

corrected with exogenous insulin therapy. A VLED greatly improved glycemia, but negative nitrogen balance persisted. The VLED was thought to be unable to completely restore protein metabolism due to (1) residual insulin resistance, and (2) insufficient dietary protein at this lowered energy intake (Gougeon et al., 1995). Therefore, it was possible to show alterations in protein metabolism in these "extreme" conditions of poorly controlled diabetes and severe energy restriction. The question remained whether protein metabolism could be normalized using a more conventional approach to diabetes treatment, as the state of protein metabolism in a type II diabetic individual receiving dietary and pharmacological interventions had not yet been studied. The quantification of the state of protein metabolism in this situation would have major significance within the clinical realm.

The proposed study centres on the effect of improved diabetes control achieved by dietary (low-energy diet, 4200-6300 kJ/d (1000-1500 kcal/d)) and pharmacological (oral hypoglycemic agent, Gliclazide) interventions on protein metabolism in an obese type II diabetic study group. The latter is being assessed by daily nitrogen balances, 24hr urinary 3-methylhistidine excretion, and changes in lean body mass determined with bio-electrical impedance analysis and cumulative nitrogen balance. The study presented herein, may help define the level of diabetes control necessary to achieve a more favourable nitrogen balance in obese type II diabetic subjects.

2.0 *REVIEW OF THE LITERATURE*

2.1 *Etiology of Diabetes Mellitus*

The cause of diabetes is attributed to a deficiency of insulin's action at the level of the tissue. This deficiency may arise from an absolute lack of circulating insulin, pancreatic beta cells that merely do not secrete a sufficient amount, or a resistance of peripheral tissues to insulin. The complete cessation of insulin production by the beta cells is usually related to an autoimmune insult to the pancreatic beta cells in a genetically susceptible individual (Anderson, 1988). The disease usually develops before the age of 40, and is consequently known as "juvenile diabetes", insulin-dependent-diabetes mellitus (IDDM), and type I diabetes mellitus. Management of these individuals requires careful dietary intervention and insulin therapy. The etiology of type II diabetes mellitus is less clear than that of type I diabetes mellitus. Type II diabetes mellitus usually develops insidiously due to one or more factors; such as a progressive decline in the ability to maintain high insulin production, an increase in insulin resistance of the insulin-sensitive tissues (mainly muscle and fat) and/or an ability to normally suppress hepatic glucose production by insulin (ie. hepatic insulin resistance). Type II diabetes usually occurs after the age of 40, and is known as "adult or maturity-onset diabetes" and non-insulin-dependent-diabetes mellitus (NIDDM). Almost all patients with this form of diabetes are obese, and the obesity is a major contributor to the insulin resistance (Campbell & Carlson, 1993). Management of these individuals centres around drug and diet therapy. Insulin production is stimulated by some oral hypoglycemic agents (OHA), aimed at the level of the still-functioning pancreas and sensitivity is improved through weight

reduction, diet, exercise, and therapy with certain OHA.

2.2 *Protein Metabolism in Type I Diabetes Mellitus (Complete Insulin Deficiency)*

Although insulin is not the sole determinant of protein metabolism, it is a primary one (Marliss et al., 1978; Fisler et al., 1982). Insulin reduces plasma amino acids to normal levels (Luck et al., 1928; Felig et al., 1977) and decreases urinary nitrogen excretion (Sokhey et al., 1924; Atchley et al., 1933). Protein loss was found to be greater when diabetic subjects were studied while very hyperglycemic (Nair et al., 1983; Umpleby et al., 1986; Pacy et al., 1989). The initiation of insulin therapy which improved glycemia resulted in a decrease of protein breakdown (Umpleby et al., 1986; Pacy et al., 1989). It was reported that improving diabetes control with insulin administration reduced urinary nitrogen excretion as early as 1924 by Sokhey, yet little to date has been studied in this field.

With the introduction of tracer technology a small number of investigators have begun to elucidate the mechanisms surrounding insulin's role on protein synthesis and breakdown during periods of hyperglycemia and improved diabetes control. Waterlow and colleagues (1978) developed a method of continuous administration (orally or intravenously) of labelled amino acid in order to achieve a steady-state, as reflected by a constant concentration of labelled amino acid in the plasma. It is under these conditions that a simple model can be used to measure the flux, and derive conclusions about the rates of protein synthesis and breakdown. As

leucine is an essential amino acid, its only source in the fasted state is body protein. Therefore once a plateau of labelled ^{13}C-leucine enrichment occurs, this metabolic pool has two fates, synthesis and breakdown. The leucine flux through the body pool can be described by the following equation:

$$Q = S + C = B + I$$

Where Q is the rate of leucine flux, S represents protein synthesis (measured by the rate of leucine incorporation into body proteins), C is the rate of catabolism to expired $^{13}CO_2$, B is the rate of leucine release from breakdown of body proteins, and I is the leucine intake (oral or by infusion).

Three groups of investigators have studied the effects of diabetes control on protein metabolism in the type I diabetic scenario. Nair and colleagues (1983) investigated protein kinetics in 5 hyperglycemic (FPG 20.1 $\pm$ 4.2 mM) type I diabetic male subjects and two control groups (1) 5 healthy lean men and (2) 5 obese women. Rates of protein synthesis and breakdown were found to be greater in the uncontrolled diabetic group than the 2 groups when expressed as grams nitrogen or on a kg LBM basis ($P<0.01$), as the 3 groups were of similar LBM. Umpleby and co-workers (1986) studied 10 type I diabetic subjects during different levels of glycemia and included a healthy control group. FPG during insulin withdrawal (20.2 $\pm$ 1.0 mM) was significantly greater than during conventional insulin treatment (10.5 $\pm$ 1.9 mM; $P<0.001$) and insulin infusion (5.5 $\pm$ 0.1 mM; $P<0.005$). Protein synthesis was found not to change with different degrees of diabetes control and found not to be different from the healthy control subjects at any time. Rates of

protein breakdown were greater in the diabetic subjects during all degrees of diabetes control than the healthy subjects ($P<0.05$). Rates of protein breakdown were also greater while subjects were hyperglycemic during insulin withdrawal than during conventional insulin treatment and insulin infusion ($P<0.005$). Similarly, Pacy and colleagues (1989) evaluated the influence of insulin on protein synthesis and breakdown in 8 type I diabetic subjects during insulin withdrawal (FPG $= 16.4 \pm 0.5$ mM) and insulin infusion (4.6 ± 1.0 mM). Hyperglycemia was associated with a greater rate of protein breakdown ($P<0.05$). Protein synthesis however was found not to change. The studies suggest that uncontrolled diabetes is associated with an increased rate of protein breakdown (Nair et al., 1983; Umpleby et al., 1986; Pacy et al., 1989), but the the effect on the rate of protein synthesis during periods of hyperglycemia appeared to be that it was not greatly altered, in some studies.

However, an increased rate of protein synthesis has been suggested by Nair and colleagues (1983). The reported increase in protein synthesis during periods of hyperglycemia is proposed to occur at the level of the non-insulin-sensitive tissues, such as the liver (Pain & Garlick, 1974), the gut (Garrow & Hawes, 1972; Nair, 1992), and the splanchnic region (Wahren et al., 1976; Nair et al., 1995). As these tissues can make use of elevated levels of amino acids available from increased breakdown in order to synthesize proteins. Both Umpleby and coworkers (1986) and Pacy and associates (1989) were unable to detect a change in protein synthesis. The difference in the findings of these three investigators may lie within the fact that Nair and coworkers (1983) did not study the effect of improving diabetes control within

the same subject, unlike the groups of Umpleby (1986) and Pacy (1989). The three studies have however demonstrated consistent findings of increased rates of protein breakdown during periods of hyperglycemia, which results in a net negative nitrogen balance. The improvement of negative nitrogen balance observed upon improvement of glycemia with insulin therapy is the result of a decrease in protein breakdown, rather than an increase in protein synthesis.

2.3 Protein Metabolism in Type II Diabetes Mellitus (Relative Insulin Deficiency)

There are a variety of indirect indices which can provide information on *in vivo* whole-body protein metabolism in type II diabetes. We are limited to these *in vivo* indices as it is physiologically, and ethically, difficult to access individual tissue and organ metabolism in humans. Likewise, it is also difficult to separate the integrated "fed & fasted" states of metabolism. Such indices commonly used in human studies are body composition, urinary 3-Methylhistidine (3-MeHis) excretion, nitrogen balance, circulating amino acid concentrations, and whole-body amino acid and nitrogen kinetics (Bier, 1992). However, only the first three will be the focus of this thesis.

2.3.1 Body Composition

It has been shown by investigators that hyperglycemic type II diabetic subjects are not significantly different in body composition when compared to healthy

subjects of comparable body weight. Three groups of investigators have reported such findings of similar body composition in hyperglycemic Type II diabetic and obese individuals (Bogardus et al., 1986; Zawadzki et al., 1988; Welle & Nair, 1990a). The first two groups of investigators (Bogardus et al., 1986; Zawadzki et al., 1988) assessed percentage of body fat (%BF) by underwater weighing with correction for the simultaneously measured residual lung volume by helium dilution (Goldman & Buskirk, 1961), while the last group (Welle & Nair, 1990a) assessed LBM through ^{40}K counting (Forbes et al., 1986). Measuring similar quantities of LBM and fat tissues in both study groups (hyperglycemic diabetic and obese subjects) by different methods of body composition assessment demonstrates that these findings are not biased towards one measurement tool. The similarity of LBM in hyperglycemic diabetic subjects and obese subjects of similar body weight suggests that there have not been any gross alterations in nitrogen balance or body fat.

2.3.2 Urinary 3-Methylhistidine Excretion

Two studies exist in which 3-MeHis was used as an index of protein breakdown in diabetes mellitus (Huszar et al., 1982; Marchesini et al., 1992). One of these studies reports 3-MeHis excretion in type II diabetes during two periods of diabetes control (Marchesini et al., 1992). Marchesini and colleagues (1992) evaluated muscle protein breakdown in eight diabetic subjects (4 type I & 4 type II) while hyperglycemic and euglycemic. Fasting blood glucose was significantly higher during hyperglycemia when compared to improved glycemia of these diabetic subjects

($P<0.01$) and to a control group ($P<0.01$). Twenty-four hour urinary 3-MeHis excretion decreased significantly upon attainment of euglycemia in both type I and II diabetic subjects (386 $\pm$ 53 vs 231 $\pm$ 35; $P<0.01$ and 314 $\pm$ 34 vs 275 $\pm$ 28 umol/d; $P<0.05$). The improvement however was more marked in the type I diabetic subjects, thus indicating that perhaps exogenous insulin is more effective in reducing muscle protein breakdown than endogenous insulin. Both type I and II euglycemic diabetic subjects excreted higher mean levels of 3-MeHis than the control subjects, although this was not significant. These findings are concordant with the studies of Umpleby et al. (1986) and Pacy et al. (1989), in which type I diabetic subjects experienced greater rates of protein breakdown when hyperglycemic than when euglycemic. Similarly, these findings also support the observations of Nair et al. (1983) and Umpleby et al. (1986) who noted that euglycemic type I diabetic subjects are in a state of increased protein breakdown when compared to matched controls.

2.3.3 Nitrogen Balance

Three studies exist to date which report nitrogen balance data in type II diabetes upon the improvement of glycemia (Bistrian et al., 1976; Henry et al., 1986; Gougeon et al., 1994). In the first study by Bistrian and colleagues (1976), 6 type II diabetic subjects consumed a protein-sparing modified fast consisting of 1.2 to 1.4 g protein/kg ideal body weight/day. However, only on three subjects were nitrogen balance studies conducted. Due to the heterogeneous nature of the treatment each subjects received, each subject's study will be discussed briefly. The first subject was

studied over a period of 44 days, in which insulin treatment continued for 18 days until the cessation of glycosuria. Nitrogen balance was most positive for the first 18 days during which insulin treatment and a protein intake of 150 g/d was provided. Nitrogen balance became progressively less positive with the cessation of insulin treatment, followed by a reduction of protein intake to 110 g/d, and finally became negative with a protein level of 55 g/d. Euglycemia was achieved without insulin treatment, at which point the protein intake was 55 g/d. The second subject received a constant protein intake of 65 g/d for 18 days. The subject remained hyperglycemic for the first four days, during which nitrogen balance was negative. OHA therapy was discontinued on the fifth day as euglycemia was achieved, and nitrogen balance remained at equilibrium or mildly positive for the duration of the study. The third subject received a constant protein intake of 99 g/d for 15 days. Neither insulin nor OHA therapy was initiated due to the achievement of euglycemia after the first day of the study. The nitrogen balance was very positive for the first day (+9 g N/d), and then returned to levels of -0.5 to +1 g N/d. Henry and coworkers (1986) studied 10 obese type II diabetic and 5 obese individuals consuming 300 kcal, of which 120 kcal were protein, daily. As euglycemia was achieved, nitrogen balance became less negative, but remained negative for the duration of the study. Cumulative nitrogen losses over the study period were less in the diabetic group than the obese controls (-112.98 $\pm$ 12.25 vs -235.80 $\pm$ 37.60 g N; $P<0.05$). This difference can partly be attributed to the obese controls having a greater BMI and LBM, which was found to correlate strongly with total nitrogen loss ($r=0.83$; $P<0.001$). Gougeon and

colleagues (1994) performed nitrogen balance studies in 7 obese type II diabetic individuals during a weight-maintaining diet and a VLED providing 1700 kJ (400 kcal)/d diet with a constant protein intake of 93 g daily. Subjects were hyperglycemic during the weight-maintaining diet and euglycemic by the end of 4 weeks of the VLED ($P<0.001$). Upon attainment of euglycemia, both protein breakdown and synthesis decreased ($P<0.05$). Nitrogen balance was not significantly different from zero during the weight-maintaining hyperglycemic period. Upon initiation of the VLED, nitrogen balance became negative and remained so throughout the duration of the study. In contrast, a comparable obese control group was able to attain nitrogen equilibrium by the end of 4 weeks of the same VLED (Gougeon et al., 1995).

There are a variety of factors which will influence nitrogen balance, each of which will be discussed. These are (1) the energy content of the study diet, (2) the quantity and quality of protein given during the study diet, (3) the content of the pre-study diet with respect to protein and energy intake, and finally (4) the vitamin and mineral content of the study diet.

(1) Energy Intake of the Study Diet

Energy intake is an important determinant of nitrogen balance, for as energy intake decreases nitrogen balance becomes progressively more negative and as energy intake increases above the subject's requirements, nitrogen balance becomes more positive. This was noted as early as 1907 by both Benedict and Cathcart who

reported that urinary nitrogen excretion is high during the initial phase of a complete fast (Peret & Jacquot, 1972). Likewise, this was observed by Henry and co-workers (1986) and Gougeon and colleagues (1994) whose subjects, although euglycemic, remained in negative nitrogen balance during a period of inadequate energy intake (ie. VLED). Gougeon and co-workers (1994) provided a VLED to obese diabetic subjects with only 420 kJ (100 kcal) more than that of Henry and colleagues, but triple the protein intake (93 g daily). This VLED supplied by Gougeon and colleagues (1994) too was incompatible with nitrogen equilibrium, as the mean cumulative nitrogen loss for 42 days was 137 $\pm$ 12 g. The cumulative nitrogen loss reported by Henry and associates (1986) after 36 days was 113 $\pm$ 13 g, not significantly different. Fisler and colleagues (1992) compared a series of obese groups, and noted that nitrogen balance was significantly more negative in a group of obese subjects undergoing a total fast, than compared to obese subjects consuming a VLED of varying composition ($P < 0.0005$). It has been demonstrated that nitrogen equilibrium could be maintained during periods of sufficient energy intake to maintain weight (Bistrian et al., 1981; Winterer et al., 1980; Gougeon-Reyburn et al., 1989; Gougeon et al., 1992; Gougeon et al., 1994). Nitrogen balance could not be achieved with the severe energy restriction of VLED's of differing macronutrient composition (Bistrian et al., 1981; Winterer et al., 1980; Gougeon-Reyburn et al., 1989). Hoffer and colleagues (1983) demonstrated in an obese group of subjects, that whereas nitrogen balance could not be achieved and protein kinetics decreased with a VLED supplying 44 g protein and 1850 kJ (440 kcal)/d. The addition of 1670

kJ (400 kcal)/d primarily as glucose was sufficient to return these lowered rates of protein turnover to values not different from baseline, although the rates of protein breakdown remained greater than those of synthesis, and nitrogen balance remained negative. These data show that the level of energy restriction is an important determinant of protein metabolism.

(2) Protein Quantity & Quality of the Study Diet

It had been postulated and observed by Benjamin as early as 1914 that the greater the protein intake, the greater the apparent retention of nitrogen, and these findings have been repeated by several investigators (Wallace, 1959). Hoffer and colleagues (1984) observed that supplying protein at the recommended intake level of 0.8 g/kg BW/d, during a VLED, was not able to attain nitrogen equilibrium in a group of obese women. Doubling of the protein intake to 1.5 g/kg BW/d was compatible with nitrogen equilibrium, although the diets remained grossly energetically inadequate (Hoffer et al., 1984). Similar nitrogen balance results were reported in obese women consuming protein at a level of 1.5 g/kg IBW/d (Bistrian et al., 1976; Bistrian et al., 1981) and at a level of 96 g protein daily (Gougeon et al., 1995). Restoration of nitrogen balance was not immediate and required 3 weeks to return to values not different from zero (Gougeon et al., 1995). Therefore, in obese subjects, nitrogen equilibrium and protein metabolism can be restored during a VLED after a period of adaptation. In situations of total fasting nitrogen balance remains negative although nitrogenous losses decrease with time (Munro & Crim,

1988). The balance remains negative due to the absolute zero protein intake.

Although protein quantity has a well established effect on nitrogen balance, the role of protein quality, as assessed by essentiality of amino acids, remains controversial. The effects of two VLED varying only in their respective amino acid contents have been studied (Fisler et al., 1982; Gougeon et al., 1995). Fisler et al. (1982) studied nitrogen economy over a 2 month period and observed that the ability to attain nitrogen equilibrium was independent of the quality of protein received. Gougeon (1992) also demonstrated in an obese group consuming a similar VLED that once a steady-state of protein metabolism had been achieved via a high quality protein diet, substitution with a lower quality protein did not adversely affect nitrogen balance. The results of this study also suggested that perhaps the essential amino acid content of the high quality protein diet exceeded requirements or perhaps the VLED modified the rates of protein synthesis and breakdown, hence altering requirements. Gougeon and associates (1995) studied the metabolic response of two all-protein VLED's, differing only in their protein quality. It was observed that subjects remained in negative nitrogen balance during the first three weeks of the diets. Nitrogen equilibrium was achieved in the lower quality protein diet by day 21 and became negative again at day 36. The higher quality protein group achieved nitrogen equilibrium by day 27 and remained so for the remainder of the study. Cumulative nitrogen losses were not significantly different between the groups. It was concluded by Gougeon and colleagues (1995) that the higher quality protein did not offer a substantial advantage compared to a lower quality protein over a 6 week

period, with respect to protein metabolism. With prolonged use however, a VLED containing a higher quality protein may offer the advantage of maintaining nitrogen equilibrium by reducing any further increments in protein breakdown.

(3) Energy & Protein Intake Prior to the Study Period

Depletion of body protein, due to either inadequate protein or energy intake, renders the subject more efficient at retaining protein (Wallace, 1959; Bistrian et al., 1975). Human studies have reported increased protein retention in situations of chronic protein-energy malnutrition (Wallace, 1959). Equivalent protein intakes yielded different nitrogen balance responses, as previously fasting individuals retained nitrogen whereas nitrogen equilibrium was observed in subjects without prior depletion (Marliss et al., 1978). It has also been reported that the prior diet influences nitrogen excretion during the first days of total fasting (Gougeon-Reyburn et al., 1989), such that a lower nitrogen excretion is measured following a protein-free diet versus a greater nitrogen excretion following a protein containing diet (Gougeon-Reyburn et al., 1989). This decreased nitrogen excretion was suggested to be the result of a depleted labile nitrogen pool during a protein-free diet, and thereby demonstrating a decreased loss of protein with the total fast. The protein containing diet did not deplete this labile N pool, unlike the total fast, and nitrogen excretion was greater at this point (Gougeon-Reyburn et al., 1989). These two points stress the importance of obtaining information regarding dietary intake in the period prior to the study in order to truly assess whether the nitrogen balance is a response to the

study diet, or merely a repletion period for the subject.

(4) Micronutrient Intakes During the Study Period

The important roles that micronutrients play on glucose and protein metabolism has been established (Flodin, 1979; Marliss, 1979). However, there has been virtually no studies of micronutrient requirements during energy restriction, in which obese and/or diabetic subjects are in a state of abnormal protein metabolism. Zinc deficiency has been implicated in protein metabolism, as Wolman and colleagues (1978) demonstrated that patients with significant zinc losses induced lower insulin concentrations and responded with poorer nitrogen balances than patients with sufficient zinc to the identical energy and protein intakes. With the addition of zinc, insulin concentrations and nitrogen balance returned to normal values. Glucose tolerance factor was shown to be a chromium containing compound by Schwarz & Mertz in 1959 (Flodin, 1979), and chromium supplementation has demonstrated improved glucose tolerance in humans. Jeejeebhoy and coworkers (1977) furnished such evidence as chromium deficiency caused a diabetic syndrome in a patient receiving total parenteral nutrition characterized by glucose intolerance and neuropathy, however the patient produced normal levels of insulin. Upon addition of chromium to daily parenteral infusate for a period of two weeks, glucose tolerance and nerve conduction returned to normal, thus indicating that correcting chromium deficiency results in normalizing glucose tolerance hence enabling glucose to be used as an energy source as well as correcting nerve conduction.

2.3.4 *Whole-Body Protein Turnover*

Gougeon and colleagues (1994) assessed whole-body protein turnover using a tracer of [^{15}N]-glycine, which was administered orally in equal doses, every 3 hours, over a 60hr period. Quantification of the rates of protein breakdown and synthesis, from the determination of nitrogen flux, is performed using the Picou Taylor-Roberts (1969) equation. It was found that hyperglycemia was associated with greater rates of protein breakdown and synthesis than during periods of euglycemia ($P<0.05$). It was also found that rates of protein turnover, synthesis, and breakdown remained significantly elevated when compared to a matched obese control group ($P<0.05$) (Gougeon et al., 1995). The reported increase in protein synthesis seen during periods of hyperglycemia is proposed to occur at the level of the non-insulin-sensitive tissues, such as the liver (Pain & Garlick, 1974), the gut (Garrow & Hawes, 1972; Nair, 1992), and the splanchnic region (Wahren et al., 1976; Nair et al., 1995). As these tissues can make use of elevated levels of amino acids that result from increased protein breakdown in order to synthesize proteins. This group of investigators have demonstrated that both type I and II diabetes are associated with greater protein breakdown and synthesis during periods of hyperglycemia which could be decreased, but not normalized, upon the improvement of glycemia or attainment of euglycemia. These rates of protein synthesis and breakdown remained elevated when compared to a matched control group, which may also partly explain the ability to remain in better nitrogen balance. Leucine kinetics have been studied in type II diabetic subjects and it was found by Staten and associates (1986) that although

glycemia was improved through intensive insulin treatment, protein breakdown and synthesis were unaffected and not significantly different from the obese controls. Welle and Nair (1990a) found unchanged rates of protein synthesis and breakdown after both 2 weeks of glyburide treatment and 2 weeks of insulin therapy. These investigators concluded that the improvement of glycemia does not alter protein breakdown and synthesis rates. However, both groups of investigators have studied leucine kinetics in the postaborptive, fasted state. This limits the ability to draw conclusive results, for endogenous insulin secretion varies with the integrated "fed-fasted" states (Anderson, 1988). Therefore, as Gougeon et al. (1994) studied the "fed-fasted" cycle, this is most probably the true representation of daily whole-body protein turnover in hyperglycemic and euglycemic type II diabetic subjects. What these studies (Staten et al., 1986; Welle & Nair, 1990a; Gougeon et al., 1994) do suggest is that even limited amounts of circulating insulin in type II diabetes mellitus may be sufficient to prevent the gross muscle wasting and emaciation observed in uncontrolled type I diabetes.

2.4 Resting Metabolic Rate in Diabetes Mellitus

Resting metabolic rate (RMR) is defined as the amount of energy expended by an individual at rest, in a thermoneutral environment, without the effects of meal consumption and physical activity (Danforth, 1985). Increased resting metabolic rates may arise, according to Bogardus et al. (1986), from one or several mechanisms: (1) increased protein turnover, (2) increased sympathetic nervous system activity, (3) increased substrate cycling,or (4) abnormal mitochondrial oxidative-phosphorylation.

Resting metabolic rate was found to be significantly higher in uncontrolled type I diabetes than from predicted values based upon age, sex, and body surface area data ($P<0.01$) (Nair et al., 1984). Upon insulin infusion to restore euglycemia, RMR returned back to predicted values ($P<0.01$) (Nair et al., 1984). Similar responses have been reported in type II diabetes. RMR was found to be increased in hyperglycemic type II diabetic subjects, and upon attainment of euglycemia, RMR decreased (Bogardus et al., 1986; Gougeon, 1993). It has also been reported that RMR is increased in type II diabetic subjects when compared to matched obese subjects (Bogardus et al., 1986; Nair et al., 1986).

The increase in RMR is proposed to be associated with increased protein turnover, as protein synthesis and breakdown are energetically costly (Nair et al., 1984). The energy requirements, based on theoretical ATP requirements for protein synthesis in the human, have been estimated to account for 10 to 25% of RMR (Waterlow, 1984; Reeds et al., 1985; MacRae et al., 1986). Therefore as glycemia is improved, protein breakdown and synthesis will normalize, and subsequently RMR will decrease. Therefore, increased protein turnover may partly explain the increase in RMR (Welle & Nair, 1990b). Increased rates of hepatic substrate cycling have been reported in type II diabetes (Efendic et al., 1982; Sheppard et al., 1983; Zawadzki et al., 1988). Elevated levels of FFA may also indirectly increase RMR by uncoupling mitochondrial oxidative phosphorylation (Himms-Hagen, 1976), and may increase gluconeogenesis (lactate-glucose cycle) (Bogardus et al., 1984).

The RMR lowering effects of severe energy restriction have been reported

to be up to 20% and to contribute to making continued weight loss difficult over the long term period as the energy deficit becomes smaller (Bray, 1969; Apfelbaum et al., 1971; Garrow et al., 1978; Doré et al., 1982; Welle et al., 1984; Hendler & Bonde, 1988; Gougeon et al., 1994; Gougeon et al., 1995). The effects of weight reduction diets providing 4200 to 4700 kJ/d (1000 to 1100 kcal/d) on RMR are documented to have some effect (Ravussin et al., 1985; Froidevaux et al., 1993), but not as dramatic a one as that produced by VLED's. A moderate energy restriction has been documented to decrease RMR by 670 kJ (160 kcal)/d over a 10 to 16 week period of continued weight loss (Ravussin et al., 1985). This decrease measured by Ravussin and co-workers (1985) represented a 9% decrease in RMR, which was not significant when expressed on a kg LBM basis. Froidevaux and colleagues (1993) found that every kilogram of body weight lost resulted in a decrease of 24hr energy expenditure by 107 kJ/d (26 kcal/d). However, the effects of the typical "1200 kcal/d" weight reduction diet on RMR in a tightly controlled in-patient setting have not been investigated. Likewise, the effects on prolonged dieting on RMR, once the weight goal has been achieved and subjects are now consuming a weight maintaining diet, remain unknown. Foster and colleagues (1990) studied the effects of realimentation with a balanced deficit diet of 5000 kJ (1200 kcal)/d diet after 2 months of a VLED, and found that the balanced deficit diet aided in the recovery of RMR and returned RMR to values no longer significantly different from those prior to the VLED. Thus providing evidence of a metabolic advantage of weight loss from a less severe energy restriction.

2.5 *Treatment of Type II Diabetes Mellitus by Weight Reduction*

The pathogenesis of the chronic manifestations of diabetes remains controversial as metabolic, genetic, and other factors affect diabetic complications such as retinopathy, nephropathy, and neuropathy. During sustained periods of hyperglycemia, insulin-sensitive tissues such as muscle and adipose tissue are required to use sources of energy other than the unavailable glucose. Muscle glycogen is quickly depleted and muscle protein is catabolized to support gluconeogenesis. Tissues which are not insulin-sensitive are then subject to the bombardment of glucose into the cells. This excess glucose produces advanced glycation end products, which are thought to accelerate the onset of diabetes complications (Brownlee, 1994; Diabetes Control and Complications Trial Research Group, 1993). Pirart (1978) followed some 4,400 type I diabetic subjects over a period of 25 years. Although the majority of the subjects presented themselves with some form of diabetic complication, those who had maintained good average glycemic control over the years had fewer complications when compared to those who had only achieved and maintained poor control. In 30-40% of patients diagnosed with type I diabetes, overt nephropathy develops on average 15 years post-diagnosis of diabetes mellitus and progresses to advanced renal failure, necessitating the exploitation of either dialysis or renal transplant as options (Anderson et al., 1983; Mogensen & Schmitz, 1988; Reddi & Camerini-Davalos, 1990).

In the type II diabetic population 20-40% of these patients develop diabetic nephropathy (Krowleski et al., 1985). Cardiovascular disease is responsible for one-

third of all deaths among type II diabetic individuals (Hagan & Wylie-Rosett, 1989). The co-prevalence of other risk factors such as poor diabetes control, obesity, hypertension, dyslipidemia, and increased abdominal circumferences all place the type II diabetic patient at greater risk of developing complications (Kannel & McGee, 1979; Wingard et al., 1983; Balkau et al., 1993). As type II diabetes accounts for greater than 90% of all cases of diabetes (National Institutes of Health, 1987), the number of type II diabetic patients with nephropathy is much greater than in type I diabetes mellitus. The prevalence of cardiovascular disease is greater in type II diabetes than in type I, partly due to the greater incidence of this type of diabetes, but also in part due to the older age of the type II diabetic individual (Balkau et al., 1993). The most prominent result of poor diabetes control on blood lipid levels is increased serum triglycerides (TG) (Hagan & Wylie-Rosett, 1989). Normalization of plasma glucose lowers TG (Kissebah & Schectman, 1988). Weight loss concomitantly improves glycemic control, and thus weight loss also improves TG levels (Ginsberg & Grundy, 1982; Liu et al., 1985).

Very-low energy diets providing less than 3500 kJ (800 kcal)/d are widely used in weight reduction therapy. The safety of prolonged use of these diets remains under much concern, as the diets tend to be of low-nutritive value and extremely unbalanced (Sours et al., 1981; Wadden et al., 1983). Among the most common side effects of these VLED's are myocardial protein degradation, cardiac arrhythmias, electrolyte disturbances (sodium, potassium, calcium, and magnesium), and hypotension (Marliss, 1978; Wadden et al., 1983). VLED's however produce

impressive weight losses and improvement in metabolic parameters and glycemic control (Henry et al., 1991; Anderson et al., 1991; Wing et al., 1991; Anderson et al., 1992). It has been demonstrated that obesity diminishes the insulin sensitivity of both peripheral and hepatic tissues in type II diabetes mellitus (Campbell & Carlson, 1993). Therefore as obesity intensifies the insulin resistance, this indicates that energy restriction is an effective mode of treatment for type II diabetes (Campbell & Carlson, 1993).

The prevention of diabetes complications and the treatment of type II diabetes is therefore straight forward: (1) achieving and maintaining diabetes control, as indicated by euglycemia, glycated hemoglobin, and fructosamine and (2) achieving weight loss and maintaining a healthy weight. The safety of prolonged use of severely energy restricted diets to achieve the above has yet to be determined in an obese type II diabetic population. Perhaps a more moderate energy restriction deserves our attention and investigation.

3.0 RATIONALES

(1) In type II diabetes, the degree of diabetes control, as indicated by the level of glycemia, determines the magnitude of the now demonstrated derangement in whole-body "fed & fasted" protein metabolism. The abnormality in protein metabolism can be corrected by oral hypoglycemic agent therapy which improves glycemia during a weight maintaining (iso-energetic) and a low energetic diet, both which provide a generous protein intake of 1.5 g protein/kg BMI_{25}/d. In order to test for such an effect, obese type II diabetic subjects will be removed from diabetes medication to simulate poor diabetes control and provided with a weight-maintaining formula diet. Improved diabetes control will be achieved by the introduction of an oral hypoglycemic agent, sulfonylurea, while the iso-energetic diet continues for approximately 1 more week, to be followed by 4 weeks of a moderately energy restricted diet. Obese subjects will be chosen to match the diabetic subjects for variables such as age, body weight, and body composition. The obese subjects will serve as controls and will follow the identical protocol, minus the oral hypoglycemic agent. Protein metabolism will be assessed at the end of each of these 3 periods (2 for the obese subjects) through daily nitrogen balance, urinary 3-methylhistidine excretion, and changes in lean body mass determined by bioelectrical impedance analysis and cumulative nitrogen balance.

(2) Furthermore, the increased rate of protein turnover in hyperglycemic obese type II diabetic subjects is associated with an increased resting metabolic rate. This increased resting metabolic rate will be corrected (ie. normalized to the values

measured in the obese control group) as glycemia is improved by the oral hypoglycemic agent. The improvement of diabetes control will be assessed by capillary blood glucose measured a minimum of four times daily, decreased urinary glucose losses, and decreased fasting plasma glucose (drawn venously) at the end of each week of treatment. Resting metabolic rate will be measured at the end of each phase of glycemia during the iso-energetic diet.

(3) Finally, the resting metabolic rate would decrease as a result of the moderate energy restriction during the low energy diet, but not to the extent observed with very-low-energy diets. Resting metabolic rate will be measured weekly for the 28 day duration of the low energetic diet in order to determine to what degree resting metabolic rate will decrease. Determination of lean body mass as detailed in (1) will assist in attempting to conclude whether this decrease in resting metabolic rate is due to a decrease in lean body mass.

3.1 HYPOTHESES

The overall hypothesis to be tested is that in type II diabetes mellitus, the degree of diabetes control determines the magnitude of the derangement of whole-body protein metabolism.

Specific Hypotheses

3.1.1 Oral hypoglycemic agent therapy sufficient to improve hyperglycemia will improve the altered protein metabolism.

3.1.2 Moderate energy restriction combined with oral hypoglycemic agent therapy to achieve euglycemia will correct the altered protein metabolism.

3.1.3 The resting metabolic rate will be restored toward normal from elevated levels in hyperglycemic diabetic subjects related to the magnitude of improvement of glucose and protein metabolism.

3.1.4 With moderate energy restriction, the decrease in resting metabolic rate will be lesser than with severe energy restriction.

SUB-OBJECTIVE

(1) The study provided us with the unique opportunity to compare changes in body composition (LBM & TBW) as assessed by BIA and cumulative nitrogen and sodium balances, respectively.

4.0 EXPERIMENTAL DESIGN

4.1 Experimental Design

The experimental design is summarized in Figure 1. Obese type II diabetic subjects and age and weight matched obese controls were studied during two diet phases: iso-energetic (ISO), which maintained weight (ISO = Harris-Benedict x 1.5) and low-energetic (LED), which allowed for weight loss (LED = 50% of ISO). Furthermore, the diabetic subjects were studied during 2 weeks of iso-energetic diet. Prior to admission (ie. 2-3 days) and throughout the first week of the ISO diet all diabetes medication was removed to simulate poor diabetes control (FPG 10-20 mM). Oral hypoglycemic agent (OHA, Gliclazide $\pm$ Metformin) therapy was introduced during the second week of iso-energetic feeding in order to improve glycemia (FPG $<$ 10 mM) and reduce urinary glucose losses. The LED was then initiated for a period of four weeks, during which OHA treatment continued. OHA dosage was adjusted to achieve and maintain euglycemia, according to daily capillary blood glucose measurements. The obese control subjects followed the identical protocol minus the drug and received only one week of the ISO diet.

4.2 Rationale for Including Obese Subjects as Controls

Increased rates of protein turnover (Nair et al., 1983; Bruce et al., 1990) and resting metabolic rate (Jung et al., 1979; James et al., 1978; Ravussin et al., 1982) have been measured in obese individuals compared to lean individuals of similar age and gender. Type II diabetic individuals tend to be obese. Therefore to truly assess

the effects of diabetes control on protein metabolism and resting metabolic rate, subjects of similar body weight, degree of obesity, and age were recruited to act as control subjects.

4.3 Rationale for Lengths of Study Periods

The time required to reach a steady-state nitrogen balance is of utmost importance if meaningful and correct conclusions are to be drawn from nitrogen balance data. Under iso-energetic conditions, this steady-state is quickly reached in several days to one week (Marliss, 1979). This period is prolonged from 14 days to 4 weeks as energy is restricted to 1.72 MJ (400 kcal)/d (Marliss et al., 1978; Gougeon, 1992; Gougeon et al., 1994). Consideration must also be given to study diets wherein protein content of the study diets changes, for there is a lag time required for the reestablishment of nitrogen balance representative of the protein intake (Munro, 1964).

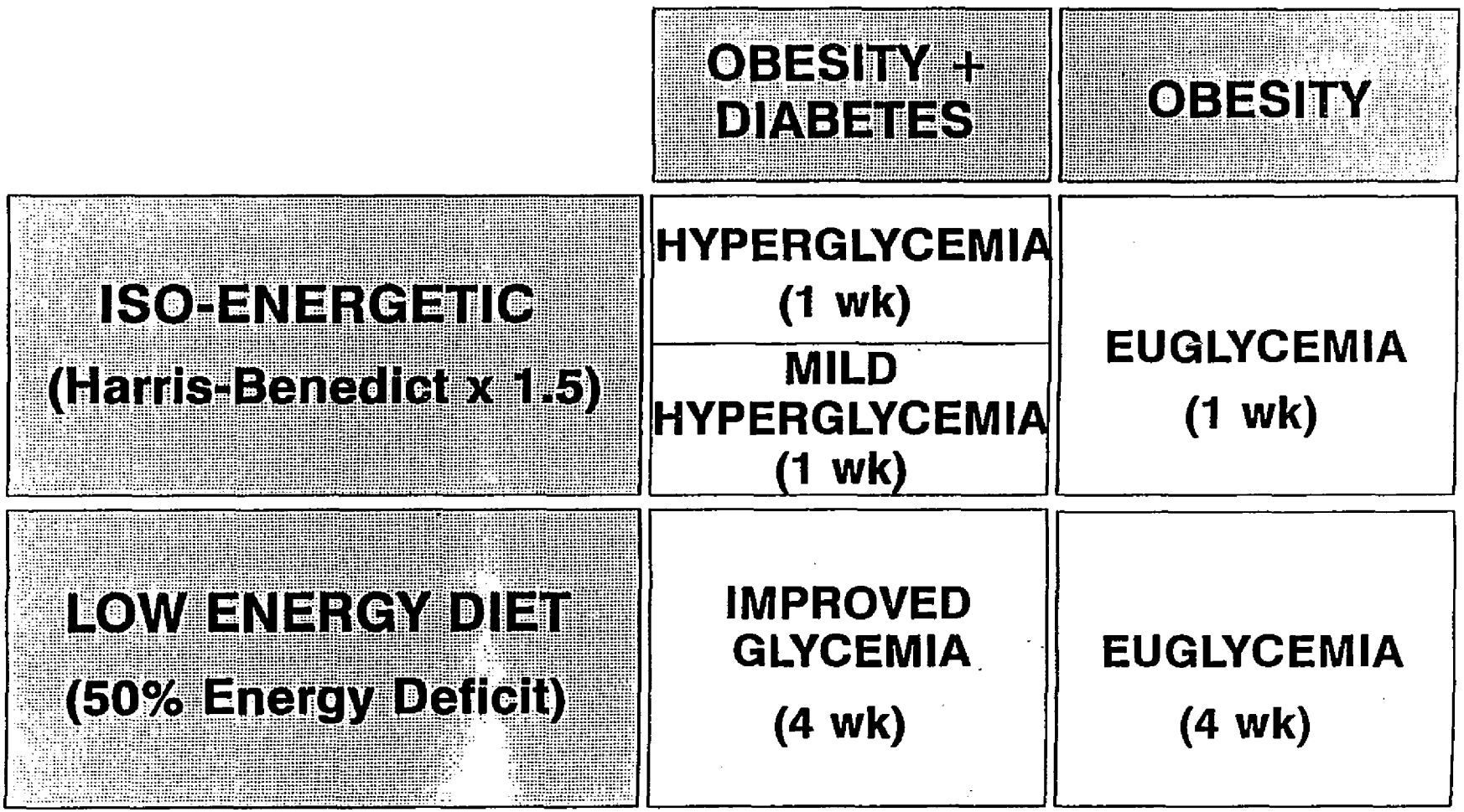


FIGURE 1 : EXPERIMENTAL DESIGN OF STUDY

5.0 RESEARCH METHODS

5.1 Subject Recruitment

The subjects were recruited through two primary methods: (1) newspaper advertisements and (2) physician referral (Appendices I - III). Subjects of either sex were carefully interviewed by the Clinical Investigation Unit (CIU) staff of the Royal Victoria Hospital (RVH) (Montréal, Québec, Canada).

5.1.1 Inclusion Criteria

Potential subjects were required to be 20 to 45 kg overweight, with a body mass index (BMI) not less than 30 kg/m^2, 18 to 60 years of age, in good general health (with the exception of obesity and diabetes), non-smoker, and have a 24hr dietary recall which did not reflect abnormally low protein or energy intakes in comparison to the intakes of the iso-energetic diet (ie. sufficient energy to maintain weight prior to admission & protein intakes that represented greater than 80% of protein provided during the iso-energetic diet). Twenty-four hour dietary recalls were performed according to standard interview procedures (Gibson, 1990), in order to ensure that study diets were not drastically different from the typical daily diets of the subjects. Dietary information was analyzed by a computer software program (The Food Processor, Nutrition and Fitness Software Version 5.01, ESHA Research, Salem, OR, U.S.A.). The nature of the study was explained to the subjects. Subjects then underwent a complete medical history and physical examination (by Dr. J. Morais or E.B. Marliss) in order to ensure that all forms of medication could be

halted during the study. At this time, chest x-rays, electrocardiograms, and urinalysis were performed and blood samples were drawn in order to assess blood chemistry (FPG, electrolytes, urea nitrogen, creatinine, serum lipids, complete blood counts) and in order to ensure for the absence of hepatic, renal, cardiovascular, pulmonary, or thyroid dysfunctions. Glycated hemoglobin and fructosamine were also measured in the diabetic subjects in order to determine the degree of previous diabetes control.

5.2 Clinical Investigation Unit - Research Facility

Upon satisfactorily meeting the inclusion criteria, the subjects were admitted to the CIU for the duration of the study. Subjects were free to withdraw from the study at any point. Subjects then signed 2 copies of a consent form as approved by the Royal Victoria Hospital's Department of Medice Ethics Committee, which also approved the study protocol, (Appendix IV) in the presence of a witness. One copy was placed in their personalized medical file which remained in the CIU, and the other was sent to medical archives. Each subject had access to a telephone and a television, as well as a refrigerator in which to store daily urine collections, and a stationary exercise bicycle. A kitchenette was available on the unit which provided the subjects with a microwave oven, freezer, and refrigerator in which the daily diets, previously prepared by the dietitian, were kept. Subjects were carefully instructed and supervised to take their meals at the specified times (8 & 11 AM, and 2, 5, 8, & 11 PM), perform 24hr urine collections (8 AM to 8 AM), and record daily body weight. Instructions regarding measurements and protocols were given, and questions

and concerns were answered and addressed throughout the entire study period. Capillary blood glucose (Accu-Chek III & Chemstrip bG systems, Boehringer Mannheim Canada, Laval, Québec) measurements were demonstrated and diabetic subjects were instructed to record these values four times daily (8 AM and 2, 5, & 11 PM). Subjects were asked to record all observations (mood, days of menstruation, water intake, bowel movements, etc...) (Appendix V).

5.3 Experimental Diets

The experimental diets are summarized in Figure 2. Upon admission to the CIU, the subjects were given a liquid formula diet calculated to provide maintenance energy requirements. Maintenance energy requirements were determined as calculation of basal energy expenditure (BEE) by the Harris-Benedict formula (Harris & Benedict, 1919), multiplied by a factor of 1.5 (Mahalko & Johnson, 1980). This intake was calculated in order to ensure weight maintenance during baseline because past experience with this calculation resulted in weight maintenance of 43 obese subjects (Gougeon-Reyburn et al., 1989; Gougeon, 1992; Gougeon et al., 1992; Gougeon et al., 1994; Gougeon et al., 1995). Although other equations to calculate BEE exist, these equations too would only yield basal requirements, and still necessitate the use of a factor to raise the energy level to maintenance requirements. The Harris-Benedict equation was therefore, in our case, the superior equation as we had previous success with this equation. This iso-energetic (ISO) period was then followed by a LED, that provided 50% of the energy required to maintain weight.

Protein levels remained constant during the entire study period, at 1.5 g/kg BMI_{25}/d. The subject's BMI_{25} was determined by measuring the subject's height and calculating the body weight which would yield a BMI of 25 kg/m^2. This allowed for determination of the protein level of the study diet, with fat and carbohydrate making up the balance in a 1:2 energetic ratio. The ISO diet was prepared using a commercial meal replacement product (Ensure, Ross Laboratories, Montréal, Québec), glucose polymer (Polycose, Ross Laboratories, Montréal, Québec), and soya oil (Table 1). Urinary glucose losses were measured daily. The energy content of these losses was replaced the following day in a 1:2 soya oil:glucose polymer ratio preparation.

The LED study diet was composed of a commercial meal replacement product (Boost, Mead Johnson Canada, Belleville, Ontario, Canada), and All Bran Cereal (Kellogg Canada Inc., Etobicoke, Ontario) and milk were consumed daily during the LED to facilitate elimination (Table 2). Additional protein was provided by all-protein formulas presented as soups and puddings (Bariatrix, Montréal, Québec), and balance of energy was supplied as during the ISO diet. A multivitamin and mineral supplement (Centrum Forte, Cyanamid Canada Inc., Montréal, Québec, Canada) was provided such that in all subjects the recommended nutrient intake (RNI) was met (Table 3).

5.4 Oral Hypoglycemic Agent

The primary oral hypoglycemic agent provided was a second generation

sulfonylurea, gliclazide (Diamicron, Servier Canada Inc., Laval, Québec). Some subjects required a second OHA, a biguanide, metformin (Glucophage, Nordic Laboratories, Laval, Québec) was provided as per the following indications. The decision to begin treatment with dual OHA therapy was based on two considerations: (1) the type of diabetes treatment the subject received prior to entrance into the study and (2) the degree of hyperglycemia during the ISO week. If subjects prior to admission were already receiving two forms of OHA treatment, then maximum doses of both gliclazide (320 mg daily) and metformin (2000 mg daily) were administered at the beginning of the ISO+OHA week. Likewise, if subjects required insulin during the ISO to maintain capillary blood glucose (CBG) in the range of 15 - 20 mM, then maximum OHA treatment was begun. As gliclazide was the primary OHA under investigation, metformin was the first OHA to be tapered. Metformin was tapered according to CBG values, which were monitored a minimum of four times daily. As subjects began to approach euglycemia (ie. CBG of approximately 6 mM), one tablet of metformin was removed daily. When subjects reached the point where they were receiving 1 tablet twice daily and CBG approached 5 mM, dosage was decreased to half a tablet twice daily. At CBG of 5 mM, metformin was discontinued. Gliclazide to this point remained at maximum doses of 320 mg daily. Sliding scale of gliclazide administration was begun when subjects were on single OHA treatment, as detailed in Table 4. Usual time of medication was 8AM and 5PM. Therefore the number of tablets (80 mg gliclazide/tablet) and times per day were dictated by capillary blood glucose levels.

Table 4 : Sliding Scale Gliclazide Administration

Capillary Blood Glucose (mM)	# of Tablets (80 mg gliclazide/tablet)
< 3.0	0
3.1 - 4.0	½
4.1 - 5.0	1
> 5.1	2

5.5 Data Collection & Experimental Protocol

The experimental protocol is schematically summarized in Figure 3. It was essential that 24hr urine collections be complete and accurate so as to capture excretion of all nitrogen, glucose, and electrolytes. Incomplete 24hr urine collections would lead to errors in daily and cumulative balances as excretion of nitrogen and electrolytes would have been underestimated. Underestimation of urinary glucose losses would have led to insufficient replacement of energy the following day and weight could not have been maintained. Completeness of urine collections was verified by daily urinary creatinine values, as creatinine does not differ more than 10% from day to day despite alterations in dietary intake (Vestergaard & Leverett, 1958; Bleiler & Schedl, 1962; Forbes & Bruining, 1976; Webster & Garrow, 1985; Waterlow, 1986). Urinary creatinine was measured using a modified Jaffe method (Heinegard & Tiderstrom, 1993) (Beckman CX4 & CX5, Brea, CA, U.S.A.), and urea by an automated Beckman (Beckman, Brea, CA), both at the RVH biochemistry laboratories. Ammonia was measured by a specific ion electrode (Orion

95-12, Cambridge, MA, U.S.A.). Once urine collections were completed, aliquots were stored at -20°C until assayed for nitrogenous components, 3-MeHis, and ketone body concentration at the McGill Nutrition and Food Science Centre (McGill University, Montréal, Québec).

5.5.1 Nitrogen Balance

Nitrogen balance summarizes the overall scheme of whole body protein metabolism. The balance is the difference between nitrogen intake (I) and nitrogen output, namely the sum of urinary (U), fecal (F), and dermal (S) losses :

$$\text{Nitrogen Balance} = I - (U + F + S)$$

If the intake exceeds the output, the individual is said to be in positive nitrogen balance, protein synthesis is greater than breakdown and implies growth. If the output exceeds the intake, the individual is in a state of negative nitrogen balance, in which protein breakdown is greater than synthesis. An individual is in nitrogen equilibrium when the output equals the intake, the rate of protein synthesis equals that of breakdown.

Nitrogen balance was calculated by subtracting the daily nitrogen losses from the daily nitrogen intake. The determination of nitrogen balance therefore requires very critical estimation of nitrogen intakes and losses. Nitrogen intakes were calculated based on the dietary protein intake, on the assumption that dietary protein contains 16% nitrogen. Nitrogen losses consisted of urinary, fecal, dermal, and miscellaneous losses. Fecal losses can either be collected, pooled, and measured as

would be urinary nitrogen losses, or a correction factor can be employed. Fecal nitrogen losses were based upon Kjeldahl determinations of previously collected weekly fecal pools of subjects (n=8) following similar ISO diets, in which a factor of 70 mg N/g N intake was established (Appendix VI). This factor was continued to be used during the LED based on two observations: (1) the essential amino acid composition of Ensure and Boost were not significantly different and (2) the number and frequency of bowel movements was not altered during ISO and LED diets. It is tedious and difficult to measure dermal losses, the custom being to either ignore these losses or to use correction factors from previous reported studies in which these losses were meticulously measured. Dermal and miscellaneous losses were set at 5 mg N/kg BW (Calloway et al., 1971).

Limitations of Nitrogen Balance

The majority of information available with respect to whole-body protein metabolism in humans has been derived from the nitrogen balance technique, now some 150 years old (Munro, 1985). This approach has provided important and invaluable data, its limitations and shortcomings have been long appreciated (Bier, 1989).

(1) The Tendency Towards Positive Nitrogen Balance

Nitrogen losses tend to be underestimated as meticulous care needs to be taken to ensure that all sources of nitrogen losses are collected. Incomplete

collections and adherence of urine and fecal losses to collections vessels are major sources of error (Bier, 1989). Unmeasured losses from skin, sweat, and blood (analytical sampling and/or menstrual) are other sources which may or may not be considerable, but are greatly variable and remain difficult to measure. There also remains the question regarding the loss of gaseous nitrogen (Bier, 1989). The nitrogen intake tends to be overestimated as dietary nitrogen is lost due to food adhering to cups, plates, and utensils. Therefore, the underestimation of output and the overestimation of intake lends itself towards a bias of positive nitrogen balance (Wallace, 1959). Subjects in our study were instructed to use drinking straws to ensure complete emptying of cups, cans, etc... To eliminate any potential errors in underestimation of urinary output, collection vessels were weighed while dry, and then when filled with a 24hr urine collection. A sample of urine was then removed and the specific gravity measured according to standard laboratory procedures. This specific gravity, along with the net weight of the 24hr urine collection, was used to determine the volume of urine output, thus reducing the possibility of vessel adherence leading to falsely lower urine volumes.

(2) Propagation of Errors in Cumulative Balance Studies

Nitrogen intake and output are relatively large numbers when compared to nitrogen balance (Bier, 1989). Any small errors in determining intake and output can therefore greatly affect the resulting nitrogen balance. These errors may be small when expressed on a daily basis. These errors are propagated over long periods of

time, and larger errors in determining cumulative nitrogen balance will ensue (Bier, 1989). This possibility was minimized as meticulous care was taken not to underestimate output or overestimate intake, as mentioned previously. Great care was taken in determining whether urine collections were complete. Furthermore, much time and energy was spent in verifying and validating the appropriateness of a newer method to measure total urinary nitrogen, to be discussed shortly.

(3) Inability of Nitrogen Balance to Offer Insight into Protein Dynamics

The nitrogen balance method offers no insight towards the internal mechanisms of protein metabolism (Bier, 1989). An observed negative nitrogen balance may be the result of increased breakdown while synthesis remains constant, or constant breakdown and decreased synthesis, or even increases in both rates of breakdown and synthesis, with breakdown increasing to a greater magnitude than synthesis. External end-point balance techniques cannot distinguish the internal mechanisms, but nitrogen balance does reflect the relative rates of protein synthesis and breakdown (Bier, 1989). Nitrogen balance, used in conjunction with measures such as body composition and urinary 3-MeHis excretion, can begin to assess the picture of whole body protein metabolism.

Kjeldahl Determination

Urinary nitrogen was analyzed by a micro-Kjeldahl method by manual digestion of samples and two controls (urine & urea) with concentrated sulfuric acid

containing SeO_2, using K_2SO_4 and $CuSO_4$ as catalysts, on an automated single-channel autoanalyzer (Technicon Analyzer, Tarrytown, NY, U.S.A.). The resulting ammonia in the digested samples, when combined with phenol, reacts with hypochlorite to form chloramine and then indophenol, using nitroprusside as a catalyst. Indophenol, which is a blue product, is assayed colorimetrically at 598 nm; its production is proportional to the ammonia, and hence nitrogen, content of the digested sample (Munro, 1964).

Chemiluminescence

The Kjeldahl method, developed in 1883, has been deemed the golden standard of nitrogen assessment, as it captured all nitrogenous products of the sample in question (Munro, 1964). The drawback however to the Kjeldahl methodology has been a tedious digestion and titration process involving much manual handling, and many sites of potential errors (Bradstreet, 1965). Chemiluminescence offers a researcher an extremely rapid and sensitive method of measuring total nitrogen in biological samples. However, being a relatively new procedure, it required validation against the Kjeldahl procedure traditionally used at the McGill Nutrition and Food Science Centre (McGill University, Montréal, Québec). Two validation studies were performed, as a means of determining the ability of chemiluminescence to (1) replace nitrogen content determination of biological samples by Kjeldahl methodology, and (2) capture all the nitrogen of the urine sample. All urine samples of this study were analyzed using chemiluminescence as indicated by the two validation studies, the results of which are presented in appendix form (Appendix VII).

Nitrogen was analyzed by the Antek Pyro-Chemiluminescent Nitrogen System (Houston, TX, U.S.A.). The basis of operation of this system involves an oxidative pyrolysis (Antek Pyroreactor 771, Houston, TX) at a temperature in excess of 1000°C, which generates nitric oxide from the chemically bound nitrogen in the sample. This nitric oxide then combines with ozone in a reaction chamber to yield metastable nitrogen dioxide. Metastable nitrogen dioxide decays back to the ground state emitting photons which are sensed by a photomultiplier tube (Nitrogen Detector 720, Houston, TX), sensitive to the longwavelength decay light typical to nitrogen (Ward et al., 1980; Antek Instruments Operation Manual, 1987; Dechert et al., 1990)

Solutions containing 1.0 to 10.0 g N/L were prepared from urea and ammonium sulfate, and a standard curve was made from each type of solution. Samples of known amounts of nitrogen were injected and nitrogen concentration was calculated by linear regression, using both urea and the ammonium sulfate standard curves. Both the urea and ammonium sulfate curves yielded similar results, and urea was chosen as the compound to serve as the standard.

Standards were prepared from analytical grade urea (ICN Biomedicals, Inc., Aurora, OH, U.S.A.) ranging from 1.0 to 10.0 g N/L. It was not necessary for standards to exceed a concentration of 10.0 g N/L, as previous determinations on a sub-sample of these subjects yielded urinary nitrogen concentrations in the order of 5 to 8 g N/L. Standards and urine samples were diluted 1:100 with water and delivered in 5 uL aliquots in duplicate into the pyrolysis chamber by a quartz boat filled with quartz wool in a water-jacketed pyrolysis tube (Antek Syringe Driver 735,

Houston, TX). If the co-efficient of variability between injections was greater than 1%, samples were re-injected. If urine was thought to be concentrated, as indicated by a urine volume < 1.5 L/d, then samples were diluted 1:200. This was done primarily to remain within the working linear range, as well as to minimize high salt concentrations which would promote corrosion of the pyrolysis tube.

As a means of verifying the constancy and reproducibility of this chemiluminescence methodology, four intra-assay controls were run with every assay, a minimum of twice daily. The controls were a urine sample, and solutions of 1.86, 5.0, and 7.5 g urea N/L. Mean co-efficients of variability for these four controls were 3.7, 2.0, 2.0, and 2.3 %, and mean measured concentrations, by chemiluminescence, for the urea solutions were 1.88, 5.07, and 7.52 g N/L respectively.

5.5.2 Urinary 3-Methylhistidine Excretion

3-MeHis was first identified as a component of human urine in the 1950's, although the source of this product was unclear (Searle & Westall, 1951; Tallan et al., 1954). However, it was a decade later that 3-MeHis was measured in the blood and urine of fasting subjects, thus indicating an endogenous origin (Asatoor & Armstrong, 1967). Evidence was provided by Johnson and co-workers (1967) indicating the presence of 3-MeHis as a part of the primary structure of contractile proteins. Therefore, as 3-MeHis could not be reutilized upon breakdown, due to the methylation of histidine that occurs post-translationally, this allowed for 3-MeHis to be used as an index of myofibrillar proteolysis (Ward & Buttery, 1978). It was

determined that greater than 90% of the total body 3-MeHis pool is of muscle protein origin (Ballard & Tomas, 1983). Much controversy has remained regarding the use of 3-MeHis as an indicator of skeletal muscle breakdown as the skin and gastrointestinal tract each contribute to the total body 3-MeHis pool by approximately 3% (Ballard & Tomas, 1983). The rates of turnover of these tissues has been cited as being 2 to 5 times that of skeletal muscle (Rennie et al., 1982), and therefore consideration must be taken in interpreting urinary 3-MeHis excretion. An estimation of the contribution of non-skeletal tissues to urinary 3-MeHis excretion was made by Afting and co-workers (1981) in totally paralyzed patient without histologically identifiable skeletal muscle. Basal urinary 3-MeHis excretion was 28% of that excreted by a group of healthy control subjects (Afting et al., 1981). Afting and colleagues (1981), Ballard and Tomas (1983), and Harris (1981) have suggested that 3-MeHis from skeletal muscle contributes approximately 75% of 3-MeHis excretion in healthy subjects.

Urinary 3-MeHis excretion was measured by reverse phase high pressure liquid chromatography (HPLC) (Wassner et al., 1980). The HPLC apparatus consisted of a M-45 Solvent Delivery System Pump, a Guard Pak uBondapak Pre-Column, a Novapak C-18 Silica Analytical Column, a Fluorescence Detector Model 420 & 420-AC, and a Waters 740 Data Module Integrator all from Waters Ltd (Milford, MA). Fluorescence detector excitation and emission filters were set at 395 and 455 nm respectively. Internal standards (25, 50, 100, and 200 umM) and 24hr urine samples were prepared according to the methodology of Wassner and

colleagues (1980), with a 23% acetonitrile solution as the mobile phase, and fluorescamine as the fluorogenic reagent (ICN Biomedicals, Inc., Aurora, OH).

5.5.3 Body Composition

It is of utmost necessity to derive accurate methods for measuring body composition if the effect of nutritional intervention is to be assessed. The selection of such a method to assess body composition depends not only on the precision and accuracy, but on cost, convenience to both the investigator and the subject, technical expertise required, and the equipment (Lukaski, 1987). LBM is most directly determined by ^{40}K counting. This measurement assumes that the average potassium concentration of LBM is constant (Gibson, 1990). It was determined, by cadaver analysis, that the potassium content of LBM was 68 mmol/kg. A range of factors, based on gender, have been used to determine LBM (Cohn et al., 1980; Womersley et al., 1972; Segal et al., 1987; Nair et al., 1988). The drawback to this procedure has been expensive operating equipment and that obese subjects have lower potassium concentrations, leading to overestimates of fat mass (Gibson, 1990; Forbes, 1990). LBM can be indirectly determined by TBW through isotopic dilution, based on the assumption that LBM contains a constant percentage of water (Sheng & Huggins, 1979). LBM is assumed to contain 73% water on average (Sheng & Huggins, 1979). However much debate remains whether this factor is appropriate for an obese population (Segal et al., 1987; Albu et al., 1989). This method is further limited by its usage of expensive isotope preparations and equipment, and lag time is required

before body composition can be determined due to the processing of biological samples. The most widely used method for determining whole-body density is by underwater weighing (Gibson, 1990). This method however requires much cooperation from the subject, and poses problems when attempting to weigh very obese subjects as additional weights are needed to completely submerge the individual as reported by Gougeon (personal communications). Equipment cost is another drawback to this method. Magnetic resonance imaging offers the investigator the ability to delineate organ size and structure, body fat distribution, total body water, and lean body mass, all without exposure to radiation (Forbes, 1990). The drawback currently is the requirement of extremely expensive equipment and technical expertise. BIA offers an investigator a subject-friendly method of determining body composition (Kushner et al., 1990). Its simplicity, non-invasiveness, and portability all make it a very desirable and clinically useful apparatus (National Institutes of Health, 1994). Its current limitation is that measurements in obese subjects have been performed on weight-stable individuals and further validations are required during periods of energy restriction. Deurenberg and colleagues (1989) compared BIA with underwater weighing for the determination of LBM during weight loss. These investigators found that BIA systematically underestimated the loss of LBM and attributed this discrepancy to the inability of BIA to detect losses of body water associated with glycogen reserves. In contrast, Kushner and associates (1990) found that BIA was a useful clinical tool to assess changes in body composition during energy restriction in situations of normal hydration status. BIA

was found to correlate strongly with determination of TBW and LBM by isotopic dilution (Kushner et al., 1990). Likewise, Gray (1988) concluded that BIA measurements accurately reflected changes in TBW in a group of obese females undergoing a 2 week total fast. Therefore in settings of normal hydration and weight stability BIA has been determined to be an accurate means of assessing body composition. Its accuracy during periods of weight loss remains to be further validated.

Bioelectrical impedance analysis (BIA) is a non-invasive mean of assessing body composition. BIA determines total body water (TBW), from which lean body mass (LBM) and body fat (%BF) are calculated. TBW is measured by introducing a small electrical current into the body and measuring the resistance to the flow of the current. Lean tissue represents a low-resistance pathway, containing large amounts of water and conducting electrolytes, whereas fat tissue is a highly resistant pathway containing low amounts of water and electrolytes.

Subjects were placed in a supine position with upper and lower limbs apart from each other and the trunk of the body. Electrodes were positioned on the dorsal surfaces of the right hand and foot, placement is crucial to the accuracy of the measurement. Two surface electrodes were cut in half longitudinally, the first was placed mid-point on an imaginary longitudinal line from the protruding bone of the wrist, bisecting the ulnar bone, the second electrode was placed just above the second knuckle of any finger, both of these cut edges of the electrodes halves faced the shoulder, a third electrode was placed anteriorly on an imaginary longitudinal line

from the ankle bone, bisecting the malleolus, and the fourth electrode was placed just above the third toe, the cut edges of the last two electrodes faced toward the thigh (Brylowski, 1992). The resistance of the body was measured from the voltage drop between the first and third electrodes. This resistance, along with the subject's height (cm) and weight (kg), was entered into sex-specific equations to determine TBW (Kushner & Schoeller, 1986; Schoeller & Jones, 1987), LBM as it is assumed to contain 73% water (Sheng & Huggins, 1979), and %BF:

$$TBW\ (F) = [(0.382 \times Ht^2/R) + (0.105 \times BW) + 8.315]/1.04$$

$$TBW\ (M) = [(0.396 \times Ht^2/R) + (0.143 \times BW) + 8.399]/1.04$$

$$LBM = TBW/0.73$$

$$\%BF = [(BW - FFM)/BW] \times 100\%$$

5.5.4 Sodium & Potassium Balances

Sodium balance was calculated as this provided information on changes in hydration status. Sodium balance is an index of the contribution of fluid gain or loss to body weight, with 150 mmol of sodium representing one kilogram of water retained or excreted, respectively (DeHaven et al., 1980). Sodium and potassium balances were calculated by subtracting daily losses from daily intakes. Daily intakes were calculated based on each individual's study diets. Daily losses included urinary and miscellaneous (skin, sweat, integumental, etc..) losses. Urinary sodium accounts for greater than 98% of sodium losses and urinary potassium for 85% of losses (Pitt, 1974), and miscellaneous losses were set at 3 mmol sodium and 2 mmol potassium

per day (DeHaven et al., 1980). Urinary sodium and potassium were measured at the RVH clinical laboratories on an automated Beckman system (Beckman, Brea, CA) by ion selective electrode methodology.

5.5.5 Blood Sampling

Venous blood samples were drawn with minimal stasis in the overnight-fasted state at the end of the ISO and ISO+OHA diet and each week of the LED.

Plasma Glucose & Metabolic Responses

Blood samples were added to red-topped vacutainer tubes without anticoagulant and analyzed for glucose, creatinine, urea, uric acid, electrolytes, and lipids using the standard automated techniques in the RVH clinical laboratory (Technicon H-I System, Tarrytown, NY).

Blood 3-Hydroxybutyrate, Plasma Insulin, and Plasma Free Fatty Acids

Blood samples were added to tubes containing either cold 10% perchloric acid or heparin and one tenth of the volume of blood as aprotinin (Trasylol, 10,000 Kallikrein inhibitor U/mL, FBA, Pointe Claire, Québec). The samples were then centrifuged at 4°C, supernatants aliquoted, and stored at -20°C. Perchloric supernatants were assayed for blood 3-hydroxybutyrate (3-OH) with enzymic micro fluorometric methods (Lloyd et al., 1978) using a Technicon Autoanalyzer II (Tarrytown, NY). Heparin and aprotinin-containing plasma was assayed for insulin and free fatty acids (FFA). Insulin was measured by single-antibody charcoal

precipitation radioimmunoassay (RIA) by use of human monocomponent insulin standards (Novo, Biospecific, Emeryville, CA) and labelled human insulin (Linco, St. Louis, MO, U.S.A.) as detailed by Marliss and co-workers (1978) based on the methodology of Herbert and colleagues (1965). Free fatty acids (FFA) were assayed by radio-chemical determination using ^{63}Ni (Dupont, Mississauga, Ontario) based on the methodology developed by Ho (1970). Plasma values were corrected for dilution by aprotinin, based on hematocrit values which were measured concurrently.

5.5.6 Anthropometric Measurements

Body Weight

Subjects weighed and recorded daily body weights themselves, under the supervision of the CIU staff, each morning in their night clothes after voiding, but before breakfast on a Scale-Tronix digital scale (Ingram & Bell-Meditron, Le Groupe Inc, Don Mills, Ont.) with accuracy of 0.1 kg.

Subject Height

Subject height was measured with the subject in a standing position using a standard mounted stadiometer. Subjects stood straight with the head positioned so as to ensure that the Frankfurt plane was horizontal. The feet were placed together, knees straight, and arms were hanging loosely at their sides, with the heels, buttocks, and shoulder blades in contact with the vertical surface of the stadiometer (Gibson, 1990).

Body Circumference

Body circumferences were measured weekly at six sites (chest, right tricep and thigh, hips, umbilical and smallest waist) of the body. Measurements required two investigators so as to ensure the measuring tape was in a horizontal plane with the floor, touching the skin, but not indenting the tissue (Lohman et al., 1988). Measurements were made with a plastic measuring tape while subjects stood up, clothed in only undergarments or hospital gowns. Hip measurements were made while subjects stood erect with arms at their sides and feet together. The measurement was made at the level of the buttocks yielding the largest circumference (Jones et al., 1986). The smallest waist was measured at the narrowest point of the upper torso, while the umbilical waist was measured at the level of the largest protrusion. The largest waist fell at either the level of the umbilicus or within four centimetres above or below this point.

5.5.7 Resting Metabolic Rate

After a training session at admission, RMR was measured at the end of each week during the entire study. RMR was measured by continuous indirect calorimetry with a Deltatrac ventilated hood metabolic monitor (Sensor Medics, Yorba Linda, CA). Upon wakening, subjects were slowly brought to a thermally neutral and quiet examination room, placed in a supine position, and allowed to rest for a minimum of 20 minutes before oxygen consumption (VO_2) and carbon dioxide production (VCO_2) measurements were begun. Subjects breathed under the plastic

canopy for twenty minutes, and the average of the last 15 minutes was used for calculation of the 24hr RMR based on the deWeir equation using the caloric equivalents of VO_2 and VCO_2 for the measured non-protein respiratory quotients and the 24hr urinary nitrogen excretion (N_u) (Deltatrac Metabolic Monitor User's Manual, Yorba Linda, CA), namely:

$$24hr\ RMR = (5.68 \times VO_2) + (1.59 \times VCO_2) - (2.17 \times N_u)$$

These measurements of oxygen consumption and carbon dioxide production also allowed for the determination of respiratory quotient (RQ) and quantities of substrates oxidized (protein (PRO), carbohydrate (CHO), and fat) according to the following formulae:

$$RQ = VCO_2 / VO_2$$

$$PRO = 6.250 \times N_u$$

$$CHO = (5.926 \times VCO_2) - (4.189 \times VO_2) - (2.539 \times N_u)$$

$$FAT = (2.432 \times VO_2) - (2.432 \times VCO_2) - (1.943 \times N_u)$$

5.6 Statistical Analysis

Sample size was calculated according to Hall (1983) based upon standard deviations (SD) of estimated nitrogen balances for each study period for each group. Both Type I and II errors were set at $P<0.05$, the difference in mean nitrogen balance (the improvement of nitrogen balance) that would be considered of interest was 1.5 g N/d. This yielded sample sizes of n = 12-15 and n = 8-12 for the diabetic and obese groups respectively.

Subject data were analyzed by a two-way ANOVA with a repeated measures design to account for individual variability (SAS software, SAS Institute, Cary, NC, U.S.A.). Results of main effects (time, treatment, time x treatment) were analyzed in the above mentioned fashion. Significant differences within each study group and between the study groups were determined using the least squares mean. It was necessary to plan these comparisons as only probabilities associated with pre-planned comparisons, using least squares means, could be used.

Remaining statistical analysis (correlations, paired & unpaired t-tests) was performed using the statistical software package Primer Biostatistics (McGraw Hill Inc., Montréal, Québec, Canada) on a Hewlett-Packard Vectra computer (Sunnyvale, CA). Linear regression analysis was used to calculate correlation coefficients. Resting metabolic rate of the two groups was also analyzed separately in a repeated measures ANOVA design and significant differences within each group were identified by the Student-Newman-Keuls test.

Significance was set at a 0.05 level. Data are presented as mean $\pm$ sem.

TABLE 1 : NUTRIENT COMPOSITION OF THE DIETARY COMPONENTS EMPLOYED DURING THE ISO-ENERGETIC DIET

NUTRIENT COMPOSITION	ENSURE[1] /235 mL	GLUCOSE POLYMER /100 g	SOYA OIL[2] /100 g
ENERGY (kJ)	1045	1590	3700
ENERGY (kcal)	250	380	884
PROTEIN (g)	8.7	-	-
CARBOHYDRATE (g)	34.1	94	-
FAT (g)	8.7	-	100
VITAMIN A (IU)	264	-	-
VITAMIN D (IU)	21.1	-	-
VITAMIN E (IU)	2.4	-	-
VITAMIN K (mg)	0.004	-	-
VITAMIN C (mg)	15.9	-	-
FOLIC ACID (mg)	0.042	-	-
THIAMINE (mg)	0.16	-	-
RIBOFLAVIN (mg)	0.18	-	-
PYRIDOXINE (mg)	0.21	-	-
VITAMIN B_{12} (mg)	0.0006	-	-
NIACIN (mg)	2.1	-	-
BIOTIN (mg)	0.032	-	-
PANTOTHENIC ACID (mg)	1.1	-	-
SODIUM (mg)	76.3	110	-
(mmol)	3.3	4.8	-
POTASSIUM (mg)	120.9	10	-
(mmol)	3.1	0.3	-
CHLORIDE (mg)	118.2	223	-
CALCIUM (mg)	55.1	30	-
PHOSPHORUS (mg)	55.1	5	-
MAGNESIUM (mg)	21.2	-	-
IODINE (mg)	0.0085	-	-
MANGANESE (mg)	0.26	-	-
COPPER (mg)	0.11	-	-
ZINC (mg)	1.19	-	-
IRON (mg)	0.95	-	0.02
MOLYBDENUM (mg)	0.008	-	-
CHROMIUM (mg)	0.0053	-	-
SELENIUM (mg)	0.0038	-	-

1 - Chocolate flavour contains 9.3 g protein & 33.8 g carbohydrate per 235mL

2 - Dubuc, M.C. & LaHaie, L.C. (1987). Nutritive Value of Foods. Ottawa, Canada.

TABLE 2 : NUTRIENT COMPOSITION OF DIETARY COMPONENTS EMPLOYED DURING THE LOW ENERGETIC DIET

NUTRIENT COMPOSITION	BOOST /235 mL	BARIATRIX[1] /pkg	BRAN & MILK[2,3] /serving
ENERGY (kJ)	980	290 - 360	745
ENERGY (kcal)	235	70 - 86	180
PROTEIN (g)	11.75	15.0	10.5
CARBOHYDRATE (g)	35.25	1.2 - 4.1	32.5
FAT (g)	5.4	0.1 -1.0	4.6
VITAMIN A (IU)	1250	-	393
VITAMIN D (IU)	47	-	124
VITAMIN E (IU)	3.5	-	0.10
VITAMIN K (mg)	-	-	-
VITAMIN C (mg)	15.04	-	1.6
FOLIC ACID (mg)	0.070	-	0.04
THIAMINE (mg)	0.4	-	0.7
RIBOFLAVIN (mg)	0.47	-	0.4
PYRIDOXINE (mg)	0.587	-	0.27
VITAMIN B_{12} (mg)	0.0009	-	0.0008
NIACIN (mg)	5.9	-	4.7
BIOTIN (mg)	0.014	-	-
PANTOTHENIC ACID (mg)	1.9	-	1.2
SODIUM (mg)	258	26 - 525	376
(mmol)	11.3	1.1 - 22.9	16.4
POTASSIUM (mg)	491	40 - 172	636
(mmol)	12.5	1.0 - 4.4	16.2
CHLORIDE (mg)	371	-	-
CALCIUM (mg)	263	132 - 270	277
PHOSPHORUS (mg)	261	131 - 176	496
MAGNESIUM (mg)	106	11 - 56	139
IODINE (mg)	0.041	-	-
MANGANESE (mg)	0.67	0.1 - 0.5	-
COPPER (mg)	0.5	0.1	-
ZINC (mg)	3.5	0.2 - 0.9	2.6
IRON (mg)	3.8	0.7 - 1.6	4.1
MOLYBDENUM (mg)	-	-	-
CHROMIUM (mg)	-	-	-
SELENIUM (mg)	-	-	-

1 - Protein supplements vary in mineral content depending on product (Soups available were cream of mushroom, chicken, & asparagus; puddings available were vanilla and chocolate)
2 - Dubuc, M.C. & LaHaie, L.C. (1987). Nutritive Value of Foods. Ottawa, Canada.
3 - One serving equals 30 g All Bran cereal & 200 mL 2% milk

TABLE 3 : VITAMIN & MINERAL INTAKES (% RNI)[1] OF THE LOW ENERGETIC DIETS PROVIDING THE LOWEST & HIGHEST LEVELS OF ENERGY INTAKE

	Low[2]	High[3]
Vitamins		
A	156 %	200 %
D	145 %	159 %
E	177 %	196 %
C	157 %	193 %
Thiamin	235 %	168 %
Riboflavin	181 %	131 %
Niacin	160 %	114 %
B6	173 %	263 %
B12	131 %	177 %
Minerals		
Calcium	183 %	248 %
Phosphorus	198 %	220 %
Iron	220 %	280 %
Magnesium	233 %	281 %
Zinc	151 %	172 %
Sodium (mg)	2229	3211
Potassium (mg)	1113	1855
Copper (mg)	1501	2501
Manganese (mg)	2010	3350

1 - Nutrition Recommendations : The Report of the Scientific Review Committee. Health and Welfare Canada. Ottawa, Ontario, Canada. 1990.

2 - The RNI of the LOW diet was for a 59 year old female.

3 - The RNI of the HIGH diet was for a 47 year old male.

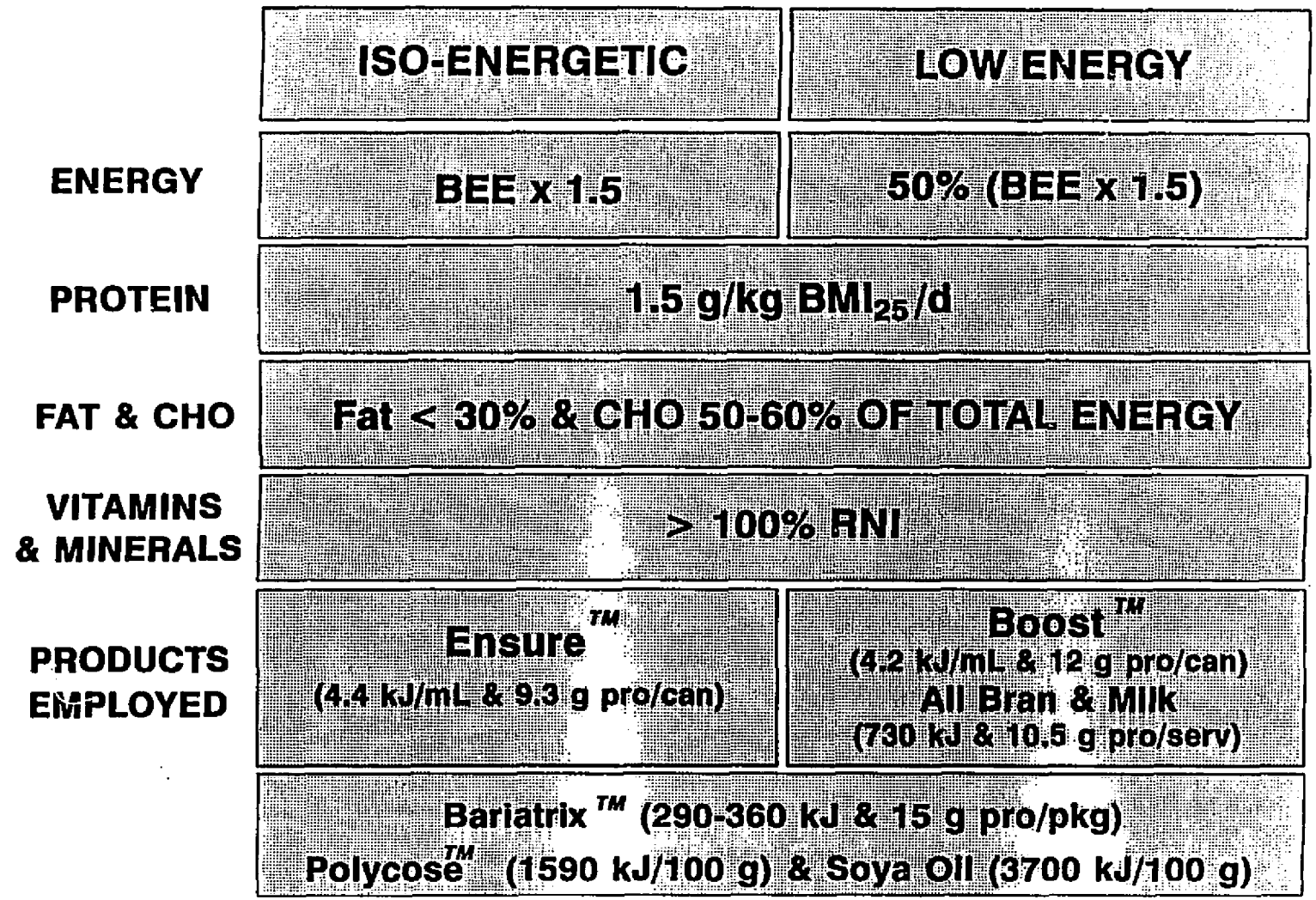


FIGURE 2 : COMPOSITION OF ISO-ENERGETIC AND LOW ENERGETIC STUDY DIETS

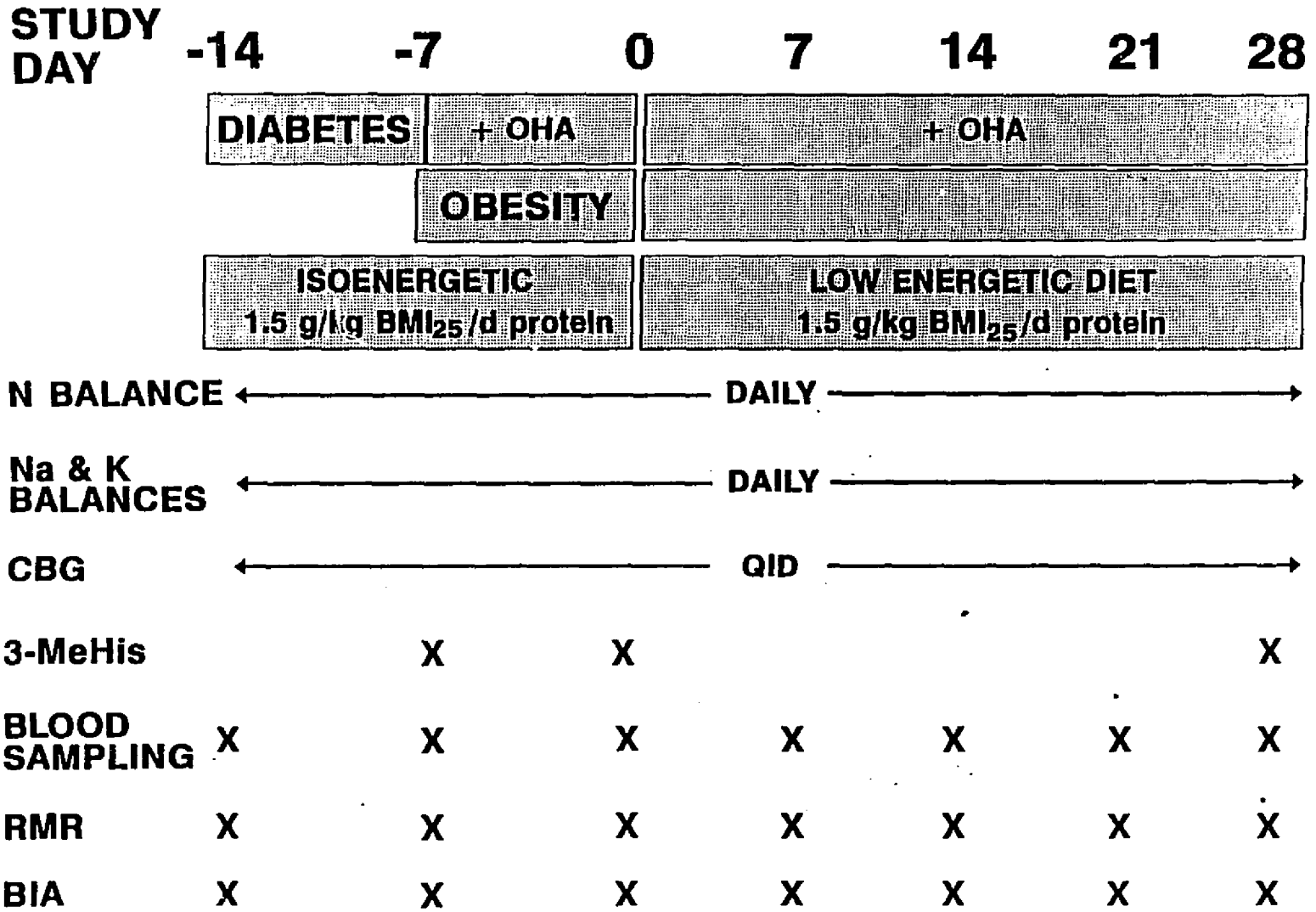


FIGURE 3 : EXPERIMENTAL PROTOCOL OF STUDY

6.0 Results

6.1 Subject Characteristics

A total of 17 subjects were recruited, however only 14 (7 type II diabetic and 7 obese subjects) were included in statistical analysis. An obese male subject elected not to continue the protocol for family reasons. Two subjects, one male diabetic and one female obese subject completed the study, but deviated from the protocol and were not entered into data analysis. The diabetic subject was not included for several reasons. This subject required exogenous insulin, metformin, and gliclazide to normalize blood glucose. Furthermore, the constancy and accuracy of his urine collections were questionable as creatinine excretion varied more than 10% from day-to-day. The most likely causes were incomplete 24hr urine collections or urine collections that spanned greater or less than 24 hours. The cumulative nitrogen balance did not resemble that of the other subjects, in that this subject was in better nitrogen balance during hyperglycemia than when glycemia was improved. Glycated hemoglobin did not improve with the study although weekly fasting plasma glucose demonstrated improved diabetes control. The purpose of this study was to assess protein metabolism during conventional treatment using diet and oral hypoglycemic agents. Therefore insulin administration and inaccurate urine collections prevented us from assessing the state of protein metabolism, using conventional diabetic therapy, in this subject. The obese female subject was not included based on a suspicious nitrogen balance response that would seem to indicate that this subject did not consume all the prescribed food. The expected nitrogen intake value was greater than the true intake. This would therefore theoretically place the subject in positive

nitrogen balance, when in reality she should be in equilibrium or negative nitrogen balance due to the decreased energy intake. During iso-energetic feeding and the first week of the LED, this subject's nitrogen balance followed the pattern of the rest of the control group. At the beginning of the second week nitrogen balance became very positive and remained so for the remainder of the study. The daily nitrogen balances of these two subjects are presented in appendix form (Appendix VIII).

The type II diabetic and obese control subjects were comparable in age, body composition, anthropometric assessment (Table 5), and dietary intake during the study (Table 6). The fasting plasma glucose however was significantly higher in the diabetic group ($P<0.0001$). Mean duration of diagnosed diabetes was 5.7 ± 1.6 yrs, with a range of 3 months to 12 years. Elevated glycated hemoglobin and fructosamine values further indicated that recent diabetes control had been poor. The range of values for non-diabetic individuals in the RVH biochemical laboratories are 4.6 to 7.5 % and 230 to 300 umol for glycated hemoglobin and fructosamine, respectively.

6.2 Clinical Course of Experimental Diets

The study diets provided fat and carbohydrate at $<30\%$ and 50-60% of total energy respectively (Table 6). The vitamin and mineral content of the LED diets providing the lowest and highest amounts of energy is detailed in Table 3. Both ISO and LED formula study diets were well tolerated, although subjects complained of boredom due to the monotonous nature of the liquid diet. Subjects did not voice or

report any feelings of hunger. No subjects experienced any adverse effects of the study protocol.

Protein and energy intakes during the pre-study period were calculated and are detailed in Table 7. Paired t-tests were used to determine if there were significantly lower pre-study intakes of protein and energy compared to the iso-energetic period. It was found that the intakes were not statistically significantly different during both of these phases. Reported energy intake did not differ significantly from that consumed during the ISO diet (9.8 $\pm$ 0.8 vs 11.1 $\pm$ 0.8 MJ; NS). However, with the exception of two subjects, the trend was to report a lower intake than what was calculated and given to maintain weight. Protein intakes prior to admission were not significantly different from those provided during the study diets (115 $\pm$ 8.0 vs 99 $\pm$ 1.7 MJ; NS). This had important implications for our study. The responses observed during the first week of iso-energetic feeding therefore reflect responses to the study and not of increased or decreased dietary protein consumption.

6.3 Anthropometric Measurements

6.3.1 Weight Patterns

During the ISO diet in which the diabetic subjects were hyperglycemic weight decreased by 1.4 $\pm$ 0.3 kg (Figure 4), significantly different from zero ($P < 0.05$). This weight loss was primarily due to the osmotic diuresis associated with urinary glucose losses which averaged 295 $\pm$ 68 mmol/d. These glucose losses were

replaced in the diet in order to stabilize weight. The glucose losses reflected 890 ± 201 kJ (213 ± 48 kcal)/d and were replaced in a 1:2 energy ratio of soya oil and glucose polymer (ie. 7.9 ± 1.8 g soya oil and 37.5 ± 8.6 g glucose polymer). Upon initiation of the OHA during ISO, urinary glucose losses decreased (52 ± 17 mmol/d; $P<0.01$)and weight was stabilized. Weight change during this period in the diabetic group was 0.3 ± 0.3 kg, no longer significantly different from zero. Mean weight decreased by 0.7 ± 0.4 kg in the obese control subjects during ISO, which was not significantly different from zero.

Weight decreased in a gradual fashion over the entire LED (Figure 4). The rate of weight loss was not different between the diabetic and obese groups (0.16 ± 0.02 vs 0.14 ± 0.01 kg BW lost/d, respectively; NS). Total weight loss was not significantly different in the diabetic and obese groups (6.5 ± 0.5 vs 5.2 ± 0.5 kg, respectively; NS), and represented losses of 6.2 ± 0.4 and 5.3 ± 0.3 % of initial body weight in the diabetic and obese study groups, respectively. The weight loss observed in the diabetic group was not skewed by the men in the group as their weight loss fell within the mean of the group. Weight loss correlated with initial body weight ($r=0.776$; $P<0.01$),initial LBM ($r=0.774$; $P<0.01$),initial RMR ($r=0.748$; $P<0.01$), and initial 24hr urinary creatinine excretion ($r=0.568$; $P<0.05$). No significant correlations were found between change in body weight and initial %BF. Change in LBM over the LED was 0.63 ± 0.64 kg and 0.23 ± 0.24 kg in the diabetic and obese study groups, respectively.

Theoretical weight loss was calculated based on the assumption that a 32.2

MJ deficit is required to achieve a 1 kg weight loss (ie. 3500 kcal/lb) and that every kilogram of LBM contains 32 g N (Hoffer, 1988). It was determined that the mean theoretical weight loss should have been 4.4 ± 0.8 & 4.4 ± 0.4 kg for the diabetic and obese study groups, respectively.

6.3.2 Body Circumferences

A paired t-test revealed that waist to hip ratios did not change over the course of the study in either study group (0.96 ± 0.04 vs 0.95 ± 0.04 (DM) & 0.83 ± 0.02 vs 0.83 ± 0.02 (OB)). There was a significant decrease in waist circumference from baseline measurements in both the diabetic and obese groups (125 ± 3 vs 120 ± 3 cm (DM) & 126 ± 3 vs 120 ± 4 cm (OB), respectively; $P<0.0001$). Waist circumference and waist to height ratios were found to correlate with %BF measured by BIA ($n=14$, $r=0.746$; $P<0.0001$ & $n=14$, $r=0.799$; $P<0.0001$, respectively).

6.4 Metabolic Responses

Statistical analyses of the results of the effects of treatment (obesity or diabetes), time [Week 1 (ISO) & weeks 2-5 (LED)], the interaction of treatment x time, and repeated measures [pt(trt)] are presented in Table 8. Weekly plasma values were within normal limits for the duration of the study for all parameters with the exception of triglycerides in the diabetic group that were elevated during ISO (Tables 9 & 10). Weekly blood samples showed no alterations in serum alkaline phosphatase, alanine aminotransferase, albumin, or red blood cell count (data not

shown). Serum creatinine and creatinine clearance remained within normal limits, did not change significantly throughout the study in either group, and was not different between the two groups at any time point (data not shown). Serum potassium, bicarbonate, magnesium, and calcium likewise did not change significantly in either group throughout the study and did not differ between the two groups at any time point. Serum sodium did not change over the study in either group, but was significantly lower at week 2 of the LED in the diabetic group than at week 3 of the LED in the obese control group. Serum chloride was significantly lower during ISO in the diabetic group than during ISO+OHA and all 4 weeks of the LED in the diabetic group and the entire study period of the obese group. Serum chloride did not change in the obese group over the study. Total cholesterol was significantly greater in the diabetic subjects while hyperglycemic during the first week of ISO when compared to the ISO+OHA period and all 4 weeks of the LED in the diabetic group. Likewise, total cholesterol decreased significantly after 2 weeks of moderate energy restriction in the obese control group. There were no significant differences in total cholesterol between the diabetic and the obese groups. Serum triglycerides decreased after one week of the ISO+OHA, and remained decreased in the diabetic group during the LED. Serum triglycerides did not change in the obese group. The two groups no longer differed in serum triglyceride concentration after the ISO+OHA week. Serum urea did not change in the diabetic group with the exception of significantly greater values at weeks 1 and 3 of the LED when compared to the ISO+OHA period and the ISO week of the obese group. Serum urea did not

change over the course of the study in the obese subjects. Serum uric acid was significantly greater during ISO+OHA and all 4 weeks of the LED in the diabetic group than compared to the ISO period in both groups and the entire LED of the obese group, in which serum uric acid remained unchanged. Blood 3-beta-hydroxybutyrate (3-OH) was measured at the end of the ISO, ISO+OHA, and weeks 2 & 4 of the LED. A significant increase in blood 3-OH concentration was observed at the end of the LED in the diabetic group when compared to both ISO periods and week 2 of the LED in the diabetic group, and all time points in the obese group. Blood 3-OH did not change significantly in the obese study group. Urinary 3-OH was undetectable in both the diabetic and obese subjects throughout the entire study. A significant reduction in hemoglobin was noted in the obese group at the end of the LED when compared to the ISO week. Hemoglobin was also significantly lower than that of the diabetic subjects during both ISO periods. Hematocrit was significantly lower at week 4 than at week 3 of the LED in the obese group only. The value measured at week 4 of the LED was also significantly lower than that of the diabetic group during ISO, ISO+OHA, and week 2 of the LED. Hematocrit and hemoglobin did not change in the diabetic group over the study period. There was a significant reduction in white blood cell count after 4 weeks of energy restriction in the diabetic group when compared to ISO and week 4 of the LED. White blood cell count was not altered in the obese group. Neutrophil count did not change in either group over the study with the exception of weeks 2 & 4 of the LED in the obese group, which were significantly lower than the values in the diabetic group during ISO and

ISO+OHA. Likewise, lymphocyte count did not change throughout the study in either group, the exception of ISO and weeks 1, 2, and 4 of the LED of the obese group being significantly lower than the diabetic group during ISO & ISO+OHA. Urinary urea concentration decreased significantly during ISO+OHA (481 $\pm$ 25 vs 412 $\pm$ 16 mmol/d; $P<0.02$) in the diabetic group and did not change significantly for the remainder of the study. No significant changes in urinary urea excretion were observed in the obese group. Urinary ammonium excretion remained unchanged in the diabetic group throughout the study, whereas a significant increase was observed in the obese group at week 4 of the LED compared to the ISO week (0.30 $\pm$ 0.04 vs 0.37 $\pm$ 0.02; $P<0.02$). The two groups were not different with respect to urinary urea or ammonium excretion at any time.

6.5 Electrolyte Balances

Cumulative sodium and potassium balances are presented for the entire obese group and only 5 of the diabetic subjects (Figure 5). Two diabetic subjects were not included as these subjects were outliers due to greatly positive sodium and potassium balances (1311 & 746 and 724 & 801 mmol respectively). The first subject was admitted at a time in the summer during which daily temperatures were in excess of 28°C. The daily sodium and potassium excretions decreased to compensate for increased alternate routes of excretion, namely the observed sweating. Incompleteness of urine collections as a reason for this drop in sodium and potassium excretion was ruled out, as creatinine levels were very steady during the studies.

Suitable values for miscellaneous losses of sodium and potassium in the situation mentioned above were not available at the present time, and therefore this subject was removed from analysis of electrolyte balances. The second subject was disregarded as this subject required anti-gout medication (colchicine) which has been reported to cause intestinal absorptive defects and increased fecal losses of sodium and potassium (Roe, 1974). These electrolyte balances were inconsistent with each subject's respective weight loss.

Cumulative sodium balance was not different from zero during the ISO period in both the diabetic and obese groups. These balances translated into water losses of approximately 180 to 380 g during the first week (-27 $\pm$ 31 & -57 $\pm$ 36 mmol, respectively, NS). Cumulative sodium balance was positive (90 $\pm$ 33 mmol) during the ISO+OHA week. This positive sodium balance indicated water retention of approximately 600 g, and was significantly different from the ISO week of the diabetic and obese subjects ($P<0.05$) and from zero. Cumulative sodium losses at the end of the LED were not different between the diabetic and obese groups (-134 $\pm$ 181 vs -241 $\pm$ 88 mmol). The sodium balance was not different from zero in the diabetic group.

Cumulative potassium balance was not significantly different from zero during the ISO period in the diabetic group. The obese controls had a balance of -30 $\pm$ 19 mmol, also not significantly different from zero. The initiation of the OHA in the diabetic group resulted in a cumulative balance of +27 $\pm$ 33 mmol, not significantly different from zero. At the end of the LED, the mean balance of the

diabetic subjects was +201 ± 149 mmol potassium and that of the obese controls was +8 ± 73 mmol. Potassium balances in both groups were not significantly different from each other, or zero, at the end of the LED.

6.6 Diabetes Control

6.6.1 Diabetes Medication

OHA therapy was initiated during ISO+OHA in all diabetic subjects, and continued throughout the LED. Four subjects required dual OHA therapy. Metformin was also given in addition to the gliclazide in four subjects during the ISO+OHA period, and continued throughout the LED as shown in Figure 6. One diabetic subject was able to discontinue all OHA treatment after 11 days of the LED as dictated by fasting capillary blood glucose values of less than 6.0 mM. The average daily doses of gliclazide at the end of the ISO+OHA week, which were 296 ± 14 mg/d, decreased to 74 ± 16 mg/d by the end of the LED ($F=19.76; P<0.0001$). A significant reduction in gliclazide dosage was observed after only 1 week of the LED diet (154 ± 38 mg/d; $P<0.05$). The dosage at week 4 of the LED was also significantly lower than that after 1 week of the LED ($P<0.05$).

Insulin was administered to 2 subjects to maintain plasma glucose levels below 20 mM. One subject required single injections of regular insulin for 4 days and the other for 1 day only. Insulin administration did not affect nitrogen balance as these days were not different from the days before or after insulin administration. To eliminate all any potential interference of insulin on the assessment of protein metabolism, urine samples of these days were not used to assess urinary 3-MeHis excretion.

6.6.2 *Fasting Plasma Glucose*

FPG decreased after 1 week of OHA therapy (12.9 ± 1.0 vs 9.0 ± 0.8 mM; $P<0.05$) and continued to decrease during the LED to concentrations near euglycemia (6.5 ± 0.7; $P<0.05$) (Figure 6). At the end of 4 weeks of energy restriction, three subjects attained euglycemia (ie. FPG < 5.8 mM), two subjects had FPG between 6.5 and 6.7 mM, and the two subjects had FPG values of 7.4 and 9.9 mM. All subjects were entered into statistical analysis as all subjects demonstrated improved diabetes control. FPG decreased by 49.3 ± 5.4 % compared to values at entrance into the study. FPG remained significantly higher throughout the study period in the diabetic group when compared to the obese controls ($P<0.05$). FPG remained within normal limits for the obese controls during both the ISO and LED diets. A paired t-test showed a significant reduction in glycated hemoglobin in the diabetic subjects. Glycated hemoglobin at entrance into the study was 11.3 ± 0.4 % and decreased to the upper end of the normal range (8.3 ± 0.4 %) by the end of the LED ($P<0.001$).

6.6.3 *Fasting Plasma Insulin*

Fasting plasma insulin (immunoreactive insulin : IRI) increased upon initiation of the OHA in the diabetic group (212 ± 13 vs 273 ± 31 pmol/L; $P<0.05$) (Table 11). IRI was significantly greater in the diabetic group during both ISO and ISO+OHA when compared to the obese group during the ISO period. IRI decreased significantly after one week of the LED in both study groups and remained

at this lower concentration. The two study groups were not significantly different in IRI at any time point during the LED.

6.6.4 Plasma Free Fatty Acids

FFA concentration at the end of the LED was not different from that during ISO (Table 11). No significant change was observed with the introduction of the OHA. Plasma free fatty acid (FFA) concentration decreased significantly in the diabetic group during weeks 1 to 3 of the LED. FFA did not change significantly over the course of the study in the obese control group. The two groups were not significantly different at any time during the study. No significant correlation was found between FFA levels and FPG or %BF.

6.7 Protein Metabolism

6.7.1 Body Composition

Initial LBM determined by BIA was compared to LBM determined by an equation, based on 24hr urinary creatinine excretion, developed for subjects consuming a creatinine-free diet (Forbes, 1988). The two equations yielded LBM measurements which were found to correlate strongly ($n=14$, $r=0.948$; $P<0.0001$), thus indicating that the initial LBM measurements by BIA were reliable and reproducible by other means. LBM measured by BIA was not significantly changed in either the diabetic and control groups from the start of the ISO to the end of the LED diets. Cumulative nitrogen balances were computed, converted into kg LBM

by a factor of 32 g N/kg LBM (Hoffer, 1988), and subtracted from initial measures of LBM by BIA. It was found that final LBM values measured by BIA correlated with those derived from cumulative nitrogen balances in both the diabetic and obese groups ($n=7$, $r=0.962$; $P<0.0001$ & $n=7$, $r=0.991$; $P<0.0001$, respectively).

Cumulative sodium balances were calculated, converted into kg TBW, subtracted from initial TBW measurements by BIA, and compared to final measurements of TBW by BIA. The final TBW values determined by BIA correlated with those from cumulative sodium balance in both the diabetic and obese control groups ($n=5$, $r=0.987$; $P=0.002$ & $n=7$, $r=0.992$; $P<0.0001$).

Percentage body fat decreased in a uniform and gradual fashion throughout the LED in both the type II and control groups. Percentage body fat was significantly lower at the end of the LED in both the diabetic and obese study groups (45.7 ± 3.0 vs 42.5 ± 3.6 %BF (DM) & 47.9 ± 1.4 vs 46.7 ± 1.5 %BF (OB), paired t-test, respectively; $P<0.05$). The diabetic group lost a significantly greater amount of body fat than the obese subjects during the LED (3.2 ± 0.8 vs 1.2 ± 0.2 %BF; $P<0.05$). Percentage body fat measured by BIA was compared to the equation of Weltman and colleagues (1988) which employs body circumferences and height as a means of determining body fat. It was found that these two methods correlated both at baseline ($n=14$, $r=0.632$; $P<0.02$) as well as after weight loss ($n=14$, $r=0.696$; $P<0.02$) (Figure 7).

6.7.2 Urinary 3-Methylhistidine Excretion

Urinary 3-MeHis excretion was higher in the diabetic group during the ISO period compared to the obese controls (3.1 ± 0.3 vs 2.2 ± 0.2 umol/kg LBM/d; $P<0.05$)(Figure 8). With OHA therapy, urinary excretion decreased to 2.5 ± 0.4 umol/kg LBM/d, and was no longer significantly higher than controls. Urinary 3-MeHis excretion did not significantly decrease with the LED in either group. Urinary 3-MeHis excretions were not significantly different between the diabetic and obese groups post LED (2.1 ± 0.4 vs 1.6 ± 0.2 umol/kg LBM/d; NS). Statistical results were not changed when 3-MeHis was expressed on a daily basis or per kilogram LBM, as LBM was not significantly different between groups and did not change significantly from the beginning to the end of the study.

5.7.3 Nitrogen Balance

The daily nitrogen balance was 0.1 ± 0.6 g N/d at the end of the ISO diet in the diabetic group and not significantly different from zero (Figure 9). Upon initiation of the OHA in the diabetic group, nitrogen balance became more positive and was significantly different from the ISO by day 6 of the ISO+OHA period (2.3 ± 0.5 g N; $P<0.05$). Nitrogen balance in the diabetic group was not different from that of the obese subjects (1.0 ± 0.2 g N/d, NS) during either of the ISO periods.

At the onset of the LED nitrogen balance became less positive, but remained positive, in the diabetic group. The diabetic subjects remained in positive nitrogen balance throughout the entire LED. In the obese control subjects, nitrogen

balance was negative during days 1 to 4, became and remained in equilibrium for the remainder of the LED. Nitrogen balance during the LED was not significantly different between the two groups, with the exception of days 1 and 25 ($P<0.05$). Nitrogen balance in the diabetic group during the last 3 days of the ISO period was significantly different from that of the last 3 days of the LED ($P<0.05$). In the obese group, nitrogen balance during the last 3 days of the ISO diet was significantly different from those of days 1, 13, 18, and 25 of the LED ($P<0.05$). Nitrogen balance in the obese group during the first 3 days of the LED was significantly different from that of day 28 of the LED ($P<0.05$).

Cumulative nitrogen gains were not significantly different between groups during ISO periods (Figure 10). Nitrogen retention occurred with the initiation of OHA therapy in the diabetic group (2.6 ± 4.5 vs 16.6 ± 2.9 g N; $P<0.05$). The obese group at the end of the LED had retained 7.2 ± 8.0 g N, whereas the diabetic subjects had retained 20.26 ± 20.3 g N. Weekly cumulative gains were not significantly different between the two groups (Figure 10). The removal of the two male diabetic subjects from cumulative nitrogen balance analysis did not alter this trend of non-significance between the two groups with respect to cumulative nitrogen balance during ISO, ISO+OHA, and the LED (paired t-test). Cumulative nitrogen balances of the 5 female diabetic subjects during ISO, ISO+OHA, and the LED were 27.4 ± 16, 1.4 ± 4.1, & 14.4 ± 2.7 g N, respectively. The change in cumulative nitrogen balance over the last 3 days of ISO to ISO+OHA was found to correlate negatively with the change in FPG at the end of ISO to ISO+OHA ($n=7$, $r=-0.765$;

P=0.045) (Figure 11a). The cumulative nitrogen balance over the LED was found to correlate with initial glycated hemoglobin values (n=7, r=0.759; P=0.045) (Figure 11b).

6.8 Resting Metabolic Rate

Resting metabolic rate in the diabetic group was significantly greater than predicted according to age, height, weight, and gender based on the Harris-Benedict equation (113 ± 4% of predicted values; P<0.01, paired t-test). A decline in RMR was noted with the improvement of glycemia during ISO+OHA (Figure 12). Although this decrease in RMR was not statistically significant, the RMR was no longer different from predicted values (105 ± 3% of predicted values; NS, paired t-test). The mean of the RMR measurements of obese control subjects was 98 ± 3% of predicted values (NS, paired t-test).

After 1 week of OHA therapy, the RMR in the diabetic group decreased to values that were no longer different from those of the obese control group, and remained not different for the duration of the LED. The RMR of the diabetic group was significantly lower at the end of weeks 3 & 4 of the LED when compared to the ISO week (P<0.05). The RMR of the obese group did not change throughout the study period. The RMR of the diabetic group during the ISO period was significantly greater than that of the obese control group (P<0.01). Statistical analysis remained the same when RMR was expressed on a kg LBM basis as LBM did not change throughout the study and was not different between the two groups. The RMR of

each group was analyzed separately (Primer Biostatistics) in a repeated measures ANOVA and significant differences within each group were identified by the Student-Newman-Keuls test. The RMR of the diabetic group was found to decrease significantly during the course of the study ($F=9.06; P<0.0001$). A significant 6% decrease in RMR was observed after the ISO+OHA week ($P<0.05$). Weeks 1 through 4 of the LED were significantly lower than during ISO in the diabetic group ($P<.05$). Weeks 3 and 4 of the LED were also found to be 10% lower than during ISO+OHA ($P<0.05$). Likewise, RMR was found to decrease by 10% in the obese group ($F=7.35; P<0.0001$). A significant reduction in RMR was observed after the second week of energy restriction in the obese group ($P<0.05$). The RMR remained at this significantly lower level for the remainder of the study ($P<0.05$). The RMR after 4 weeks of the LED in the obese group was found to be significantly lower than that after the first week of energy restriction ($P<0.05$).

No significant correlations were found between decreases in RMR and changes in BW, LBM, %BF, kg body fat, or 3-MeHis excretion during the LED. Post absorptive RQ, VO_2, or VCO_2 did not change significantly with either OHA and/or LED therapy in both groups. No significant linear correlations were observed between initial %BF or improvement of glycemia and RQ after ISO+OHA or post LED in either group. Protein, carbohydrate, and fat usage did not change significantly throughout the study in either group, and was not significantly different between study groups at any time of the study.

TABLE 5 : COMPARISON OF BASELINE ANTHROPOMETRIC, BODY COMPOSITION, & FASTING PLASMA GLUCOSE MEASUREMENTS OF STUDY SUBJECTS

	Diabetes	Obesity	*P-value*[1]
Sex	2M ; 5F	0M ; 7F	*na*
Age (yrs)	51 (2)	49 (4)	0.669
Anthropometry:			
Weight (kg)	106 (5)	96 (7)	0.276
Height (cm)	163 (3)	160 (2)	0.400
BMI (kg/m^2)	39 (2)	38 (3)	0.681
W:H (Male)	1.07 (0.03)	[2]*na*	*na*
(Female)	0.96 (0.03)	0.97 (0.01)	0.757
Waist Circumference (cm)	126 (4)	125 (3)	0.930
Body Composition:			
LBM (kg)	57 (3)	51 (3)	0.138
%BF	46 (4)	48 (1)	0.571
Diabetes Control:			
Duration of Diabetes (yrs)	5.7 (1.6)	*na*	
Glycated Hemoglobin (%) [3][4.6 - 8.5%]	11.3 (0.4)	*na*	
Fructosamine (umol/L) [230 - 300 umol/L]	320 (18)	*na*	
FPG (mM) [3.9 - 5.8 mM]	**12.9 (1.0)**	**5.3 (0.2)**	**0.001**

Mean (sem)

1 - Study groups were compared by un-paired t-tests (Primer Biostatistics)

2 - Not applicable (na) for obese control group

3 - Values in [] represent normal ranges for non-diabetic subjects in the RVH biochemistry laboratories

TABLE 6 : MACRONUTRIENT COMPOSITION OF ISO-ENERGETIC AND LOW ENERGETIC STUDY DIETS

	Diabetes	Obesity	*P-value*[1]
Isoenergetic Diet:			
Energy Intake (MJ/d)	11.5 (0.5)	10.5 (0.5)	0.812
Protein Intake (g/d)	101 (3)	97 (2)	0.404
Protein (% of kcal)	15 (1)	16 (1)	0.493
Carbohydrate (% of kcal)	57 (1)	55 (1)	0.183
Fat (% of kcal)	28 (1)	29 (1)	0.493
Low Energetic Diet:			
Energy Intake (MJ/d)	5.7 (0.3)	5.1 (0.3)	0.150
Protein Intake (g/d)	102 (3)	98 (2)	0.289
Protein (% of kcal)	30 (1)	32 (1)	0.183
Carbohydrate (% of kcal)	54 (1)	53 (1)	0.477
Fat (% of kcal)	18 (1)	17 (1)	0.260

Mean (sem); n= 7 DM & 7 OB

1 - Study groups were compared by unpaired t-test (Primer Biostatistics)

TABLE 7 : COMPARISON OF DIETARY INTAKES OF PROTEIN & ENERGY DURING THE PRE-STUDY AND ISO-ENERGETIC DIET

	Protein Intake (g/d)		Energy Intake (kJ/d)	
	Pre-Study	ISO Diet	Pre-Study	ISO Diet
Type II Diabetes Mellitus				
#1	[1]*na*	110	*na*	12.3
#2	110	87	9.0	9.4
#3	109	95	8.8	11.3
#4	180	102	16.1	11.9
#5	105	99	10.8	11.9
#6	149	101	10.0	10.1
#7	100	111	9.0	13.3
Obesity				
#1	*na*	103	*na*	11.1
#2	130	103	9.4	10.0
#3	96	103	7.4	11.0
#4	107	103	7.9	11.0
#5	121	95	5.9	9.5
#6	78	95	9.1	13.1
#7	90	97	14.4	10.5
Mean (n=12)	115	99	9.8	11.1
SEM	8.0	1.7	0.8	0.3
P-value[2]	0.079		0.157	

1 - Not available (na) and therefore not included into statistical analysis

2 - Paired t-test (Primer Biostatistics)

TABLE 8 : STATISTICAL ANALYSIS RESULTS OF WEEKLY SERUM SAMPLES

Parameter	[1]P-value (F-value) [2]Trt	[3]Tm	[4]Tm x Trt	[5]Repeated
S. Na^+	0.1126	0.3678	0.9922	0.0028 (6.52)
S. K^+	0.5144	0.4160	0.4452	0.0649
S. Cl^-	0.2686	0.0152 (3.37)	0.6402	0.8129
S. HCO_3^-	0.6644	0.0838	0.4334	0.1416
S. Mg^{2+}	0.1096	0.4689	0.3485	0.1671
S. Ca^{2+}	0.0938	0.6613	0.5005	0.2424
Cholesterol	0.0001 (26.79)	0.0001 (7.02)	0.5120	0.0001 (12.75)
Triglycerides	0.2572	0.0015 (5.02)	0.2760	0.0254 (3.92)
S. urea	0.0032 (9.45)	0.5486	0.9766	0.0003 (9.57)
S. creatinine	0.3867	0.9461	0.9347	0.5904
S. uric acid	0.4855	0.0364 (2.75)	0.2521	0.0183 (4.29)
[6]Blood 3-OH	0.1575	0.0160 (4.68)	0.2971	0.9218
Hemoglobin	0.0169 (6.05)	0.3714	0.9982	0.0018 (7.04)
Hematocrit	0.0860	0.2164	0.8726	0.0165 (4.41)
[7]WBC	0.0273 (5.13)	0.1821	0.9612	0.0121 (4.77)
Neutrophils	0.0332 (4.76)	0.2187	0.9553	0.1060
Lymphocytes	0.2994	0.8562	0.6487	0.0045 (5.94)

Two-way ANOVA with repeated measures design (SAS Software); n=7 DM & 7 OB
1 - Values in table are P-values; F-values are in parentheses when significant
2 & 3 - Treatment (diabetes or obesity) & Time [week 1 (ISO) & weeks 2-5 (LED)]
4 - Time x Treatment (Interaction of main effects)
5 - Repeated Measures
6 & 7 - 3-hydroxybutyrate & White Blood Cell count, respectively

TABLE 9 : SERUM ELECTROLYTE AND LIPID RESPONSES TO ISO-ENERGETIC & LOW ENERGETIC DIETS

		ISO	ISO+OHA	LED			
				1	2	3	4
Serum Na^+ (mM)	DM	138 ± 1 AC	140 ± 1 ABC	140 ± 1 ABC	139 ± 1 AB	140 ± 1 ABC	139 ± 1 ABC
[1][136 - 145]	OB	140 ± 1 ABC		141 ± 1 BC	141 ± 1 ABC	142 ± 1 AC	141 ± 1 ABC
Serum K^+ (mM)	DM	4.2 ± 0.1	4.4 ± 0.1	4.4 ± 0.1	4.3 ± 0.1	4.2 ± 0.1	4.1 ± 0.1
[3.7 - 5.1]	OB	4.2 ± 0.1		4.1 ± 0.1	4.2 ± 0.1	4.1 ± 0.1	4.1 ± 0.1
Serum Cl^- (mM)	DM	101 ± 1 A	104 ± 1 B	104 ± 1 B	104 ± 1 B	105 ± 1 B	104 ± 1 B
[100 -109]	OB	104 ± 1 B		106 ± 1 B	105 ± 1 B	106 ± 1 B	106 ± 1 B
Serum HCO_3^-(mM)	DM	28 ± 2	26 ± 1	26 ± 1	26 ± 1	25 ± 1	25 ± 1
[18 - 30]	OB	26 ± 1		27 ± 1	26 ± 1	26 ± 1	25 ± 1
Serum Mg^{2+} (mM)	DM	0.8 ± 0.1	0.8 ± 0.1	0.9 ± 0.1	0.9 ± 0.1	0.8 ± 0.1	0.9 ± 0.1
[0.75 - 1.25]	OB	0.9 ± 0.1		0.8 ± 0.1	0.9 ± 0.1	0.9 ± 0.1	0.9 ± 0.1
Serum Ca^{2+} (mM)	DM	2.4 ± 0.1	2.4 ± 0.1	2.4 ± 0.1	2.4 ± 0.1	2.4 ± 0.1	2.4 ± 0.1
[2.12 - 2.62]	OB	2.3 ± 0.1		2.3 ± 0.1	2.4 ± 0.1	2.4 ± 0.1	2.3 ± 0.1
[2]T. Chol. (mM)	DM	5.5 ± 0.5 A	4.3 ± 0.2 BC	4.6 ± 0.2 CD	4.3 ± 0.2 C	4.3 ± 0.2 C	4.1 ± 0.2C
[3.6 - 6.2]	OB	5.2 ± 0.4 A		5.0 ± 0.2 ABDE	4.8 ± 0.2 DE	4.6 ± 0.2 EC	4.4 ± 0.2EC
Triglycerides (mM)	DM	2.5 ± 0.4 A	1.9 ± 0.2 B	1.5 ± 0.3 B	1.4 ± 0.2 B	1.3 ± 0.2 B	1.3 ± 0.2B
[0.6 - 2.3]	OB	1.5 ± 0.2 B		1.0 ± 0.1 B	1.3 ± 0.1 B	1.2 ± 0.1 B	1.1 ± 0.1B

Two-way ANOVA with repeated measures design. Differences were determined using Least Squares Means.
Data presented are least squares means ± sem, n=7 DM & 7 OB.
Means with a common upper case letter are not significantly different.
1 - Normal ranges for RVH laboratories in [] & 2 - Total Cholesterol

TABLE 10 : METABOLIC & HEMATOLOGICAL RESPONSES TO ISO-ENERGETIC & LOW ENERGETIC DIETS

		ISO	ISO+OHA	LED			
				1	2	3	4
S. Urea (mM)	DM	5.5 ± 0.3 AB	4.9 ± 0.2 A	6.0 ± 0.2 B	5.9 ± 0.4 AB	6.0 ± 0.5 B	5.8 ± 0.3 AB
[1][2.1 - 7.5]	OB	5.0 ± 0.3 A		5.5 ± 0.5 AB	5.3 ± 0.5 AB	5.6 ± 0.5 AB	5.7 ± 0.5 AB
S. Uric Acid (uM)	DM	286 ± 20 AC	317 ± 18 B	367 ± 23 B	361 ± 22 B	372 ± 22 B	374 ± 28 B
[147-353]	OB	253 ± 15 AC		285 ± 19 C	275 ± 17 C	267 ± 18 C	257 ± 17 C
Blood 3-OH (uM)	DM	93 ± 17 A	89 ± 21 A	*na*	213 ± 74 A	*na*	436 ± 157 B
	OB	33 ± 6 A		*na*	107 ± 21 A	*na*	150 ± 37 A
Hemoglobin (g/L)	DM	130 ± 4 A	126 ± 4 A	124 ± 4 ABC	128 ± 4 ABC	128 ± 3 ABC	125 ± 4 ABC
[120 - 160]	OB	125 ± 2 AB		120 ± 3 BC	123 ± 3 ABC	123 ± 3 ABC	120 ± 2 C
Hematocrit (%)	DM	39 ± 2 A	38 ± 1 A	37 ± 1 AB	38 ± 1 A	38 ± 1 AB	37 ± 1 AB
[37 - 47]	OB	38 ± 1 AB		36 ± 1 AB	37 ± 1 AB	37 ± 1 AB	35 ± 1 B
[2]WBC (10^9/L)	DM	7.5 ± 0.5 A	7.1 ± 0.6 AB	6.7 ± 0.7 AB	7.0 ± 0.6 A	6.6 ± 0.5 AB	6.0 ± 0.6 B
[4 - 11]	OB	6.3 ± 0.6 AB		5.8 ± 0.4 B	5.6 ± 0.5 B	5.8 ± 0.5 B	5.3 ± 0.5 B
Neutrophils (10^9/L)	DM	4.5 ± 0.5 A	4.1 ± 0.4 A	3.9 ± 0.4 AB	4.1 ± 0.5 AB	3.8 ± 0.4 AB	3.4 ± 0.4 AB
[1.6 - 7.7]	OB	3.9 ± 0.4 AB		3.5 ± 0.4 AB	3.1 ± 0.4 B	3.3 ± 0.4 AB	3.0 ± 0.4 B
Lymphocytes (10^9/L)	DM	2.2 ± 0.2 A	2.2 ± 0.2 A	2.1 ± 0.2 AB	2.1 ± 0.2 AB	2.0 ± 0.2 AB	1.9 ± 0.2 AB
[0.8 - 4.4]	OB	1.7 ± 0.2 B		1.7 ± 0.1 B	1.8 ± 0.1 B	1.9 ± 0.2 AB	1.7 ± 0.2 B

Two-way ANOVA with repeated measures design. Differences were determined using Least Squares Means.
Data presented are least squares means ± sem, n=7 DM & 7 OB.
Means with a common upper case letter are not significantly different.
1 - Normal ranges for RVH laboratories in [] & 2 - White blood cell count

TABLE 11 : FASTING PLASMA INSULIN & FREE FATTY ACIDS (FFA) DURING ISO-ENERGETIC & LOW ENERGETIC DIETS

		ISO	ISO+OHA	LED 1	LED 2	LED 3	LED 4
Fasting Plasma Insulin							
(pmol/L)	DM	212 ± 13 A	273 ± 31 B	157 ± 20 CF	161 ± 15 DE	122 ± 8 DEF	120 ± 7 E
	OB	173 ± 16 C		134 ± 10 DEF	124 ± 9 DEF	141 ± 14 CDEF	124 ± 9 EF
(uU/mL)	DM	29 ± 2 A	38 ± 4 B	22 ± 3 CF	22 ± 2 DE	17 ± 1 DEF	17 ± 1 E
	OB	24 ± 2 C		19 ± 1 DEF	17 ± 1 DEF	20 ± 2 CDEF	17 ± 1 EF
Plasma FFA							
(umol/L)	DM	1087 ± 94 AC	1016 ± 61 A	721 ± 53 B	733 ± 40 B	767 ± 119 BC	1016 ± 68 AC
	OB	978 ± 37 AC		857 ± 111 AC	882 ± 79 AC	806 ± 107 AC	933 ± 88 AC

Two-way ANOVA with repeated measures design. Differences were determined using Least Squares Means.
Data presented as least squares means ± sem, n=7 DM & 7 OB.
Means with a common upper case letter are not significantly different.

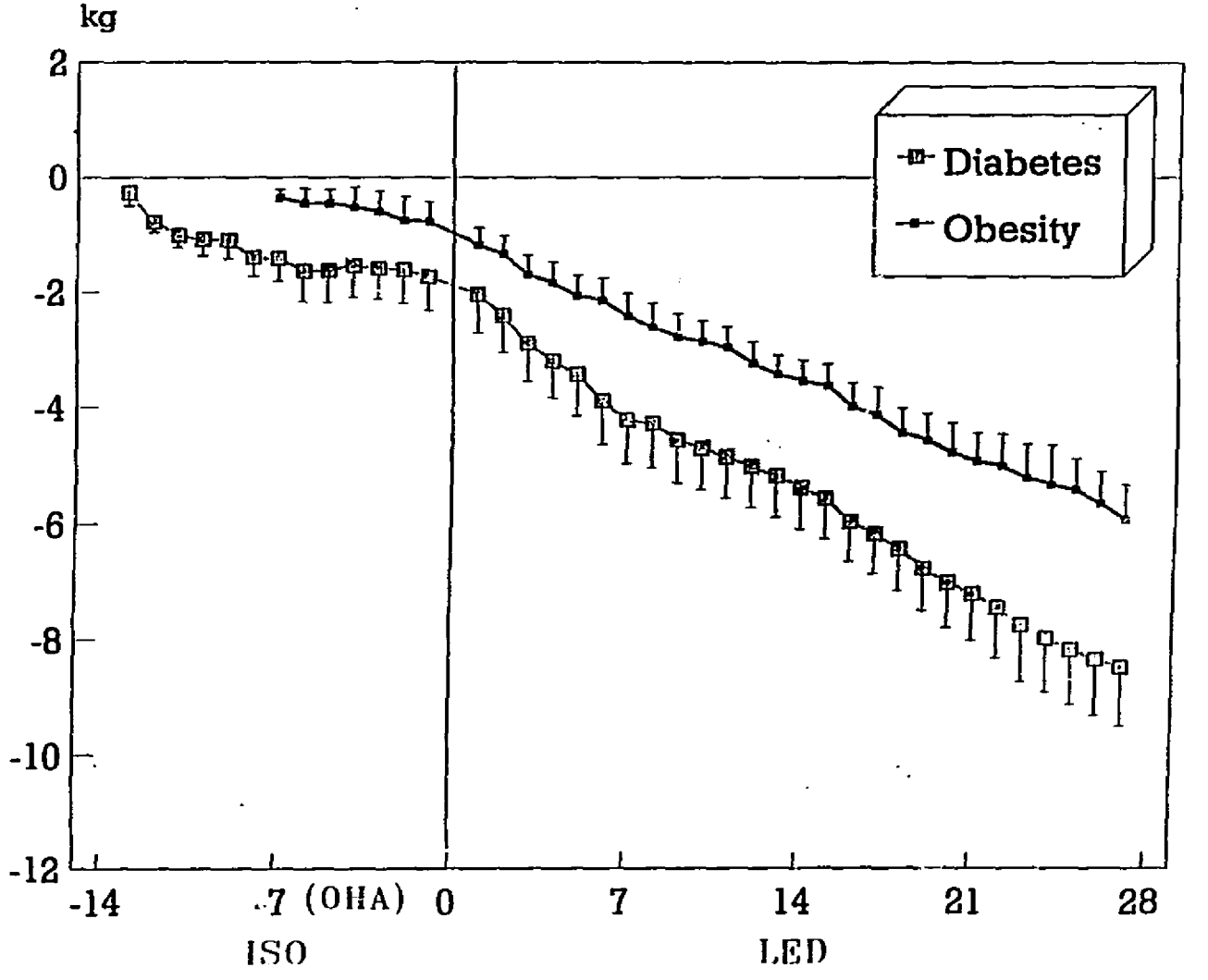


FIGURE 4 : WEIGHT LOSS PATTERNS OF ISO-ENERGETIC & LOW ENERGETIC DIETS (n = 7 DM & 7 OB)

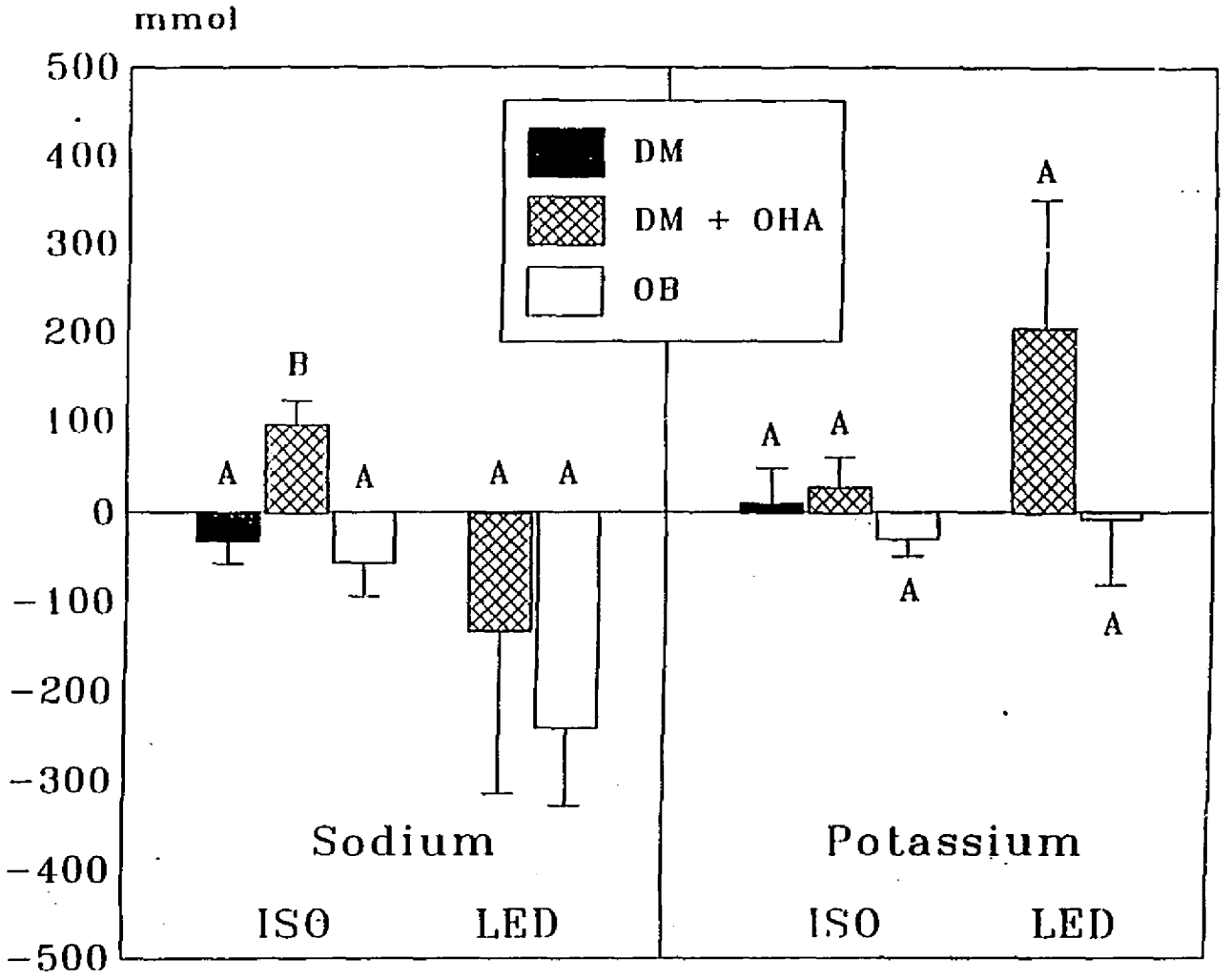


FIGURE 5 : CUMULATIVE SODIUM & POTASSIUM BALANCES OF ISO-ENERGETIC & LOW ENERGETIC DIETS (Two-Way ANOVA; Data presented as least squares mean ± sem; n = 5 DM & 7 OB. Means with a common letter are not different at $P<0.05$ during each phase of the study).

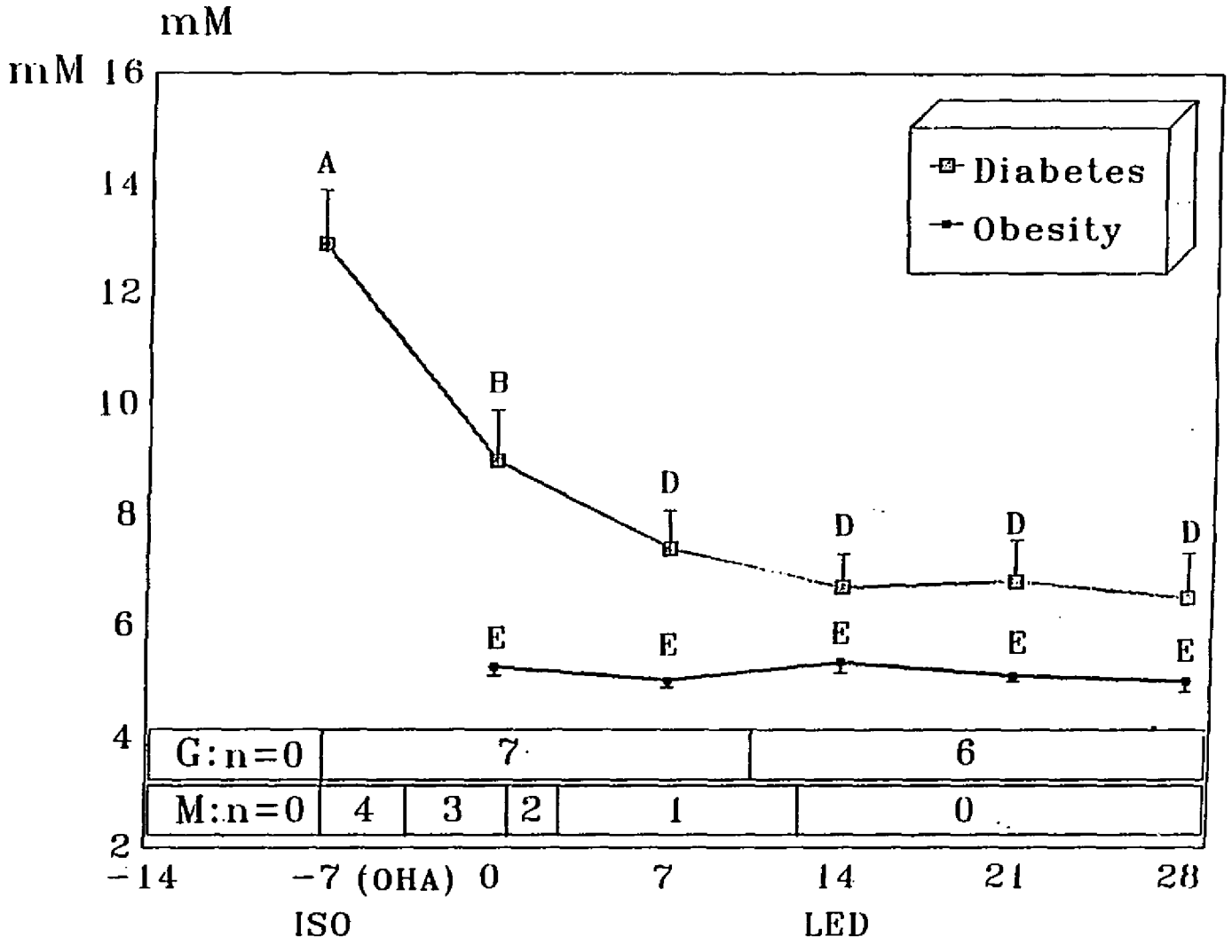
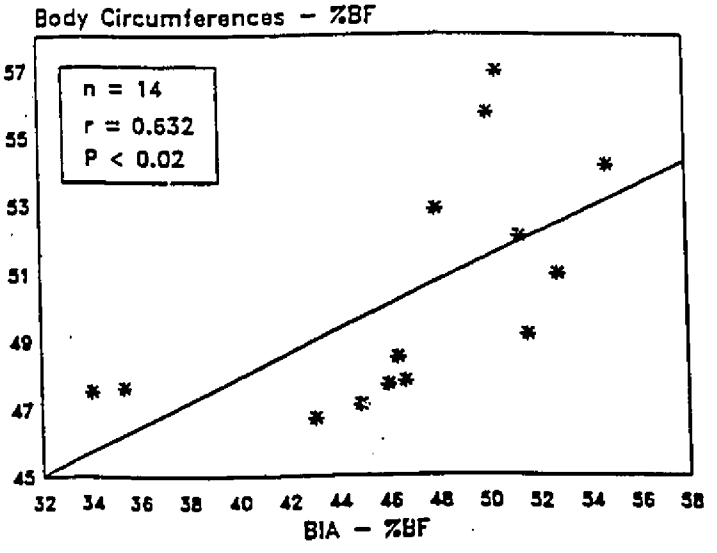


FIGURE 6 : FASTING PLASMA GLUCOSE MEASUREMENTS OF ISO-ENERGETIC & LOW ENERGETIC DIETS
(Two-Way ANOVA; Data presented as least squares mean ± sem; n = 7 DM & 7 OB. Means with a common letter are not different at $P<0.05$. G & M : number of patients receiving gliclazide & metformin, respectively).

(a)



(b)

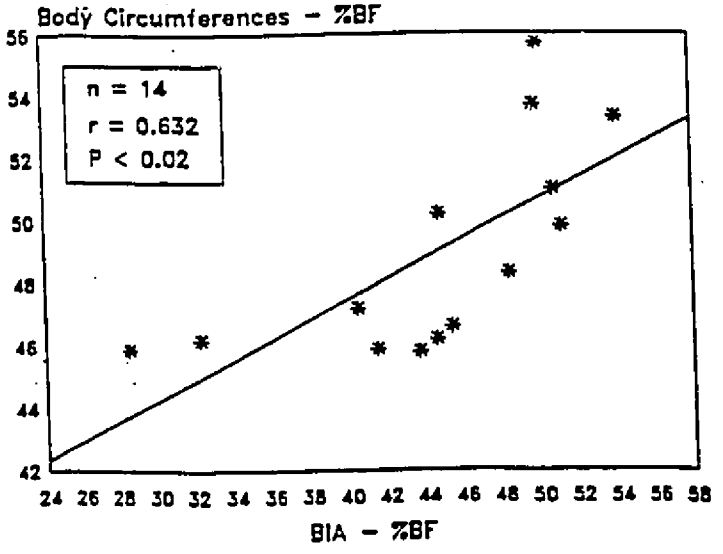


FIGURE 7 : CORRELATION BETWEEN DETERMINATION OF PERCENTAGE OF BODY FAT BY BIA VERSUS BODY CIRCUMFERENCE MEASUREMENTS (a) DURING ISO-ENERGETIC & (b) POST LOW ENERGETIC DIETS

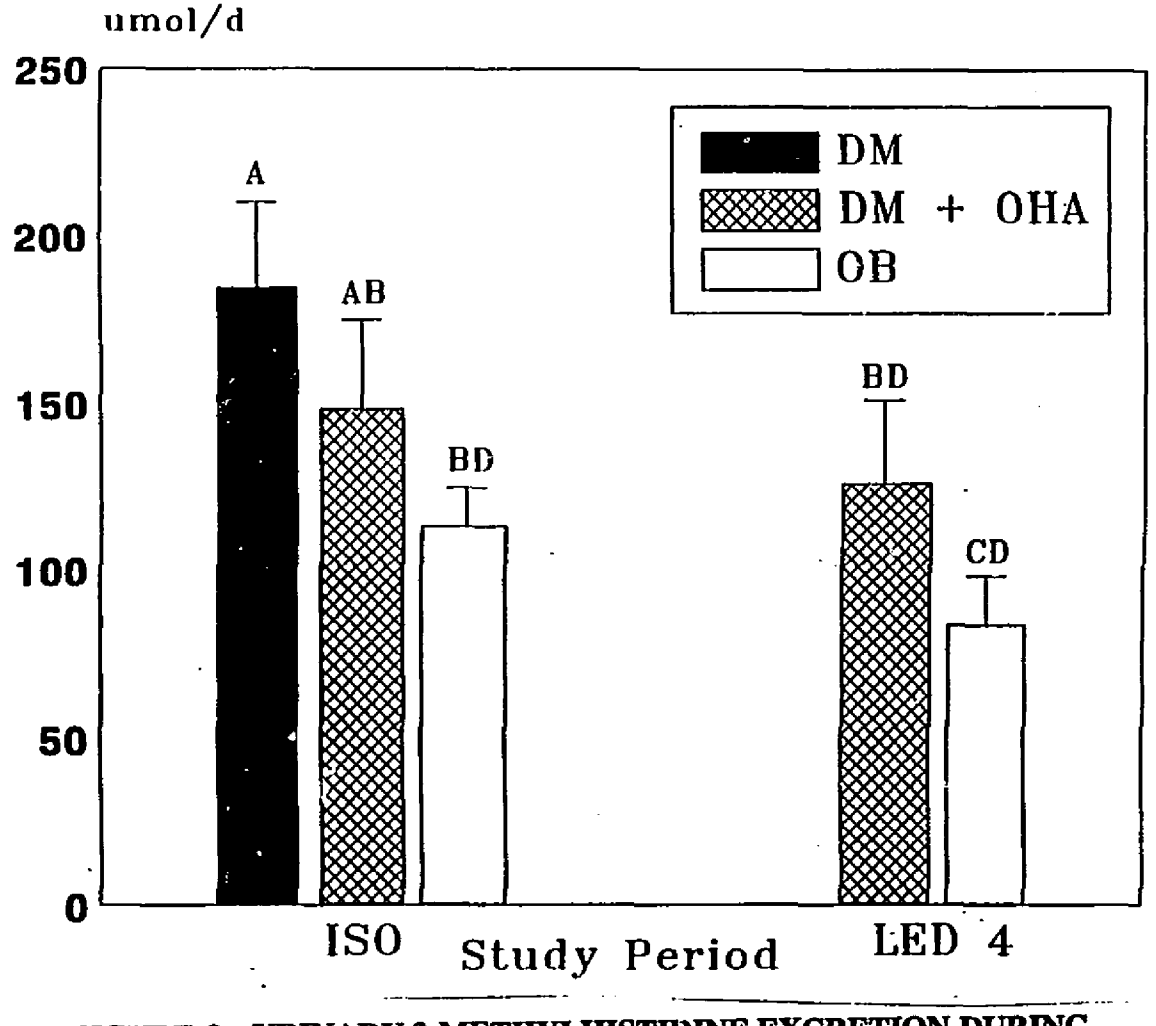


FIGURE 8 : URINARY 3-METHYLHISTIDINE EXCRETION DURING ISO-ENERGETIC & WEEK FOUR OF LOW ENERGETIC DIETS

Two-Way ANOVA; Data presented as least squares mean ± sem; n = 7 DM & 7 OB
Means with a common letter are not different at $P<0.05$

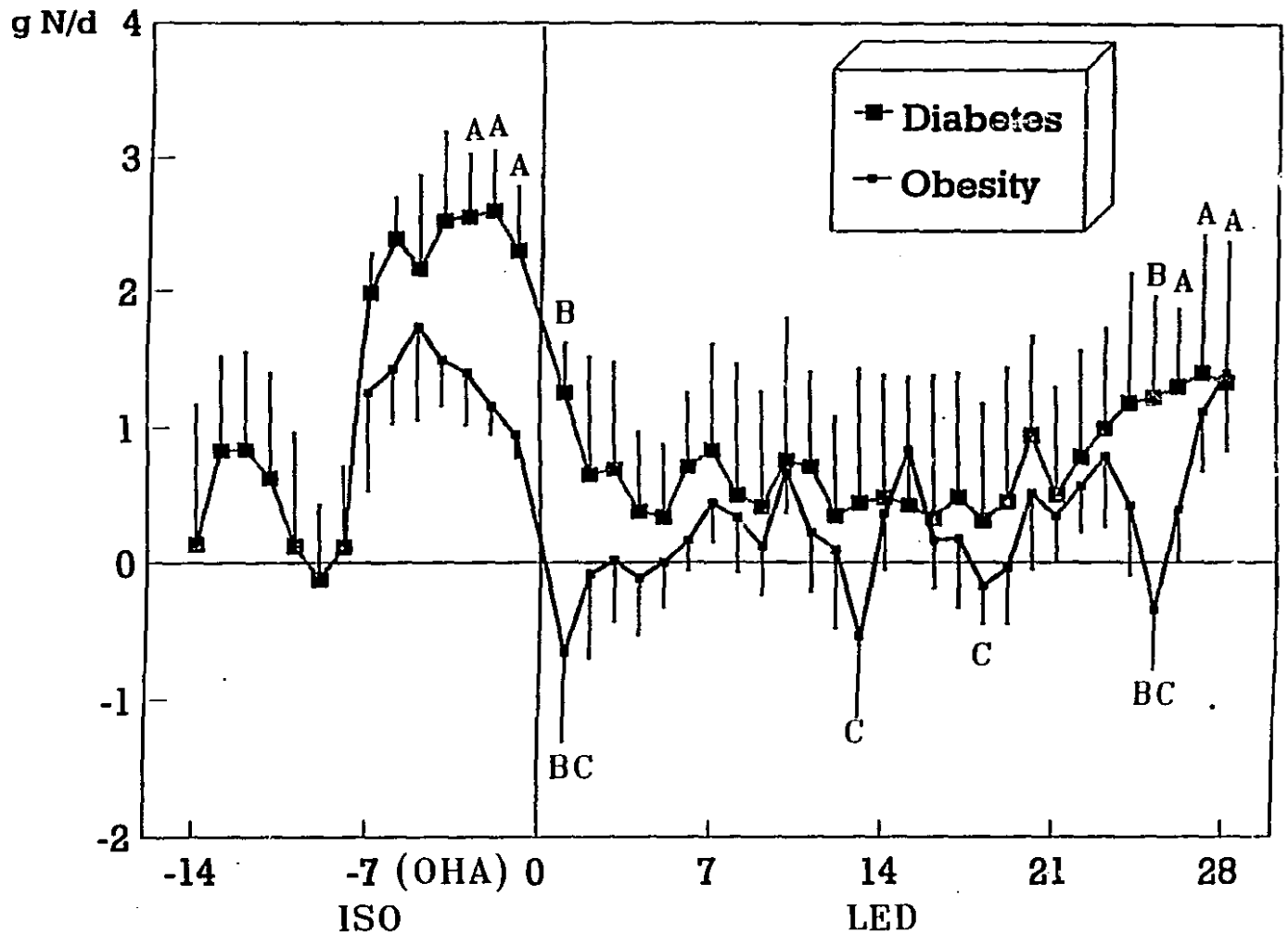


FIGURE 9 : DAILY NITROGEN BALANCE OF ISO-ENERGETIC & LOW ENERGETIC DIETS

Two-Way ANOVA; Data presented as least squares mean ± sem; n = 7 DM & 7 OB

A : Significantly different ($P<0.05$) from DM during ISO 5 to 7

B : Significantly different between DM & OB at identical time point only

C : Significantly different from OB during LED 26 to 28

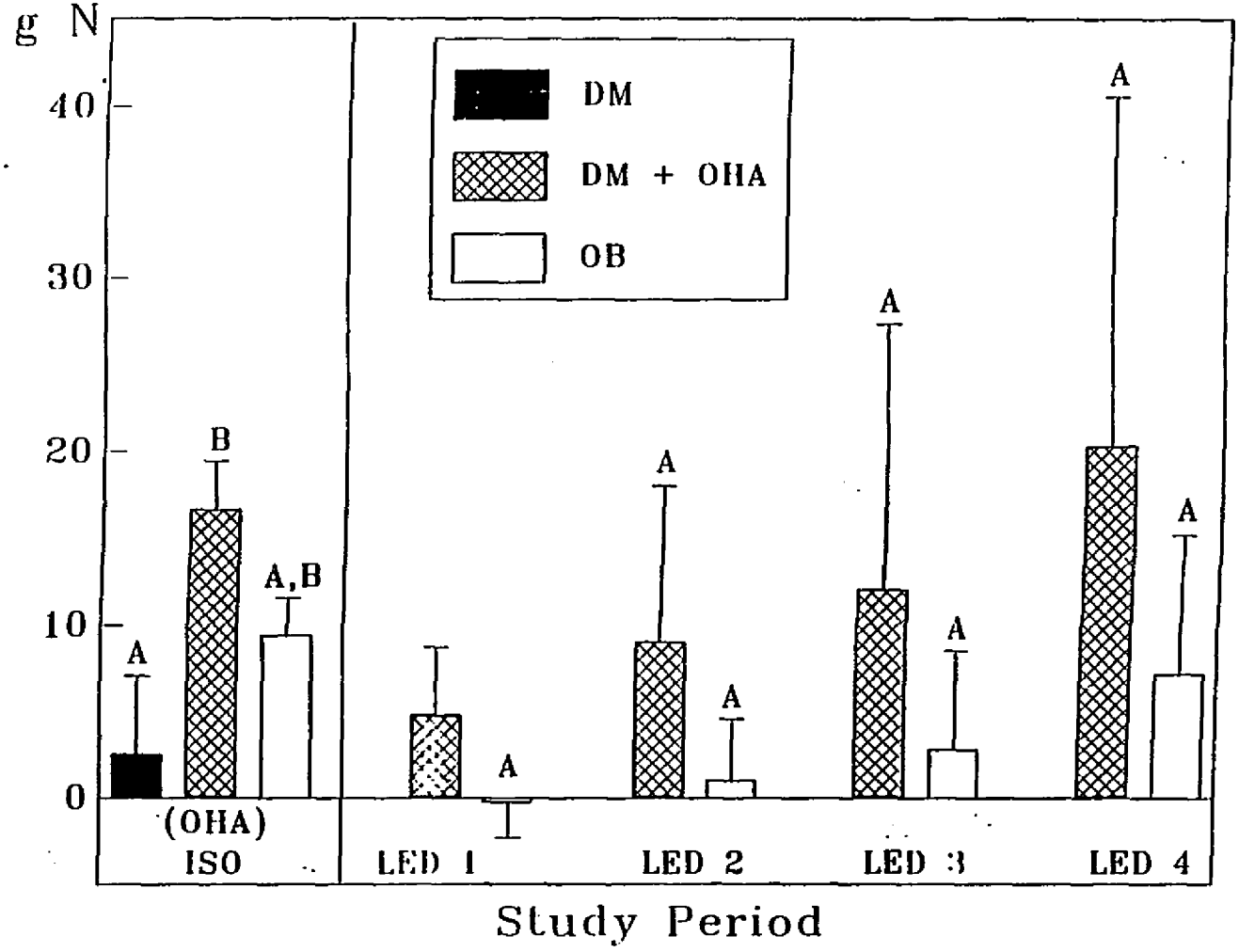


FIGURE 10 : CUMULATIVE NITROGEN BALANCE OF ISO-ENERGETIC & LOW ENERGETIC DIETS (Two-Way ANOVA; Data presented as least squares mean ± sem; n = 7 DM & 7 OB. Means with a common letter are not different at $P<0.05$at each week of the study).

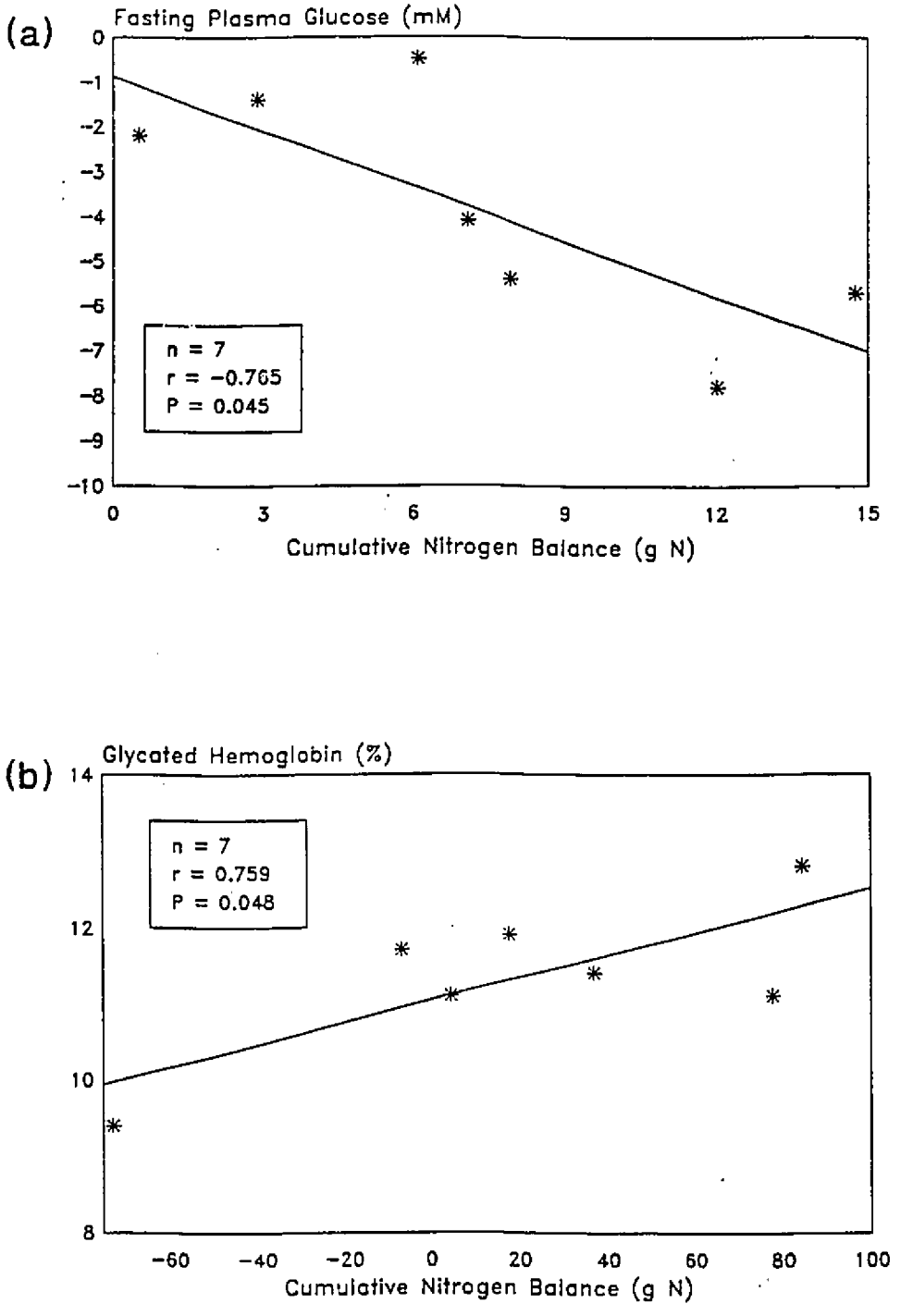


FIGURE 11 : CORRELATION BETWEEN (a) CHANGES IN CUMULATIVE NITROGEN BALANCE AND CHANGE IN FASTING PLASMA GLUCOSE DURING ISO-ENERGETIC DIET WITH & WITHOUT ORAL HYPOGLYEMIC AGENT THERAPY (DM ONLY) & (b) CUMULATIVE NITROGEN BALANCE AND GLYCATED HEMOGLOBIN PRIOR TO STUDY (DM ONLY)

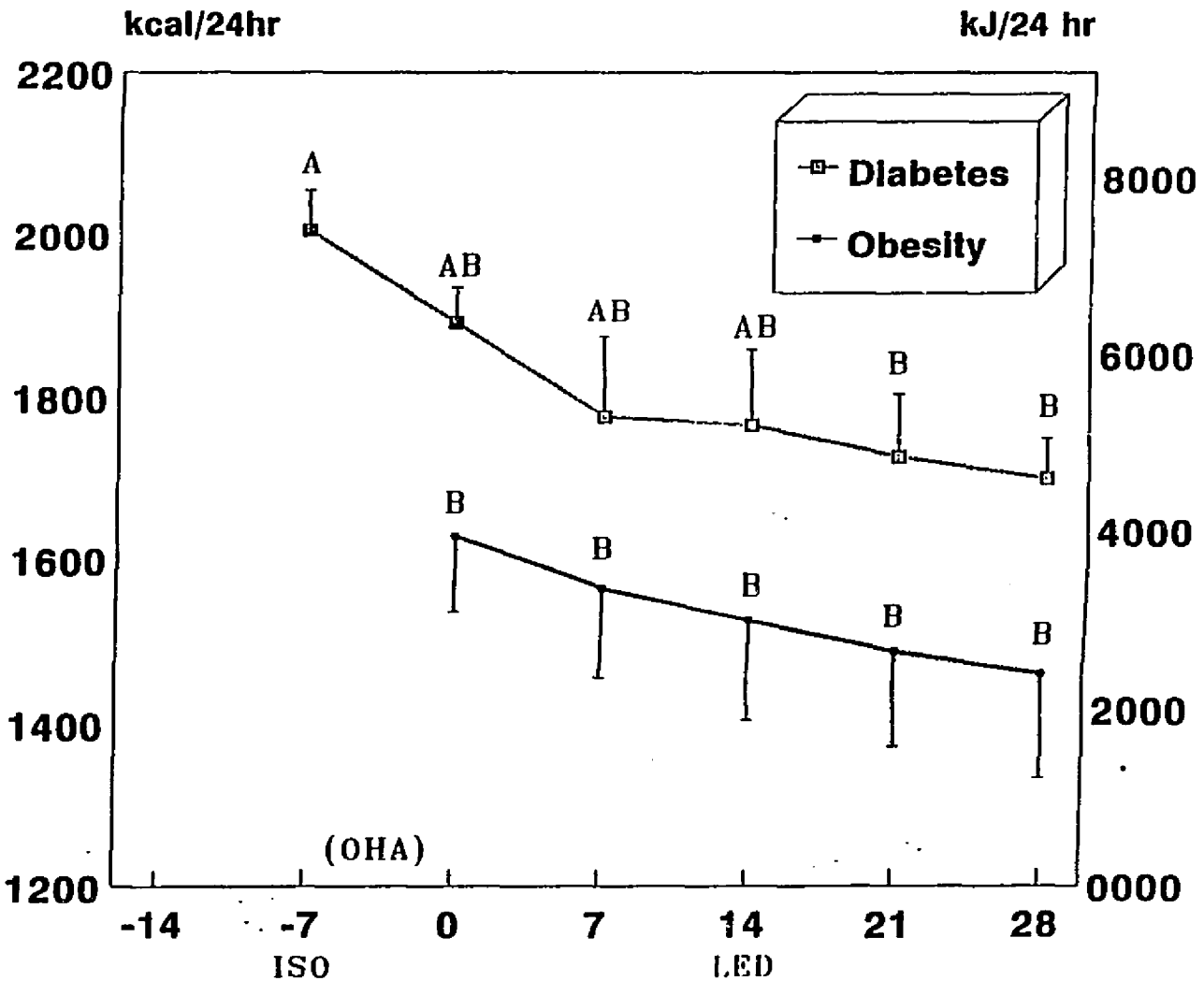


FIGURE 12 : RESTING METABOLIC RATE MEASUREMENTS OF ISO-ENERGETIC & LOW ENERGETIC DIETS (Two-Way ANOVA; Data presented as least squares mean ± sem; n = 7 DM & 7 OB. Means with a common letter are not different at $P<0.05$).

7.0 DISCUSSION OF RESULTS

The primary question addressed by this study was whether improving diabetes control would modify the abnormal protein metabolism observed in type II diabetic individuals. It was hypothesized that the degree of diabetes control determines the magnitude of abnormality of protein metabolism in obese type II diabetic individuals, and that the improvement of diabetes control would tend to return protein metabolism to that of an obese control group. This study assessed the state of protein metabolism in obese type II diabetic subjects while very hyperglycemic and mildly hyperglycemic during an iso-energetic diet and after 4 weeks of moderate energy restriction. It was found that: (1) a mild improvement of glycemia through treatment by gliclazide, which induced a significant increase in insulin secretion, (± metformin) resulted in a significant retention of nitrogen while at the same energy intake, (2) hyperglycemic diabetic subjects were in nitrogen equilibrium with the provision of generous protein intakes, (3) nitrogen equilibrium was maintained during a moderate energy restriction, also with the provision of generous protein intakes, and (4) the magnitude of cumulative protein retention during the LED was correlated with prior diabetes control.

Although insulin concentrations were slightly, but significantly greater in the diabetic subjects during ISO compared to the obese control group, one week of gliclazide treatment was sufficient to increase plasma levels further. Though insulin was higher, it was still insufficient to overcome the insulin resistance and normalize glycemia. This increase in endogenous insulin secretion, consistent with the action

of gliclazide (Campbell et al., 1991; Chiasson et al., 1991), was associated with protein retention during a period of lesser hyperglycemia. The initial glycated hemoglobin and the change in FPG correlated with nitrogen retention, thus indicating that poor diabetes control previous to exposure of generous protein intake will determine to what degree nitrogen will be retained. It has been reported that hyperglycemic type II diabetic subjects demonstrate accelerated rates of protein turnover (Gougeon et al., 1994). This accelerated rate of protein turnover may cause a protein depletion, thus rendering the subject more avid to retain nitrogen. This has been demonstrated in patients with protein-energy malnutrition (Wallace, 1959). As the subjects of the current study were matched for body composition and LBM, it is unlikely that the observed protein retention is a function of body protein status. Nor is it a function of decreased protein intake prior to the study, as a correlation between pre-study protein intake expressed as a percentage of the protein intake of the study versus the cumulative nitrogen balance over both the ISO and the LED was not significant in either the diabetic or the obese group. The positive nitrogen balance indicates that the relative rate of protein synthesis was greater than that of breakdown. This is similar to an observation made previously by Gougeon et al. (1994), in which improved glycemia was associated with a greater decrease in breakdown than synthesis, leading to a greater net protein synthesis. Our study further supports the hypothesis of Staten and associates (1986) and Welle and Nair (1990a) that even limited amounts of circulating insulin are sufficient to prevent the massive muscle wasting observed in type I diabetes mellitus, a situation of complete insulin deficiency.

Likewise our study indicates that prolonged hyperglycemia is not associated with gross alterations in nitrogen balance as the diabetic and obese subjects appear similar in body composition compartments, as indicated by %BF, waist circumference, and waist to hip ratios, which has been previously reported (Bogardus et al., 1986; Zawadzki et al., 1988; Welle & Nair, 1990a). However, body composition measurements are gross and imprecise, whereas the change in nitrogen balance was small, suggesting a subtle but nonetheless potentially important abnormality of nitrogen metabolism.

While the diabetic subjects were withdrawn from diabetes treatment, nitrogen balance remained in equilibrium despite hyperglycemia. Three factors have been proposed to be responsible for this observation. The first was that subjects were receiving sufficient energy to maintain weight, along with generous protein intakes. In previous studies where type II diabetic subjects were hyperglycemic and in negative nitrogen balance, energy and/or protein intakes were inadequate to support nitrogen equilibrium (Bistrian et al., 1986; Henry et al., 1986; Gougeon et al., 1994). Gougeon and co-workers (1994) had provided a similar iso-energetic diet with a constant protein intake of 80 g daily for 7 days to obese type II diabetic subjects withdrawn from diabetes treatment. The nitrogen balance during the last day of the iso-energetic diet was not significantly different from zero, thus indicating that nitrogen equilibrium can be achieved during hyperglycemia when energy and protein intakes are sufficient (Gougeon et al., 1994). The increased positive nitrogen balance observed in the present study is therefore most likely the result of the higher protein intake, individualized for each subject. Secondly, the level of hyperglycemia in this

study was lower than that observed by Gougeon et al. (1994) (15 $\pm$ 2.5 vs 12.9 $\pm$ 1 mM). It has been reported in type I diabetic individuals that even mild improvements of glycemia resulted in a less negative nitrogen balance (Umpleby et al., 1986). Therefore it is quite likely that lesser hyperglycemia would be associated with a lower protein breakdown in the type II diabetic population as well. Finally, the subjects in our study consumed 6 equal meals per day. It was suggested that the protein sparing effect of meal frequency is due to the lack of amino-acid storage capacity (Antoine et al., 1984). Antoine and colleagues (1984) reported a lesser nitrogen loss ($P < 0.05$) when 6 small frequent meals are consumed, rather than 3 larger ones, providing the same total daily energy content (Antoine et al., 1984). This has also previously been reported by Leverton & Gram (1949) with 2 versus 3 meals, Wu & Wu (1950) with 2 versus 4 meals daily, Durrant and associates (1980) with 3 versus 5 meals daily, and Garrow and colleagues (1981) with 1, 3, and 5 meals daily. Meal frequency may therefore explain the nitrogen equilibrium measured during ISO in the diabetic subjects. By the same token, the frequency of the meals may have further potentiated the nitrogen retention during ISO+OHA. Likewise, meal frequency may also explain the positive nitrogen balance observed in the obese control subjects during ISO.

Nitrogen equilibrium was quickly restored during the moderate energy restriction, suggesting that when the energy restriction is not as severe as with a VLED, it requires a shorter period of adaptation even if protein intakes are similar, such as in a study by Gougeon and associates (1995). In the present study, the

diabetic subjects were in positive nitrogen balance, whereas the obese control subjects remained in nitrogen equilibrium. Henry and colleagues (1986) found similar results of significantly lesser negative daily nitrogen balance in diabetic than obese subjects consuming a VLED for a period of 36 days ($P < 0.05$). However, in contrast to the present study, the cumulative nitrogen losses measured by Henry and colleagues were significantly greater in the obese group ($P < 0.05$). This could be explained by the fact that the obese subjects in the study by Henry and co-workers had significantly greater body weights, and thereby significantly greater quantities of LBM. In contrast, Gougeon and colleagues (1994) did not measure more favourable nitrogen balances in the diabetic subjects consuming a VLED; an obese control group comparable in all aspects receiving the same VLED was in fact in better nitrogen balance (Gougeon et al., 1995).

The increased excretion of urinary 3-MeHis, an index of myofibrillar proteolysis, indicates a greater rate of its breakdown during periods of poor diabetes control. This is consistent with findings in the literature of total protein catabolism in type I diabetes (Nair et al., 1983; Umpleby et al., 1986; Pacy et al., 1989) and type II diabetes (Marchesini et al., 1982; Gougeon et al., 1994). After one week of improved glycemia by OHA therapy, a 32% reduction in 3-MeHis excretion was observed. Other investigators have also found that a mild improvement in type I diabetes was associated with a reduction in protein breakdown (Umpleby et al., 1986). A decrease from 314 ± 34 to 275 ± 35 umol/d ($P < 0.05$)in 3-MeHis excretion was observed upon improvement of glycemia in the study by Marchesini and co-

workers (1982). The reduction in urinary 3-MeHis excretion in our study upon improvement of glycemia, represents daily values that are significantly less than that of Marchesini and colleagues (1982). The differences between the present study and that of Marchesini and associates may be explained by two factors: (1) the 3-MeHis content of the study diet and (2) the means by which glycemia was improved. Marliss and associates (1979) studied the effects of varying energy and protein (beef & poultry) intakes on 3-MeHis excretion and found that 3-MeHis excretion correlated strongly with protein intake ($r=0.93$; $P<0.001$), thus stressing the importance of quantifying dietary 3-MeHis intakes if meaningful conclusions are to be drawn from its excretion. In the study by Marchesini et al. (1982), mentioned previously, subjects were consuming 150 g/d of lean beef, which provided from 1.8 to 13.3 umol methylhistidine per gram protein (Asatoor & Armstrong, 1967; Rangeley & Lawrie, 1976; Block et al., 1985). In the present study, subjects consumed a meat-free diet that did not contain sources of 3-MeHis. The only non-formula sources of protein were the milk and cereal which were consumed daily. Milk was found not to contain 3-MeHis or methylhistidine derivatives (Block et al., 1985). Likewise, bread was also found not to contain methylhistidine, and therefore it is quite unlikely that the bran cereal provided to our patients was not methylhistidine free (Block et al., 1985). It could be argued that meaningful conclusions regarding 3-MeHis excretion could still have been drawn from the present study had either of these two products contained methylhistidine derivatives, as all subjects received the same quantity of milk and cereal throughout the entire LED. Furthermore, as the study was tightly controlled

for intake, the dietary methylhistidine intake could have been calculated if required. The second factor for the differences in methylhistidine values with the improvement in glycemia possibly lies within the means by which each study improved glycemia. In the present study, dietary treatment remained unchanged, and thus the only difference was the improvement of glycemia by the OHA. Marchesini and colleagues (1982) measured 3-MeHis excretion as subjects entered the study with poorly controlled diabetes. Glycemia was improved by OHA therapy along with diet therapy of 26-30 kcal/kg BW, translating into energy and protein intakes of 7500 to 8800 kJ (1800 to 2100 kcal) and 150 g protein from lean beef daily. Therefore, the decrease in excretion may be the result of improved diabetes control and/or the result of moderate energy restriction. The possibility that energy restriction was responsible for lower 3-MeHis excretion is consistent with the findings of Winterer and co-workers (1980). Decreases in 3-MeHis excretion during a period of energy restriction have been reported by Winterer and co-workers (1980). In contrast, a VLED was found not to change urinary 3-MeHis excretion (Garlick et al., 1980). Garlick and co-workers (1980) attributed this to a sufficient protein intake that was able to maintain normal rates of protein turnover. Our study supports that of Garlick and associates (1980) as 3-MeHis excretion remained unchanged with the LED, indicating the protein intake was sufficient and energy restriction was not as severe.

The mild improvement in glycemia within 1 week of OHA therapy was associated with a significant reduction ($P < 0.05$) in RMR, to values that were no longer different from those of the obese controls. Increased RMR may arise from

one or several mechanisms, namely increased protein turnover (Nair et al., 1984), increased sympathetic nervous system activity (Bogardus et al., 1986), increased substrate cycling (Efendic et al., 1982; Sheppard et al., 1983; Zawadzki et al., 1988)), and/or abnormal mitochondrial oxidative-phosphorylation (Bogardus et al., 1986). The increased RMR during periods of hyperglycemia can partly be explained by the increased protein breakdown and synthesis, as a decrease in protein breakdown was associated with improved glycemia. Increased rates of protein synthesis have been reported to occur during periods of hyperglycemia at the level of the splanchnic tissues (Wahren et al., 1976; Nair et al., 1995), gut (Garrow & Hawes, 1972), and the liver (Pain & Garlick, 1974). Increased rates of hepatic substrate cycling have been reported in hyperglycemic type II diabetes (Efendic et al., 1982; Sheppard et al., 1983; Zawadzki et al., 1988). It has also been demonstrated that endogenous glucose production rates, but not glucose recycling rates, in obese type II hyperglycemic diabetic subjects approach that of obese non-diabetic subjects as glycemia is improved (Zawadzki et al., 1988). This was similarly observed in our study, although indirectly. During periods of hyperglycemia, the net conversion of glucogenic amino acids into glucose occurs very actively. The deamination of these amino acids results in large amounts of urea, which is then excreted into the urine (Lehninger, 1982). The total body pool of urea was unchanged, but a significant decrease in urinary urea was observed. It therefore goes to reason that hepatic gluconeogenesis was reduced, an energetically costly process. Increased sympathetic nervous system activity has not been reported in diabetic subjects (Bogardus et al., 1986), but when hyperglycemia

is severe, especially with ketoacidosis, norepinephrine increases (Christenson et al. Elevated levels of FFA have been postulated to indirectly increase RMR by uncoupling mitochondrial oxidative phosphorylation (Himms-Hagen, 1976). This however is not likely to be the case as plasma FFA concentrations were not significantly greater in the diabetic subjects than in the obese controls during the ISO diet. Plasma concentrations however do not reflect production and clearance rates which may very well be elevated. Increased rates of FFA turnover have been reported in android obesity (Björntorp, 1987), and may be associated with increased gluconeogenesis (Bogardus et al., 1984). As the subjects from both groups were similar in waist circumference and waist to hip ratio and displayed android obesity, it is unlikely that one group would exhibit greater rates of turnover because of differences in fat distribution. The acetyl CoA formed from fatty acid oxidation will condense with oxaloacetate to enter the citric acid cycle if fat and carbohydrate degradations are appropriately balanced. Hepatic acetyl CoA faces a different fate in situations where fat breakdown predominates. Two such situations exist in our study: (1) the state of poor diabetes control and (2) the moderate energy restriction. Firstly, if carbohydrate is unavailable, or improperly used, the concentration of available oxaloacetate is decreased as it is being used to form glucose. Secondly, with energy restriction wherein fat breakdown predominates, FFA concentrations increase due to increased rates of lipolysis. This was observed in our study by decreases in %BF during the LED. These two situations caused elevations in acetyl CoA which can either lead to increased hepatic ketone production or make use of the pyruvate,

from the breakdown of muscle, to form lactate leading to the re-conversion of glucose at the hepatic level. This second option is an energetically costly, yet futile, cycle as glucose from the muscle is being used to make glucose in the liver. Therefore, it seems reasonable to conclude that as the diabetic and obese subjects were matched, the increased RMR observed during periods of hyperglycemia can be explained in part by increased rates of protein breakdown, increased rates of protein synthesis, and hepatic cycling. As glycemia improved, rates of protein turnover would be less elevated, contributing to the observed decrease in RMR.

Resting metabolic rate has been reported to decrease by up to 20% during severe energy restriction (Bray, 1969; Apfelbaum et al., 1971; Garrow et al., 1978; Doré et al., 1982; Welle et al., 1984; Hendler & Bonde, 1988; Gougeon et al., 1994; Gougeon et al., 1995). Foster and colleagues (1990) observed that realimentation after a VLED with a balanced deficit diet aided in the recovery of RMR, thus providing evidence of a metabolic advantage of weight loss with a less severe energy restriction. The decrease is commonly attributed to a reduction in the active tissue of the body (Shetty, 1990). Since LBM did not change significantly throughout the course of the study, one could expect that RMR would not change either, thus suggesting that an energy restriction which does not induce a loss of LBM would not induce a decrease in RMR. However, RMR was found to decrease by 10% in both the diabetic group (from ISO+OHA) and in the obese group (from ISO) after 4 weeks of moderate energy restriction, but this did not reach statistical significance. When the RMR data for the present study are analyzed in a two-way ANOVA with

repeated measures design, the decreases are found not to be statistically significant using the least-squares means method. In contrast, when the data are analyzed separately for the diabetic and the obese group, and the differences within each group identified by the Student-Newman-Keuls test, significant differences are detected. This difference in statistical findings can be attributed to the difference in statistical procedures. Firstly, the two-way ANOVA allows for a greater number of subjects to be entered into analysis, and thereby increasing the statistical power. Secondly, the obese subjects served as controls for the diabetic group and therefore these control subjects need to be entered into analysis. Thirdly, the least squares means were only used to identify significant difference if the ANOVA yielded a significant F-value. This is commonly referred to as the protected least squares means method (Snedecor, 1980). The protected least squares means has a good control of Type I errors, more so than the Student-Newman-Keuls method (Snedecor, 1980). A Type I error is the probability of asserting that a difference exists when no such difference exists (Mendenhall, 1975; Snedecor, 1980; Ferguson, 1981). The protected least squares means has been considered to be a close competitor for the Duncan method for multiple comparisons, requiring just slightly more effort (Snedecor, 1980). Ferguson (1981) ranks the Duncan, Student-Newman-Keuls, Tukey, then Scheffé methods of multiple comparisons from low to high, in terms of per-comparison Type II error. A Type II error is the probability of asserting there is no difference when in fact a difference does exist (Mendenhall, 1975; Snedecor, 1980; Ferguson, 1981). It therefore stands to reason that the differences detected by

the Student-Newman-Keuls may perhaps be type I errors.

A more moderate energy restriction has been shown to produce a decrease in RMR as well, but not one as dramatic as that produced by VLED's (Ravussin et al., 1985; Froidevauz et al., 1993). The decrease observed in our study is similar to the ones reported in the literature (Ravussin et al., 1985; Froidevaux et al., 1993). RMR was found to decrease by 10% in both study groups from the last day of iso-energetic feeding to the end of the LED. This represented decreases of 795 & 703 kJ (190 & 168 kcal)/d over a 4 week period, or 112 & 135 kJ (27 & 32 kcal) for every kilogram of body weight lost in the diabetic and obese groups, respectively. Decreases of 670 kJ (160 kcal)/d have been documented over a 10 to 16 week period of continued energy restriction (Ravussin et al., 1985), which represented a decrease of 9% which was not significant when expressed on a kg LBM basis. Froidevaux and colleagues (1993) found that every kilogram of body weight lost resulted in a decrease of 24hr energy expenditure by 107 kJ/d (26 kcal/d). Two factors other than a loss of LBM can contribute to a decrease in RMR: (1) a decrease at the periphery in the conversion of T_4 to triiodothyronine (T_3), with increases in reverse T_3 due to a decreased metabolic clearance (Shetty et al., 1979) and (2) a metabolic adaptation to energy restriction which is less clearly understood (Shetty, 1990; Hendler & Bonde, 1988). It has been shown regularly that energy restriction induces a reduction in the metabolically active form of thyroid hormone, namely T_3 (Gelfand & Hendler, 1989; Hendler & Bonde, 1988). The concept however that T_3 holds a dominant role in this decrease in RMR remains controversial. Phinney and co-workers (1988) found such

a fall in T_3 that corresponded to decreases in RMR, whereas Barrows & Snook (1987) & Hendler & Bonde (1988) both were unable to find such a correlation. It appears that dietary carbohydrate plays an important role in blunting the decrease in T_3 levels during weight reduction (Spaulding et al., 1976; Azizi, 1978; Hendler et al., 1986; Mathieson et al., 1986). Therefore, a small decrease in thyroid hormone activity could have been expected during the current study, but not one as large during an all-protein VLED, thereby partly explaining the differences in the magnitude of such a decrease in RMR. What remains unknown are the effects of a moderate energy restriction on RMR once the energy restriction is halted and subjects begin a weight maintaining diet. Foster and colleagues (1990) studied the effects of realimentation with a balanced deficit diet of 5000 kJ (1200 kcal)/d diet after 2 months of a VLED, and found that the balanced deficit diet aided in the recovery of RMR and returned RMR to values no longer significantly different from those prior to the VLED. These observations provide evidence of a metabolic advantage of weight loss from a less severe energy restriction. Thus, it is likely that the RMR would return to baseline values observed in the diabetic subjects during ISO+OHA and the obese subjects during ISO.

The improvement of diabetes control has been documented to improve lipid profiles (Hagan & Wylie-Rosett, 1989). Individuals with type II diabetes often have elevated triglyceride concentrations. This is usually attributed to the overproduction and defective lipolysis of triglycerides. Overproduction may be the result of increased FFA and glucose concentrations, both which stimulate triglyceride synthesis.

Defective lipolysis may result from a relative insulin deficiency, as insulin potentiates lipoprotein lipase production. It has also been reported that energy restriction will lower serum cholesterol concentrations (Hagan & Wylie-Rosett, 1989). Similarly in our study, 1 week of OHA therapy was sufficient to induce decreases in triglyceride and cholestrol concentrations in the diabetic study group. Total cholesterol concentration decreased with moderate energy restriction in the obese group.

The LED was not as ketogenic as the VLED's previously used in the treatment of diabetes and/or obesity. Blood 3-OH concentrations during the moderate energy restriction were significantly lower than those reported in the literature during a severe energy restriction (Gougeon et al., 1992; Gougeon, 1992; Gougeon et al., 1994). Urinary ammonium excretion and serum bicarbonate have been shown to increase in response to ketosis to play the role of a buffer in assisting the excretion of the ketoacids (Gougeon & Marliss, 1989). These increases were not observed in the present study, however serum uric acid increased in the diabetic group over the LED. This is proposed to be associated with the increased 3-OH concentrations, as these two acids compete at the level of the kidneys for excretion (Gougeon & Marliss, 1989).

The effects of nutrition on maintaining immune function have been documented (Corman, 1985); the effects of energy restriction however have not. It has been reported that obese subjects consuming an all-protein VLED demonstrates small but significant decreases in leukocytes, neutrophils, lymphocytes, and monocytes (Field et al., 1991). Lymphocyte count was found to return to baseline values after

4 weeks of the VLED (Field et al., 1991) In contrast, once leukocyte, neutrophil, and monocyte counts decreased, the values remained at these levels (Field et al., 1991). Wing and co-workers did not observe a decrease in WBC during a total fast of 10 to 14 days duration (1983). Leukocyte count was found to decrease in situations of prolonged fasting (3 - 9 weeks), primarily due to the significant decrease in neutrophils (Drenick & Alvarez, 1971). However, the effect of decreases in these parameters that remain within normal limits on immune function is not known (Field et al., 1991). Field and co-workers (1991) concluded that the study did justify the use of caution in the prolonged usage of severely energy restricted diets that cause protein-energy malnutrition and could further compromise immune function. In the current study, lymphocyte, neutrophil, and WBC counts remained within normal limits during the entire study. Furthermore, subjects did not experience protein-energy malnutrition as subjects remained in nitrogen equilibrium, the weight loss being due primarily to losses in adipose tissue. However, these parameters were only measured for a period of 4 weeks of moderate energy restriction, thus indicating that some caution be exerted if moderate energy restriction is to be prolonged.

It has been reported than men subjected to energy restriction will lose more weight than women. This is attributed to the greater amount of LBM commonly found in men (Cohn et al., 1984). Weight loss and nitrogen balance were therefore prone to be skewed by the inclusion of 2 men in the diabetic group and none in the obese control group. The men were included into analysis as (1) both the diabetic and obese groups were comparable in body weight and LBM (2) their weight patterns

fell into the range of weight loss of the diabetic group, (3) likewise, cumulative nitrogen balances were analyzed with and without the 2 male subjects and non-significance was not altered. Furthermore, it was very difficult to recruit subjects for a minimum of 5 weeks of in-patient hospitalization. The current study forms a smaller part to a larger on-going study, in which both diabetic and obese male subjects will continue to be recruited. Prior to data analysis for future publications, it may be necessary to separate the men and women subjects of both group for several reasons. The first is that a man and a woman of the same height will receive the identical protein intake. It is well documented however that men have greater quantities of LBM than women (Cohn et al., 1984), even though LBM is related to the cube of height (Forbes, 1988). It stands to reason that the men will receive a lower proportion of protein to total energy intake and this may tend to a less favourable nitrogen balance. Energy requirements calculated for weight maintenance will be different for a man and women of equal weight, height, and age. A man of 45 years, 90 kg, and 170 cm would require, according to our calculations 11.5 MJ (2765 kcal)/d, a woman would require 10.0 MJ (2400 kcal)/d. The men when subjected to a 50% energy deficit will undergo a greater negative energy balance, and lose more body weight.

Nitrogen flux has been reported to be unchanged during either the luteal or follicular phase of the menstrual cycle by Gougeon and coworkers (1995) and protein turnover was observed to be unaffected by the menstrual cycle (Garrel et al., 1985). Nitrogen retention has been shown to be greater during the follicular phase

and excretion during the luteal phase (Calloway & Kurzer, 1982). Twelve female subjects participated in the study of which 2 diabetic and 2 obese control subjects had regular menstrual cycles. Daily nitrogen balance was not different between the luteal and follicular phases of the menstrual cycle. Cumulative nitrogen balance similarly is not affected by the menstrual cycle for two reasons; (1) firstly daily nitrogen balance was not affected and (2) had daily nitrogen balance been affected by the menstrual cycle, the effects of the menstrual cycle would not have been assessed as the LED covered a period of 28 days, one complete menstrual cycle of follicular and luteal phases. Likewise, it has been reported that RMR increases during the follicular phase of the menstrual cycle (Solomon et al., 1982), as well as 24hr energy expenditure by 8 to 16% (Webb, 1986) due to increased levels of progesterone from the corpus luteum. Other investigators however were unable to detect any changes in RMR due to the menstrual cycle (Gougeon et al., 1995). Likewise in our study, we were unable to detect any increases in RMR during the follicular phases of the four women studied.

We were able to show that the changes in body composition (LBM & TBW) measured by BIA were consistent with those measured by cumulative nitrogen and sodium balances. Likewise, in the diabetic group during ISO when cumulative sodium balance was negative. BIA detected less TBW than during the ISO+OHA in which rehydration occurred. BIA determinations in the current study were able to detect small losses of LBM and TBW over the 4 week LED. These findings are in contrast to those of Deurenberg and colleagues (1989), who reported that BIA

measurements after weight loss overestimates LBM. This was attributed to the inability of BIA to detect changes in TBW associated with glycogen depletion. However, the likelihood that the subjects of that study exhibited glycogen depletion is debatable, as subjects consumed 4200 kJ (1000 kcal)/d diet over an 8 week period. The mean weight loss was 10.0 $\pm$ 2.8 kg, loss of LBM was measured to be 2.3 $\pm$ 1.7 kg by densitometry and 0.6 $\pm$ 1.9 kg by BIA. Deurenberg and co-workers (1989) stated that BIA demonstrated that 94 $\pm$ 17% of the weight loss was due to loss of fat mass, and further stated that this was an unrealistically high percentage. As no details are provided regarding the experimental diet or nitrogen balance, it is difficult to determine whether these subjects were in nitrogen equilibrium, and hence the composition of weight loss. In the current study, nitrogen equilibrium was maintained, and therefore the weight loss is primarily fat tissue, our BIA measurements consistently showed a reduction in %BF. The use of the equation by Kushner and associates (1990) demonstrated that BIA was able to detect changes in TBW, LBM, and %BF that are to be expected during moderate energy restriction.

There appears from preliminary analysis to be a "dose-response" relationship between plasma glucose levels and the derangement of protein metabolism. Control of glucose metabolism is a complex process, and resistance to insulin affects different aspects of glucose metabolism (a known consequence of type II diabetes). Therefore, these abnormalities in glucose and protein metabolism should be quantified in situations of type II diabetes, as well as normal and impaired glucose tolerance.

8.0 SUMMARY & CONCLUSIONS

Diabetes mellitus has long been identified as a disease of altered carbohydrate metabolism, the hallmark of this disease being hyperglycemia. However, aberrations in lipid and protein metabolism exist in this condition as well. Prolonged hyperglycemia in the type II diabetic population does not appear to result in the gross muscle emaciation observed in type I diabetes due to the low levels of circulating insulin. Glycemia was improved after 1 week of gliclazide (± metformin) treatment which induced a significant increase in insulin secretion. The improvement of glycemia with OHA was associated with an increased nitrogen retention, decreased 3-MeHis excretion, and normalization of RMR to values observed in the obese control group. A moderate energy restriction with continued OHA usage over a period of 4 weeks induced weight loss, primarily due to adipose tissue, and normalized glycemia in most subjects. Nitrogen balance was not different between the diabetic and obese subjects during this period. Resting metabolic rate decreased over the four week period of moderate energy restriction, on the average by 10%, however this did not reach statistical significance in either study group. The present study therefore indicates that the conventional treatment of type II diabetes mellitus consisting of OHA and dietary intervention was able to improve nitrogen balance and normalize urinary 3-MeHis excretion in this disease.

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APPENDIX I

Are You Overweight? Do You Have Diabetes?

The McGill Nutrition Center is conducting a research study that offers:

- weight loss
- improved diabetes control
- follow-up treatment

If you are 18-60 years old, 45-100 pounds overweight and willing to stay in the Royal Victoria Hospital for 5-7 weeks please call 842-1231 local 5378, 4127, or 5877

APPENDIX II

McGill Nutrition and Food Science Centre
Centre de nutrition et des sciences de l'alimentation de l'Université McGill

(514) 843-1665 Fax: (514) 982-0893

PROTEIN METABOLISM IN TYPE II DIABETES

Dear Colleague,

We are currently recruiting obese volunteers for a research project investigating the effects of diabetes control on protein metabolism in Type II diabetes. We are rather desperate for suitable subjects and greatly appreciate any assistance you may be able to provide in referring patients from your practice.

To be eligible, subjects must be:

- diabetic and 40 to 100 lbs overweight
- 18 to 60 years of age
- non-smokers (or willing to quit)
- free of other serious illnesses
- able to remain at the Royal Victoria Hospital for 6 weeks

STUDY PROTOCOL

WEEK	1	2	3 to 6
STUDY DIET	ISO-ENERGETIC (Weight Maintaining)		VERY LOW or LOW ENERGY DIET (25 - 50% of iso-energetic)
DIABETES THERAPY	None	Oral Hypoglycemic Agent or Insulin	Oral Hypoglycemic Agent or Insulin
GLYCEMIA	Moderate Hyperglycemia	Mild Hyperglycemia or Euglycemia	Euglycemia

BENEFITS

- Dietetic consultation
- Weight loss
- Improved diabetes control
- Blood glucose normalization

Any interested subjects are welcomed to contact the Clinical Investigation Unit of the Royal Victoria Hospital at #842-1231 extension 5378 or 5877 or leave a message at 4127 between 8AM and 4PM.

It is also clearly understood that should any of your patients elect to participate in the study, upon completion they will return to the care provided by you at your clinic.

Should you require any further information, please do not hesitate to contact us as #843-1665.

Réjeanne Gougeon, Ph.D. Karin Styhler P.Dt. Errol B. Marliss, M.D.

Postal Address: Crabtree Nutrition Laboratories.
Royal Victoria Hospital, 687 Pine Avenue West, Montreal, Quebec, Canada H3A 1A1
Adresse postale: Laboratoires de nutrition Crabtree.
Hôpital Royal Victoria, 687 ouest, avenue des pins, Montréal, Québec, Canada H3A 1A1

DO YOU HAVE DIABETES?

DO YOU HAVE SOME WEIGHT TO LOSE?

The McGill Nutrition and Food Science Centre is looking for volunteers to participate in a research project on diabetes and low-calorie weight reducing diets.

If YOU are

- diabetic & overweight by 40 to 100 lbs
- 18 to 60 years of age
- non-smoker (or willing to quit)
- free of other serious illnesses

You are welcomed to participate in a 6 to 7 week in-hospital study at the Royal Victoria Hospital. For further information please call # 842-1231, extension 5378 or 5877 or leave a message at 4127 between 8AM and 4PM.

BENEFITS

- Therapeutic support & lifetime follow-up
- Dietetic consultations
- Weight loss
- Improved diabetes control
- Blood glucose normalization

#842-1231 ext 5378 or 5877 4127 (message)

#842-1231 ext 5378 or 5877 4127 (message)

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#842-1231 ext 5378 or 5877 4127 (message)

APPENDIX IV

CONSENT FORM

PROTEIN METABOLISM STUDY IN TYPE II DIABETES

I have been invited by Dr. E.B. Marliss and/or Dr. R. Gougeon to take part in a study of the effect of diabetes (and high blood glucose) on metabolism of protein during a weight maintaining and (if I am overweight) a weight reducing diet. My participation may require a total stay in hospital of up to 45 days and will include:

1) Admission to the Clinical Investigation Unit of the Royal Victoria Hospital where routine admission examinations will be carried out: history and physical examination, standard blood and urine tests, chest X-ray and electrocardiogram (unless performed prior to admission).

2) A period of 17 days during which I shall be given a liquid formula diet in amounts sufficient to meet my requirements in energy (calories) and nutrients, with no diabetes medication or insulin from days 1-7 followed by oral hypoglycemic agent therapy during days 7 to 17, to improve my blood glucose. Depending on the severity of my diabetes, I may receive such medication in small doses the first 1-7 days, and larger doses thereafter. My blood glucose will never be allowed to rise to levels at which I am very uncomfortable or that are considered dangerous for the 7-day period.

3) For the subsequent 4 weeks, I shall receive a low energy high protein liquid formula diet taken as 6 meals per day at designated times. I will also take a vitamin and mineral tablet and water for a total of 1500 to 2500 ml (50-80 ounces) of fluid per day.

   The oral hypoglycemic agent will be gradually decreased (and discontinued if possible) in a manner required to maintain normal blood glucose during the low energy diet.

4) On 3 occasions, that is at the end of weeks 1 and 2 and during the sixth week, I shall take part in a test that will last 60 hours. The test will start at 8:00 a.m. At that time I shall be given an amino acid dissolved in 5 ml of water. I understand that this amino acid called ^{15}N-glycine is an isotope (a special form of the amino acid) and is without risk since it is found in low concentrations in foods usually eaten; also this isotope is widely used in research tests such as this one. It is not radioactive. Every 3 hours for the next 60 hours I shall consume 5 ml (1 teaspoon) of the solution of glycine. At the same time I shall be asked to urinate in special containers that will be used for 3-hour urine collections only. A nurse and the investigator will be assisting me with this test. I understand that this procedure will cause my sleep to be interrupted every three hours over two consecutive nights, three times during my stay.

On 3 occasions, on the second day the 3 studies with ^{15}N-glycine, an intravenous catheter will be inserted in my arm for blood sampling during the day. 7 mL of blood will be taken every 3 hours before meals from 8 AM to 8 PM.

5) During my stay in hospital, I shall participate in a weekly group session for training in behavior modification. These sessions will focus on the teaching of principles to bring about changes in my life style and attitudes towards food, my body, my relationships and my commitment to exercise. I will be invited to attend these weekly sessions which continue after discharge.

6) Every weekday I shall participate in a physical conditioning session that includes warming up, stretching, and cooling down exercises, as well as a 40-minute session on a stationary bicycle, programmed to sustain a tolerable effort or a brisk walk on the hospital grounds.

7) All my urine will be collected in special containers provided for me. These urine collections must be carefully and completely done. I will be weighed each morning in bed clothes after voiding. My blood pressure and heart rate will be checked by the nurses. I will check my urine for glucose and ketones every morning. Blood tests will be done on a weekly or twice-weekly basis. I will be taught the methods for measuring my own blood glucose using reagent strips and a reflectance meter (e.g., Chemstrips and Accuchek meter) and will make such measurements up to 6 times per day during the ^{15}N-glycine studies. This involves a fingerprick using a lancet, which causes mild discomfort.

8) At the beginning and once a week thereafter, circumference of my waist and hips, my body fat content by bioelectrical impedance, non-invasive, harmless methods, which takes 5 minutes and consists in putting electrodes on my hand and foot while I am in a supine position will be measured.

9) I shall be asked to complete a self-rating symptoms scale that lists positive feelings, as well as problems and complaints that I may experience.

10) Every morning I will blow into a "diet monitor" machine to measure my breath acetone levels.

11) Every week, my resting metabolic rate will be measured in the study room of the Nutrition Centre. I will be asked to breathe under a clear plastic canopy that covers my head and shoulders for 20 minutes. On 3 occasions, the thermic effect of a meal will be measured. I will be given food, and will remain underneath the canopy for 6-7 hours or less, with 20-minute breaks every hour. During that time an intravenous catheter will be inserted for blood sampling.

The risks involved in consuming the diets and in blood sampling are considered to be minimal when carefully selected patients are studied in this manner in hospital. I may experience some symptoms associated with the diets, including light headedness, changes in my bowel movements, or coldness, but these are usually mild and temporary. The intravenous catheter used is the standard kind used in the

ments up to 6 times per day during the ^{15}N-glycine studies. This involves a fingerprick using a lancet, which causes mild discomfort. This will allow the investigator to compare how blood glucose evolves during the day in obesity with and without diabetes.

8) At the beginning and once a week thereafter, circumference of my waist and hips, and my body fat content will be measured by bioelectrical impedance, a non-invasive, harmless method, which takes 5 minutes and consists in putting electrodes on my hand and foot while I am in a supine position.

9) I shall be asked to complete a self-rating symptoms scale that lists positive feelings, as well as problems and complaints that I may experience.

10) Every morning I will blow into a "diet monitor" machine to measure my breath acetone levels.

11) Every week, my resting metabolic rate will be measured in the study room of the Nutrition Centre. I will be asked to breathe under a clear plastic canopy that covers my head and shoulders for 20 minutes. On 2 occasions, the thermic effect of a meal will be measured. I will be given food and will remain underneath the canopy for 6-7 hours or less, with 20-minute breaks every hour. During that time an intravenous catheter will be inserted for blood sampling.

The risks involved in consuming the diets and in blood sampling are considered to be minimal when carefully selected patients are studied in this manner in hospital. I may experience some symptoms associated with the diets, including light headedness, changes in my bowel movements, or coldness, but these are usually mild and temporary. The intravenous catheter used is the standard kind used in the case of hospitalized patients. There may be slight pain or discomfort while introducing them. There is always a slight risk of bruising with any blood test. The amount of blood drawn over the entire study will not exceed that in an ordinary blood donation.

12) During the study, I shall stay on the ward unless given a special pass to leave for short periods.

Although the diet for weight reduction are expected to benefit me, the other procedures, including the 7 days on a weight maintaining diet, are not expected to provide any direct benefit to me. However, it is hoped that the information obtained will lead to the advancement of scientific knowledge. The data obtained will be treated confidentially and it will not be possible to identify me personally in any publication of the results. Any questions I may have about the diet and the study results will be answered.

I _____ **consent to be a subject in this project.**

I am free to withdraw from the study at any time. Early termination of this project, for any reason, will not compromise my medical care.

Dated at Montreal, this _____ day of _____ 19__.

WITNESS _____ SUBJECT _____
(signature) (signature)

INVESTIGATOR _____
(signature)

CONSENT FORM
PROTEIN METABOLISM STUDY IN TYPE II DIABETES:
OBESE NONDIABETIC SUBJECT

I have been invited by Dr. E.B. Marliss and/or Dr. R. Gougeon to take part in a study of the effect of diabetes (and high blood glucose) on metabolism of protein during a weight maintaining and (if I am overweight) a weight reducing diet. My participation, as an obese control subject without diabetes, and with normal blood sugar levels, may require a total stay in hospital of 35 days and will include:

1) Admission to the Clinical Investigation Unit of the Royal Victoria Hospital where routine admission examinations will be carried out: history and physical examination, standard blood and urine tests, chest X-ray and electrocardiogram (unless performed prior to admission) and a glucose tolerance test for which I'll be given 75g of glucose, a catheter will be inserted and blood sampling will be taken at 0, 30, 60, 90, 120 and 180 minutes.

2) A period of 7 days during which I shall be given a liquid formula diet in amounts sufficient to meet my requirements in energy (calories) and nutrients.

3) For the subsequent 4 weeks, I shall receive a low energy protein liquid formula diet taken as 6 meals per day at designated times. I will also take a vitamin and mineral tablet and water for a total of 1500 to 2500 ml (50-80 ounces) of fluid per day.

4) On 2 occasions, that is at the end of week 1 and during the fourth week, I shall take part in a test that will last 60 hours. The test will start at 8:00 a.m. At that time I shall be given an amino acid dissolved in 5 ml of water. I understand that this amino acid called ^{15}N-glycine is an isotope (a special form of the amino acid) and is without risk since it is found in low concentrations in foods usually eaten; also this isotope is widely used in research tests such as this one. It is not radioactive. Every 3 hours for the next 60 hours I shall consume 5 ml (1 teaspoon) of the solution of glycine. At the same time I shall be asked to urinate in special containers that will be used for 3-hour urine collections only. A nurse and the investigator will be assisting me with this test. I understand that this procedure will cause my sleep to be interrupted every three hours over two consecutive nights, twice during my stay.

   On 2 occasions, on the second of the the 2 studies with ^{15}N-glycine, an intravenous catheter will be inserted in my arm for blood sampling during the day. 7 mL of blood will be taken every 3 hours before meals from 8 AM to 8 PM.

5) During my stay in hospital, I shall participate in a weekly group session for training in behavior modification. These sessions will focus on the teaching of principles to bring about changes in my life style and attitudes towards food, my body, my relationships and my commitment to exercise. I will be invited to attend these weekly sessions, which continue after discharge.

6) Every weekday I shall participate in a physical conditioning session that includes warming up, stretching, and cooling down exercises and a 40-minute session on a stationary bicycle programmed to sustain a tolerable effort.

7) All my urine will be collected in special containers provided for me. These urine collections must be carefully and completely done. I will be weighed each morning in bed clothes after voiding. My blood pressure and heart rate will be checked by the nurses. I will check my urine for ketones every morning. Blood tests will be done on a weekly basis. I will be taught the methods for measuring my own blood glucose using reagent strips and a reflectance meter (e.g., Chemstrips and Accuchek meter) and will make such measure-

case of hospitalized patients. There may be slight pain or discomfort while introducing them. There is always a slight risk of bruising with any blood test. The amount of blood drawn over the entire study represents an ordinary blood donation.

There is a risk of low blood glucose with the oral agent therapy, but my blood glucose will be monitored to prevent hypoglycemia and I will, at all times, be under the supervision of medical and nursing personnel.

12) During the study, I shall stay on the ward unless given a special pass to leave for short periods.

Although the diet for weight reduction and the oral agent therapy are expected to benefit me, the other procedures, including the 17 days on a weight maintaining diet, are not expected to provide any direct benefit to me. However, it is hoped that the information obtained will lead to the advancement of scientific knowledge. The data obtained will be treated confidentially and it will not be possible to identify me personally in any publication of the results. Any questions I may have about the diet and the study results will be answered.

I _____ **consent to be a subject in this project.**

I am free to withdraw from the study at any time. Early termination of this project, for any reason, will not compromise my medical care.

Dated at Montreal, this _____ day of _____ 19__.

WITNESS _____ SUBJECT _____
(signature) (signature)

INVESTIGATOR _____
(Signature)

R00140jb
16/2/95

NAME: R.V.H. Unit No. C.I.U. File No.

DATE	DAY OF WEEK	STUDY DAY & DIET	WT. (KG)	TEMP	WATER INTAKE *=500ml	LAST A.M. URINE	BOWEL MOVE-MENTS	15-N GLYCINE	BODY MSR	EXER.	B.P./PULSE A.M.	B.P./PULSE P.M.	COMMENTS

APPENDIX VI : Determination of Factor for Fecal Nitrogen Losses

Objective

To derive a factor for fecal nitrogen losses for subjects consuming the liquid formula diet of this study.

Materials

Weekly pooled fecal collections for nine subjects consuming a similar iso-energetic diet consisting of Ensure, Polycose, and soya oil, providing a constant 80 g of protein (12.8 g nitrogen) daily.

Procedure

Weekly collections for each subject were pooled, freeze dried, and finely ground. Approximately 25 mg was then analyzed, in duplicate, for nitrogen by Kjeldahl digestion as discussed in section 5.5.1. Another Kjeldahl digestion, in duplicate, was performed if the values per subject were greater than 10% different. Values were then divided by 7 to yield daily nitrogen content.

Results

Subject	Mean Daily Nitrogen Content of Fecal Collection	Co-efficient of variability
1	1.315	5.3 %
2	0.709	5.9 %
3	0.112	13.9 %
4	1.275	5.8 %
5	0.694	5.6 %
6	0.489	2.3 %
7	0.815	2.3 %
8	1.552	0.6 %
9	0.631	1.0 %
Mean	n=9 : 0.844 g nitrogen daily	
	n=8 : 0.935 g nitrogen daily	

Discussion of Results

The subjects in our study received protein intakes at levels individually determined on an ideal body weight basis. Therefore, it was decided to express our factor on a "g N intake" basis. If all 9 values were taken, this factor became 0.066 g fecal nitrogen per g nitrogen intake. The samples analyzed for subject number 3 were greatly variable and lower than the other subjects. In order to determine how great of an effect this subject had on the final factor, subject 3 was removed from calculation. This yielded a factor of 0.073 g fecal nitrogen per g nitrogen intake.

Conclusion

As the two factors (0.066 & 0.073) were similar, a factor of 0.07 g or 70 mg fecal nitrogen per gram nitrogen intake was established. This factor would in our study then account for fecal nitrogen losses of 0.98 to 1.25 g nitrogen per day, based on the lowest and highest protein intakes of 87.5 g (14.0 g nitrogen) and 111.3 g (17.8 g nitrogen) daily.

APPENDIX VII : Determination of the ability of Chemiluminescence to measure the nitrogen content of biological samples

Objective

(1) To determine the ability of chemiluminescence to replace the traditional Kjeldahl methodology in measuring the nitrogen content of biological samples.

(2) To determine if chemiluminescence has the ability to capture all nitrogenous components of urine (ie. urea, creatinine, ammonium, and uric acid).

Materials

Aliquots of 24hr urine collections of subjects participating in this study.

Procedure

For objective (1) : this validation entailed 73 urine samples that were analyzed for total nitrogen content by chemiluminescence and Kjeldahl methodologies, as described in section 5.5.1.

For objective (2) : the second validation entailed 146 urine samples that were analyzed for total nitrogen content by chemiluminescence and compared to the summed nitrogen content of urea, creatinine, ammonium, and uric acid. Urea and creatinine were measured by standard automated techniques in the clinical biochemistry laboratories of the Royal Victoria Hospital. Ammonium was measured at the McGill Nutrition and Food Science Centre by ion specific electrode methodology. Uric acid was not measured, and a factor of 0.2 g uric acid nitrogen was used. Mean percent differences were calculated and correlation were performed.

Results

Validation (1) $n = 73$ samples; $r = 0.765$; $P < 0.0001$
mean % difference $= 1.5 \pm 0.7$ %
Chemiluminescence yielded greater values

Validation (2) $n = 146$ samples; $r = 0.815$; $P < 0.0001$
mean % difference $= 3.6 \pm 0.9$ %
Chemiluminescence yielded lower values

Discussion of Results

Both validation studies indicate that chemiluminescence yields similar results to both Kjeldahl methodology and the summaion of measured nitrogenous components of urine. Chemiluminescence was expected to yield greater values than Kjeldahl method, as the latter requires much manual manipulation and hence many potential sites for error. Chemiluminescence was also expected to yield lower values than the sum of the nitrogenous components, for each individual component will have some margin of error associated with it, and thereby errors will be summed as components are summed.

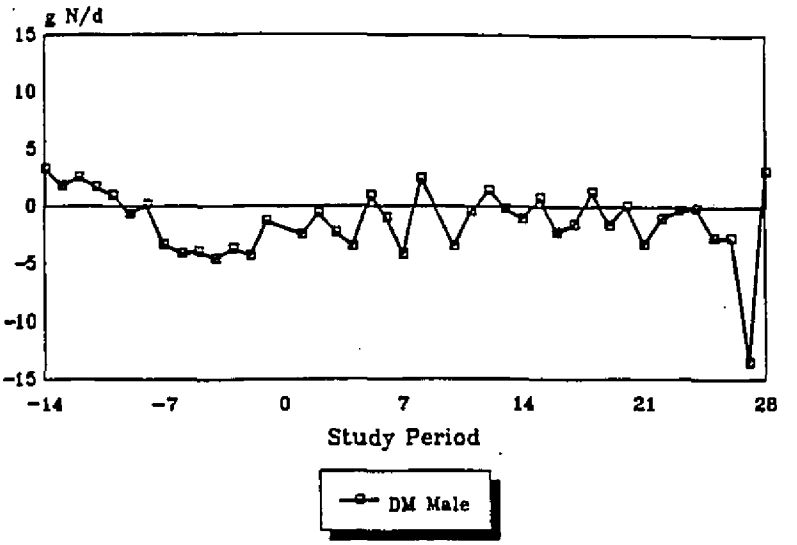
Conclusion

It was decided, based on the two validation studies, that chemiluminescence could be used as the method by which total urinary nitrogen was measured.

APPENDIX VIII

Daily Nitrogen Balance : Non Compliant Subjects

(a)



(b)