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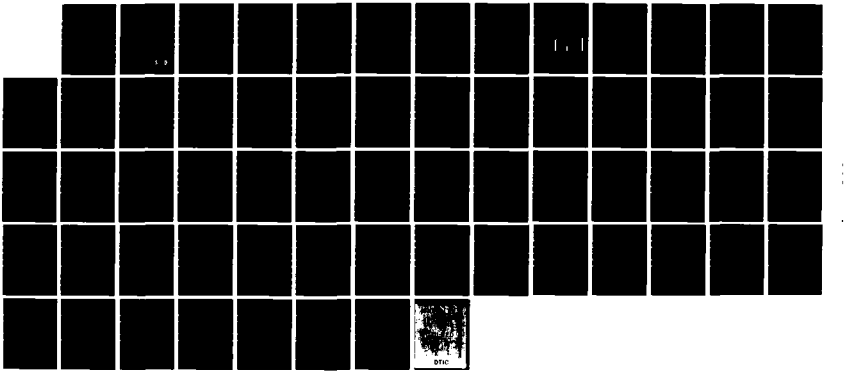

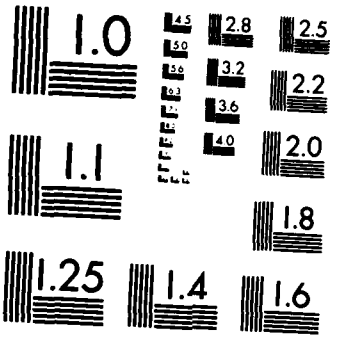

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Investigation of Intermediary Metabolism and Energy Exchange Following Human Trauma

Annual Report

John M. Kinney, M.D.

July 1979

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John M. Kinney, M.D.
DA 49-193MD-2552

PROGRESS REPORT

I. MUSCLE COMPOSITION IN INJURY, SEPSIS AND DEPLETION

Kinney J.M., Askanazi J., First P., Elwyn D.H. and Gump F.E.

A. INTRODUCTION

1. Objective

The overall objective of this project is to explore the changes in intracellular amino acid pattern, energy intermediates and water, and electrolytes of human muscle tissue associated with injury, sepsis and depletion. The specific objectives of the past year have been to examine the influence of operative trauma (total hip replacement) on muscle amino acids and to determine the influence of the type of hypocaloric nutrition and of enforced bed rest on the "pattern of injury" which was found.

It was formerly thought that the weakness and easy tiring of the surgical patient was the result of muscle wasting which was the reflection of a single metabolic response to stress. Hence, the only question at issue was to determine the amount of the metabolic response which was present in any given individual. It is now evident that there is no single response to stress as judged by muscle composition, but rather, a series of differing metabolic responses, depending upon the clinical situation. Therefore, any given surgical patient probably represents a blend of stimuli (injury, infection, starvation, bedrest, etc.) and it seems probable that the appropriate nutrition for an acutely ill patient in the future will depend upon understanding the particular metabolic blend of that patient at the time of his convalescence when his nutritional therapy is being planned.

The nitrogen metabolism of the injured or septic patient has suggested the possibility of an increase in cytoplasmic amino acids in muscle which are not efficiently used for protein synthesis. If the catabolic state is characterized by increased levels of free amino acids in muscle cells, perhaps this initiates a series of changes which result in increased hepatic synthesis of urea.

However, the weakness and fatigue of surgical convalescence may also be related to abnormalities in glycolysis or the TCA cycle and the associated production of high energy bonds. High energy phosphates, such as ATP and phosphocreatine (PC) are the immediate sources of energy in muscle cells. The formation of ATP in cells requires energy derived mostly from the oxidation of foodstuff. Oxygen as a final electron acceptor is utilized by all cells with the exception of those which lack a suitable electron transport system such as macrophages and erythrocytes.

These pathways of ATP resynthesis are used simultaneously by the muscle and are in equilibrium with the rate of ATP breakdown. This equilibrium in different pathological conditions is dependent on the rate of energy expenditure in the muscle cell. The only substrate which can be

used for resynthesis of ATP in all body cells in glucose. However, the active glucose store - glycogen in muscle and liver and extracellular glucose - is also very limited as an energy source. Without gluconeogenesis the store will be quickly exhausted. Gluconeogenesis is of special importance in carbohydrate depletion, starvation or severe catabolism, as during severe catabolic states it appears that gluconeogenesis is accelerated.

Introductory information on muscle samples has been obtained in man under anesthesia at the time of operative procedures. However, such techniques are not suitable for repeated study in the same individual. An ideal technique would be one which could be repeated in the same individual without discomfort or risk and on which a variety of important metabolic parameters could be assayed on relatively small amounts of muscle tissue. A technique fulfilling these advantages became available when Bergstrom introduced the percutaneous needle biopsy technique in Sweden. This methodology has now been studied in over 12,000 cases with a vanishingly low complication rate and has yielded a large amount of information. The safety and lack of discomfort of the method has been well illustrated by the fact that both cross country and downhill ski races in Sweden were utilized for the analysis of muscle biopsies in both the leg and the arm of participants. This procedure was performed just before, midway, and at the end of each athletic event without interfering with competitive performance.

The biopsy technique was originally used for studies of intracellular water and electrolyte metabolism in muscle tissue. It was, however, soon apparent that this method could also be used for the study of other tissue constituents. In the last ten years, various aspects of carbohydrate, lipid, protein, and mineral metabolism under normal and pathological conditions have been studied. The method has also proved to be suitable for the study of muscle morphology by light and electron microscopy.

The technique of obtaining samples and the diagnostic and research opportunities offered by this technique has been extensively discussed in two review articles:

1. Edwards RH: Percutaneous Needle Biopsy of Skeletal Muscle in Diagnosis and Research. LANCET 2, 593, 1971

2. Bergstrom J. Percutaneous needle biopsy of Skeletal Muscle in Physiological and Clinical Research. SCAND J CLIN LAB INVEST 35, 609, 1975

Many studies of the metabolism of injury have utilized patients undergoing upper abdominal operations, often gastroduodenal procedures, as a model for study. We believe that such operations represent relatively limited degrees of tissue trauma and therefore, we have selected total hip replacement as an operation which has both extensive soft tissue and bony dissection as a model of human trauma for metabolic studies. The following material presents in summary fashion our studies of the muscle and plasma free amino acid pattern taken preoperatively and again on the fourth postoperative day. The patients who received only 5% dextrose and water for their postoperative nutrition were then compared with normal control

subjects on four days of bed rest receiving either 5% dextrose and water or a regular house diet. Subsequent studies are then reported on patients undergoing hip replacement and receiving either 70 g of amino acids/day as their only nutrition, or both that amount of amino acids and 90 g/day of dextrose.

MUSCLE and PLASMA AMINO ACIDS AFTER INJURY: THE ROLE OF INACTIVITY

A prominent feature of the postinjury state is increased nitrogen excretion which is felt to reflect mainly the breakdown of muscle protein. This breakdown is in excess of what could be explained on the basis of inactivity and starvation, however, both may play a partial role. Practically every condition associated with weight loss and nitrogen wasting is thought to be associated with a translocation of amino acids from muscle to liver. The mechanism and significance of these changes are incompletely understood. The percutaneous muscle biopsy technique of Bergstrom has been used extensively to study changes in muscle composition with various disease states and exercise. Studies have indicated that the intracellular amino acid pattern of muscle differs from plasma and that various disease states are associated with their own specific patterns (i.e. uremia, starvation, lactic acidosis) in muscle.

Vinnars et al, have recently suggested that a unique pattern may be associated with injury. The classical work of Deitrick suggests inactivity to be associated with muscle wasting even if nutrition is adequate, while semistarved but active muscle is preserved. Although muscle wasting during severe injury or infection is greater than could be accounted for on the basis of inactivity and starvation both may play a role in the amino acid patterns obtained.

This study compares plasma and muscle amino acid changes of patients undergoing total hip replacement whose sole nutrient was 90 grams of dextrose/day to normal subjects on 1) bed rest plus a regular diet and 2) bed rest plus 90 g of dextrose/day only.

This study is part of an investigation of whether injury is associated with a unique amino acid pattern in muscle and plasma using the total hip replacement as a model for injury. The role of inactivity is examined as a factor in producing the changes observed with injury and associated bed rest. Nineteen preoperative patients and 16 normal subjects received a muscle biopsy following an overnight fast. Blood for plasma amino acids was drawn at the time of the biopsy. Seven patients (Group I) were treated with 90 g/day of dextrose for the first four postoperative days following which a second biopsy was performed. Eight normals were placed on four days of strict bed rest. Four (Group II) received a regular diet and four (Group III) received 90 g of dextrose/day I.V. as the sole nutrient. Both Groups I and III showed increases in valine, leucine and isoleucine in both muscle and plasma on the postoperative biopsy. The postoperative pattern differed from that observed in either group of normal subjects in that significant decreases occurred in muscle glutamine and histidine, plasma alanine, lysine and glycine. Phenylalanine, tyrosine, methionine and threonine were increaded in muscle postoperatively while only phenylalanine

was increased in either Group II or III of the normal subjects. Plasma phenylalanine increased in the patients while remaining unchanged in normal subjects. The pattern reported here for the patient group differs from that reported for other catabolic states (uremia, starvation, etc.), as well as inactivity with or without partial starvation. This study suggests that injury in the form of a total hip replacement is associated with a unique amino acid pattern of muscle and plasma which differs from that observed in other catabolic states. Bed rest plus partial starvation causes a pattern with certain similarities but cannot account for the postoperative changes observed.

The branched chain amino acids, valine, leucine and isoleucine, showed marked increases in the postoperative period in muscle and lesser increases in plasma, as shown in Fig. 1. The postoperative changes for muscle were also notable for the striking decrease in glutamine while the plasma showed a major decrease in alanine, as shown in Fig. 2. Each of the figures include the findings with normal subjects on enforced bed rest and the same nutritional intake for four days. The influence of bed rest is evident in changes which tend to move in the same direction as those of the operative trauma but not to the same degree.

Fig. 1

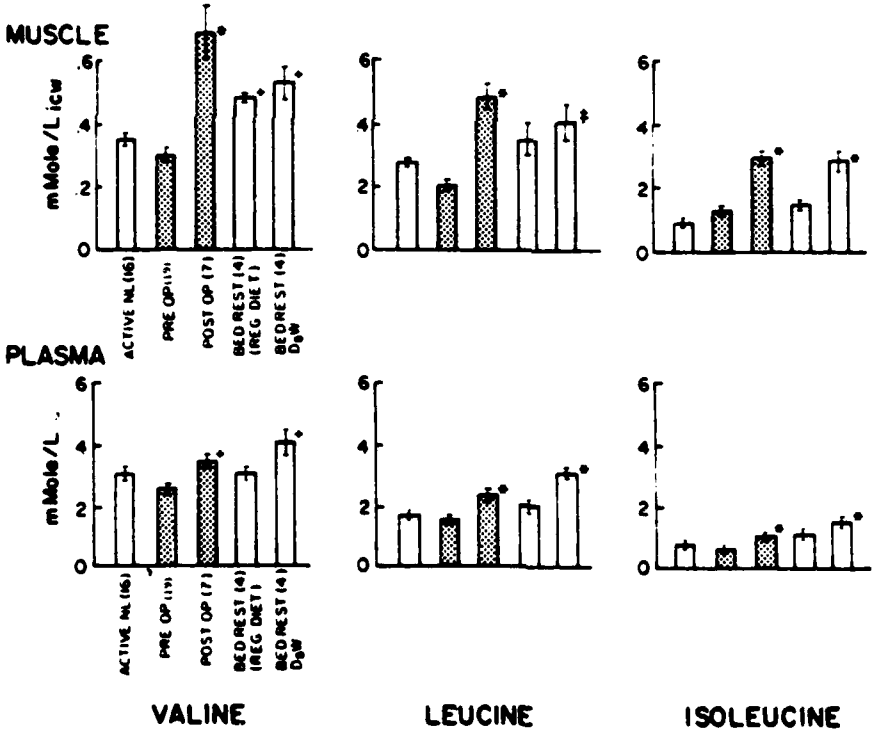


FIG. 1. *p < .001. †p < .01. ‡p < .05.

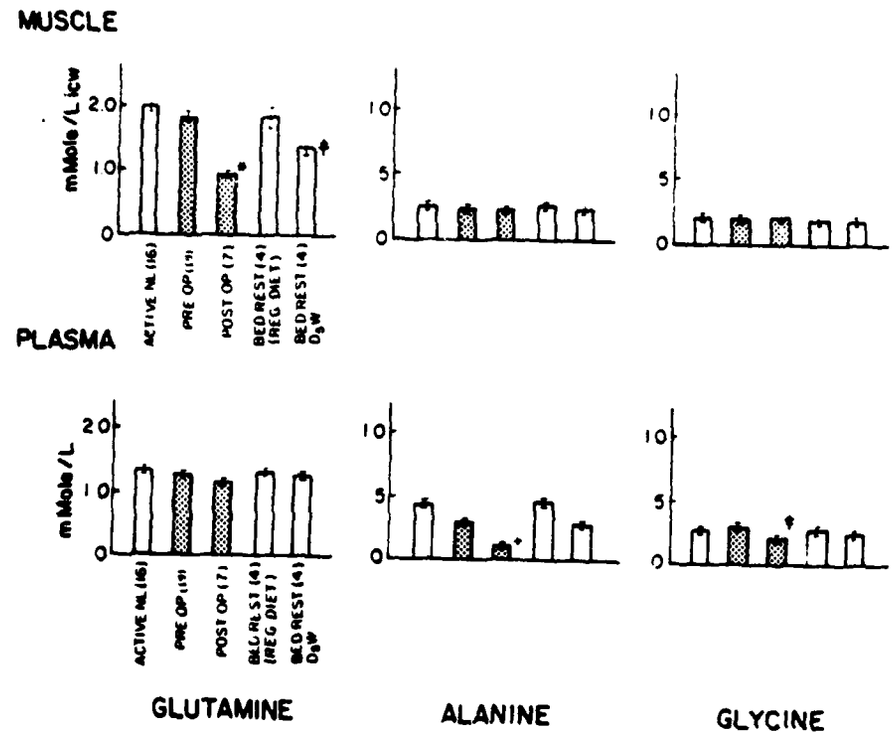


FIG. 3. *p < 0.001. †p < 0.01. ‡p < 0.05.

Fig. 2.

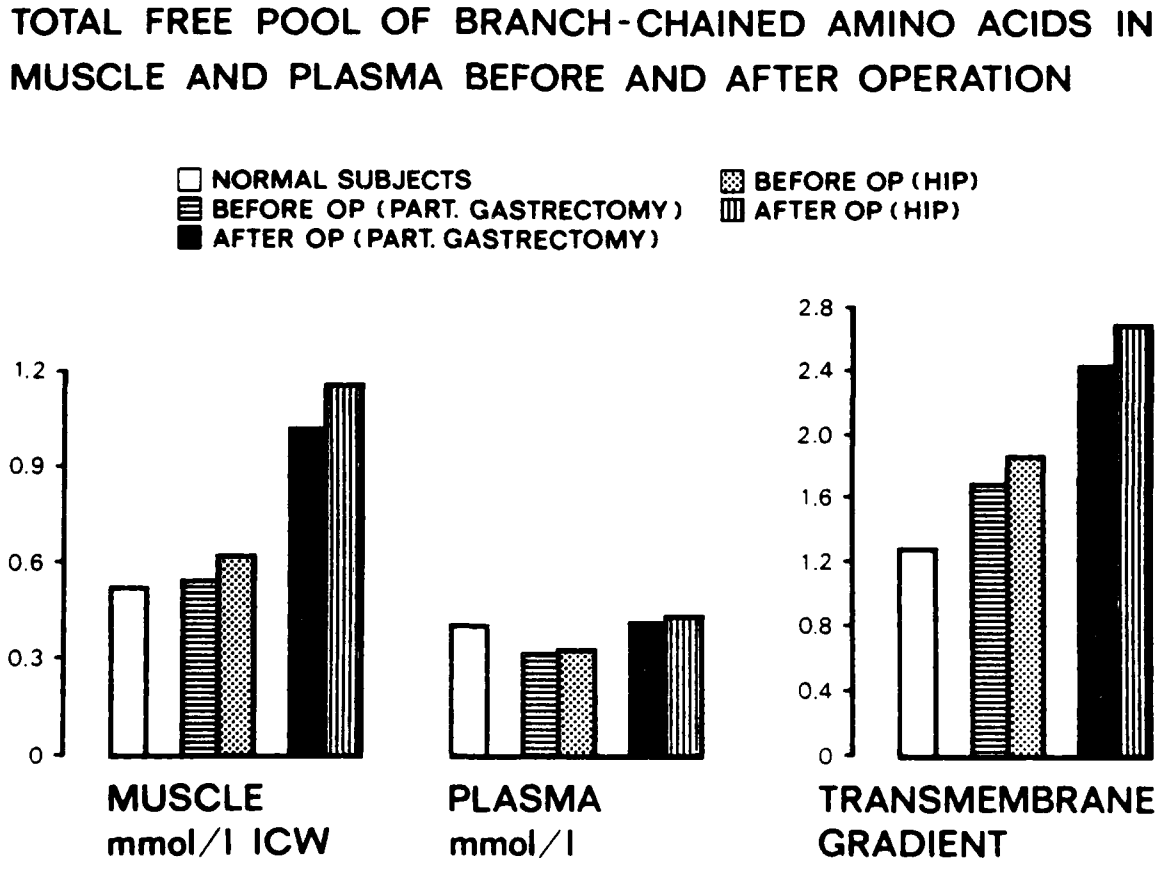
We are pleased to find that despite our early methodological problems, our initial data on the hip patients receiving carbohydrate alone was in close agreement with data on partial gastrectomies receiving similar intake in Sweden. The values for the total free pool of branched-chain amino acids of the Columbia patients are compared with the Swedish patients in Fig. 3, on the following page.

MUSCLE AND PLASMA AMINO ACIDS AFTER INJURY: II. INFLUENCE OF HYPOCALORIC DEXTROSE VS AMINO ACID INTAKE

The initial changes in the free amino acids of muscle and plasma, after the operation of total hip replacement, were found after four days of post-operative nutrition that was limited to 5% dextrose and water (90 g/day). There was the possibility that certain of the changes were the result of having given no amino acid intake during the postoperative period. Therefore, the original series of 7 patients receiving only dextrose postoperatively were compared with the findings from 7 more patients who received 3.5% amino

Fig 3

(Muscle and plasma amino acids after injury: The role of inactivity)



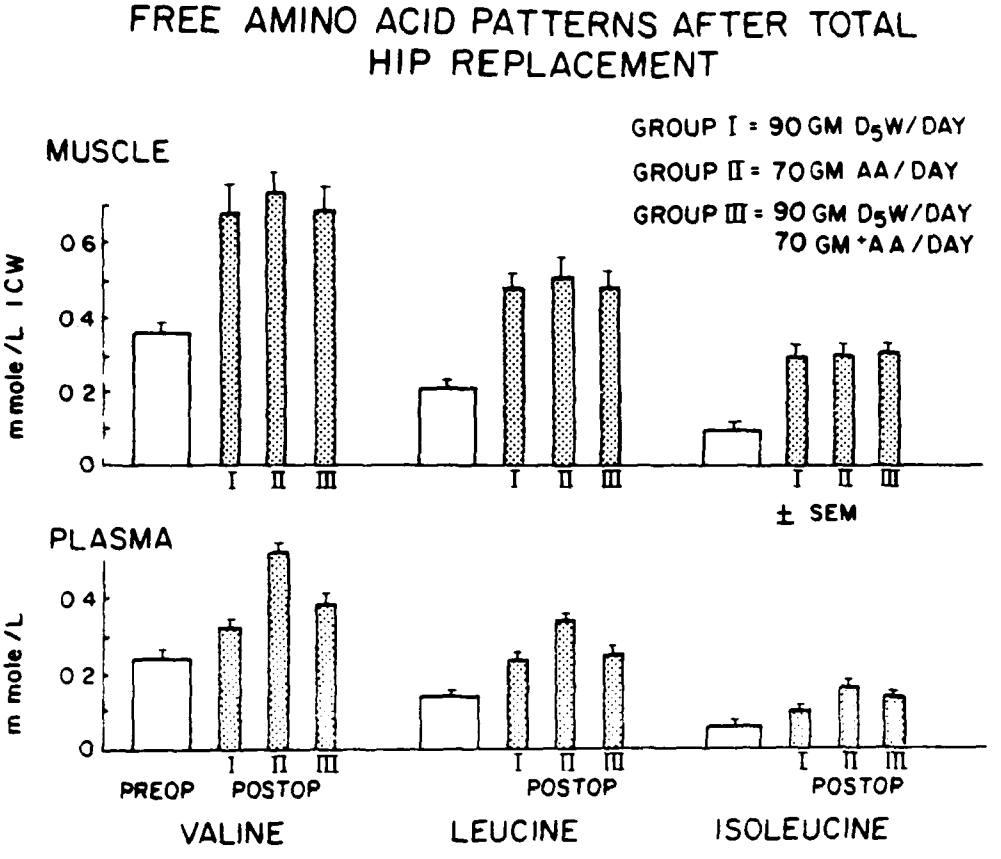


Fig. 1.

acids (70 g/day), and 8 more patients who received 90 g/day of dextrose together with 70 g/day of amino acids. All of the patients had the same operative procedure and were identical in their postoperative care except for difference in peripheral intravenous nutrition.

Material and Methods

Twenty-two patients undergoing total hip replacement were hospitalized on our metabolic research unit for 2 days prior, and 5 days after, operation. The study was approved by the Institutional Review Board. The nature, purpose and possible risks involved in the muscle biopsy technique and associated with the investigation were explained to the patients and their voluntary written consent was obtained. All patients were active and healthy except for the pain and disability associated with bony degeneration of the hip. All patients were on a regular diet prior to the study and appeared to be well nourished. Routine chemical analyses of plasma (SMA-6, SMA-12), analyses of urine, blood chemistry, EKG, chest x-ray and CBC

revealed normal findings. There were no evidences of diabetes, cardiac insufficiency, or kidney, liver or thyroid diseases. On the morning of the operation, 19 of the 22 patients had a percutaneous muscle biopsy (vida intra) after induction of pentothal anesthesia. Following the operation, each patient was kept 2-3 hours in the recovery room and then returned to our surgical metabolism unit (SMU) where the assigned nutritional regimen was started.

A repeat biopsy was performed on the morning of the fourth post operative day in the post absorptive state. The infusion was changed to normal saline at 40 ml/hr, eight hours prior to the second biopsy. Local anesthesia (xylocaine 1%) was used, confined to the skin only.

Muscle Biopsy and Analyses

The muscle biopsies were taken from the lateral portion of the quadriceps femoris muscle, about 15-20 cm above the knee. The wet biopsy material was dissected carefully to remove visible fat and connective tissue. The material was then divided into four portions. Two smaller samples (10-15 mg) were used for determination of water, fat and electrolytes and the other two (15-20 mg) were used for measurements of free amino acids.

The calculation of extra - and intracellular water was based on the chloride method according to Nernst's equation, assuming a normal resting transmembrane potential of -87.2 mv. Knowing the total water and chloride content in plasma, one can calculate the extracellular concentration of chloride and the extra - and intracellular water volumes. The intracellular concentration of each individual amino acid was calculated by substracting the free extracellular part from the total amount, assuming the plasma concentration to be equal to the concentration in the interstitial fluid. In this calculation, it was also assumed that none of the amino acids (except tryptophane) was bound to proteins. Plasma free and muscle free amino acids were determined after precipitation with sulphosolicylic acid by applying an automated amino analyzer.

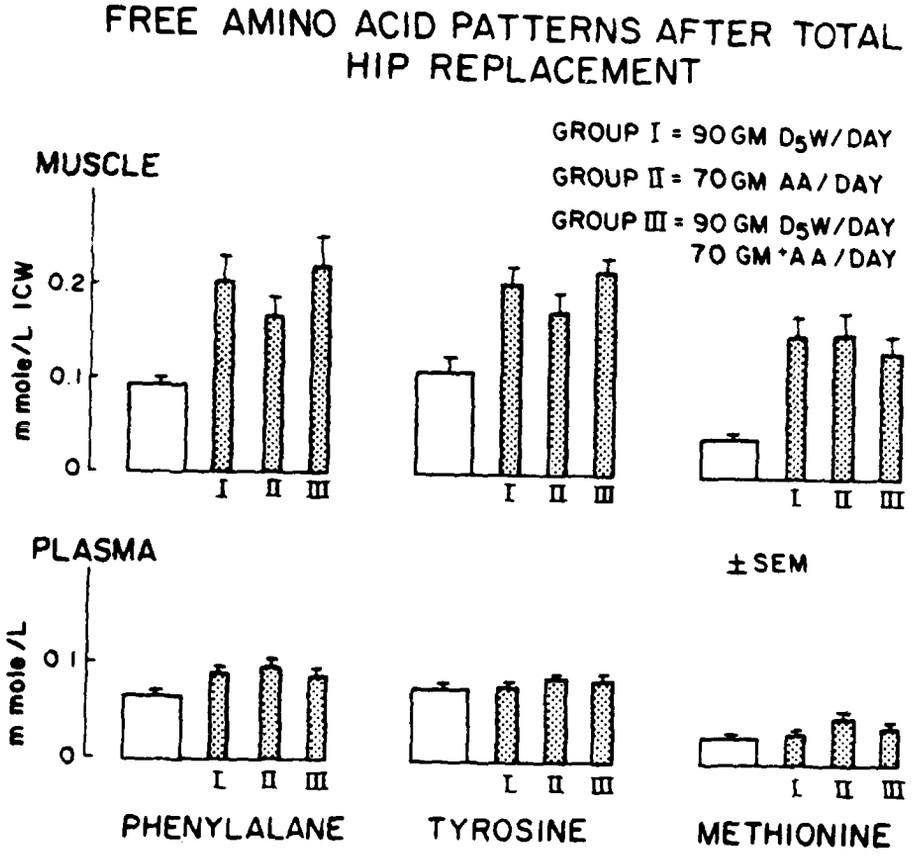
Statistics

Statistical calculations were carried out by using a Prime 300 computer, applying routines derived from Scientific Subroutine Package Library (SSPL). Results are expressed as mean values ± SEM. Statistical significance was assessed by both unpaired and paired t-test. The preoperative values in patients' material were compared with a normal material consisting of male subjects only, in the age range of 21 to 45. Only muscle lysine was found to be significantly elevated in the patient group. A comparison between the three preoperative groups revealed no significant differences. Hence, all the preoperative data are combined into one group, and unpaired t-tests were used to compare the three postoperative groups to the preoperative controls. Paired t-tests within respective groups showed essentially the same course of significance, although with less degree due to the limited number of observations.

RESULTS

The concentration of branched chain amino acids (valine, leucine, isoleucine) in muscle was increased, compared to preoperative values, in all three groups to approximately equal levels (Table 1, Fig. 1), as did the aromatic amino acids (phenylalanine, tyrosine), as seen in Fig. 2.

Fig 2



The corresponding plasma concentrations are increased (Table 2) but the elevation observed four valine and leucine in Group II was greater than those found in Group I and III, while the elevation in isoleucine was simililar in all 3 groups.

Glutamine revealed a profound decrease in muscle postoperatively with no corresponding alteration in plasma (Fig. 3, Table 1). Alanine levels in muscle decreased only in Group II, while the plasma concentrations were declined in all 3 groups. In contrast plasma glutamine concentrations were decreased in Group I and were unaltered in other groups. Glycine levels increased in muscle in Groups II and III (Fig. 3, Table I). There was also a decrease in muscle lysine and arginine in both groups receiving amino acids (Fig. 4). In plasma only the lysine concentration (Fig. 4) was found to be decreased and only in Group I ($p < .05$).

As a consequence of the postoperative changes in plasma and muscle concentrations, many of the resultant muscle/plasma ratios were found to be significantly changed as indicated in Table III. It is important to note that the basic amino acids exhibited decreased gradients, while the neutral amino acids showed a tendency toward increased muscle/plasma ratios.

The elevated levels of the branched chain amino acids both in muscle and plasma was a consistent finding in this investigation, in good agreement with results found in Swedish patients in the postoperative state or suffering from severe injury or burns.

High plasma phenylalnine levels are frequently found in catabolic situations as in this study. It is suggested that this increment might be due to increased release of phenylalanine from skeletal muscle. There is also the possibility that the high concentration of aromatic amino acids and methionine is at least partly dependent on impaired liver function.

The profound decrease (about 50%) in muscle glutamine concentration was consistent in all three groups. This finding seems to be one of the most typical features of post injury, which is important because glutamine contributes about 50% of the total free amino acid pool. In agreement with the present findings, intracellular glutamine was found to be decreased 4 days after colectomy when patients received about 40% of a normocaloric TPN. Also in severe multiple trauma and burns a 50% intracellular glutamine depletion was found.

The most striking finding in this study was that the "pattern of trauma" was maintained irrespective of nutritional regimens, thus in general there were no major differences between the postoperative groups. This would suggest that the hormonal milieu seen in the injury state causes changes which nutrition can only affect in minor ways. Exceptions were muscle, lysine and arginine where amino acid seem to be influenced by glucose but not by amino acid administration. In contrast, several plasma free amino acids were altered in the three different nutritional groups. Carbohydrate administration either with or without amino acids was associated with lower plasma levels for the BCAA than patients receiving amino acids alone, though this was not reflected in muscle.

Fig 3

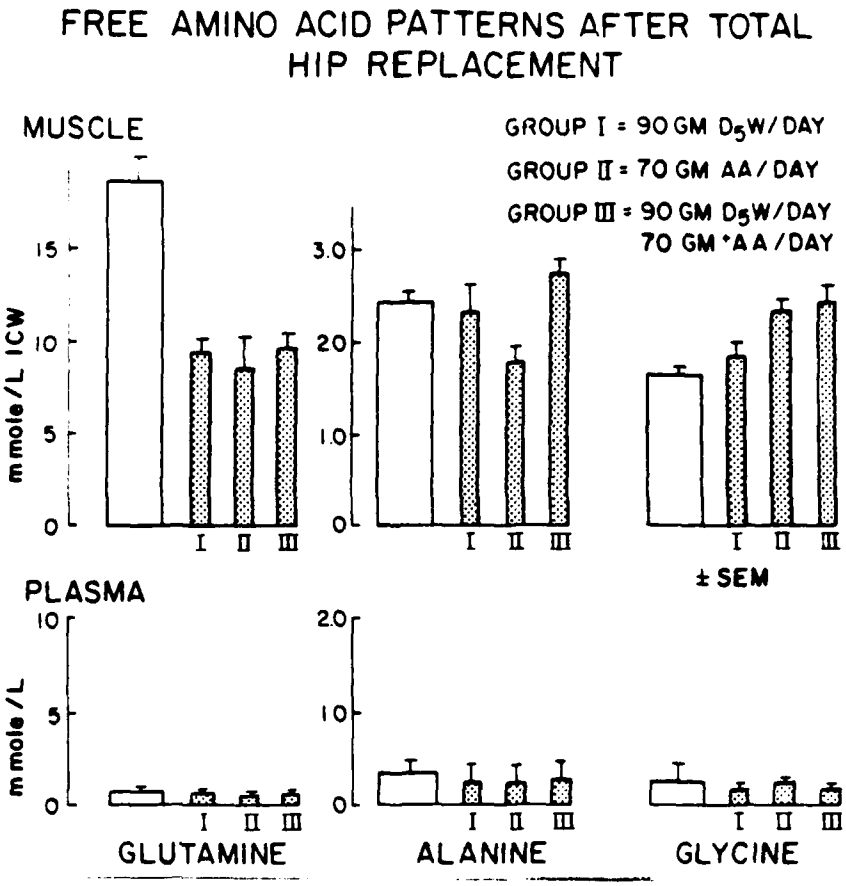
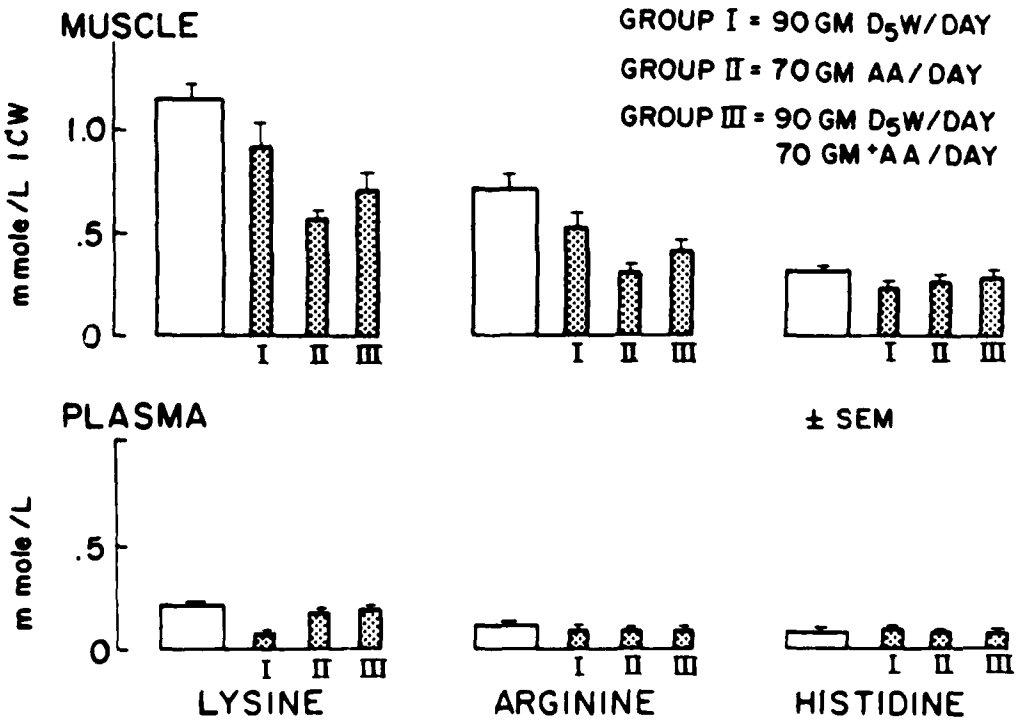

FREE AMINO ACID PATTERNS AFTER TOTAL HIP REPLACEMENT
MUSCLE
GROUP I = 90 GM D5W/DAY
GROUP II = 70 GM AA/DAY
GROUP III = 90 GM D5W/DAY 70 GM *AA/DAY
m mole/L ICW
15
10
5
0
3.0
2.0
1.0
I II III
± SEM
PLASMA
m mole/L
10
5
0
2.0
1.0
GLUTAMINE
ALANINE
GLYCINE

Fig 4


FREE AMINO ACID PATTERNS AFTER TOTAL HIP REPLACEMENT
MUSCLE
GROUP I = 90 GM D5W/DAY
GROUP II = 70 GM AA/DAY
GROUP III = 90 GM D5W/DAY 70 GM *AA/DAY
m mole/L ICW
1.0
.5
0
I II III
± SEM
PLASMA
m mole/L
.5
0
LYSINE
ARGININE
HISTIDINE

MUSCLE AMINO ACIDS BEFORE AND 4 DAYS AFTER TOTAL HIP REPLACEMENT

	5% D/W		3.5% AA		5% D/W & 3.5% AA	
	Before	After	Before	After	Before	After
LYS	1.416±0.375	1.280±0.575	1.206±0.374	.525± .136****	1.213±0.249	0.752±0.296**
VAL	0.345±0.058	0.524±0.118***	0.407±0.123	.702± .118***	0.392±0.092	0.768±0.156***
LEU	0.199±0.084	0.478±0.076****	0.212±0.023	.495± .132***	0.240±0.140	0.539±0.077*
ILE	0.127±0.045	0.242±0.068***	0.111±0.047	.288± .082****	0.101±0.038	0.320±0.061****
THR	0.736±0.299	0.897±0.249	0.715±0.319	.879± .285	0.982±0.298	1.246±0.285
TYR	0.085±0.034	0.152±0.060****	0.107±0.038	.165± .059	0.137±0.067	0.208±0.039*
PHE	0.079±0.032	0.159±0.039**	0.094±0.025	.168± .058**	0.092±0.032	0.208±0.079*
MET	0.050±0.025	0.124±0.033**	0.033±0.017	.147± .059**	0.044±0.024	0.137±0.066
GLN	10.072±7.533	7.240±2.603	15.818±3.571	7.096±2.609****	18.541±3.475	9.702±3.101**
GLU	3.337±0.987	1.817±1.059	3.900±1.271	3.266±1.438	4.241±0.973	3.811±0.779
ALA	1.861±0.727	2.469±0.456	2.352±0.304	1.762±0.479*	2.865±0.710	2.956±0.319
GLY	1.155±0.456	1.575±0.440	1.823±0.555	2.279±0.442	1.732±0.398	2.503±0.291**
SER	0.983±0.218	1.066±0.283	1.017±0.608	1.296±0.535	1.039±0.425	1.408±0.195
ARG	0.622±0.257	0.842±0.155	0.747±0.398	0.259±0.085*	0.726±0.121	0.418±0.191*
HIS	0.383±0.111	0.398±0.198	0.292±0.081	0.243±0.110	0.375±0.098	0.273±0.049

Significance of difference between pre- and postoperative patients. * $p<0.05$, ** $p<0.025$, *** $p<0.01$, **** $p<0.001$.

TABLE I.

PLASMA AMINO ACIDS BEFORE AND 4 DAYS AFTER TOTAL HIP REPLACEMENT

	5% D/W		3.5% AA		5% D/W & 3.5% AA	
	Before	After	Before	After	Before	After
LYS	0.140± .027	0.157±0.074	0.223±0.034	0.174±0.038****	0.220±0.046	0.211±0.046
VAL	0.195±0.046	0.210±0.082	0.225±0.076	0.506±0.071****	0.261±0.063	0.367±0.089**
LEU	0.088±0.018	0.161±0.056**	0.155±0.045	0.348±0.072****	0.149±0.036	0.247±0.066***
ILE	0.040±0.012	0.077±0.032**	0.068±0.016	0.151±0.038***	0.067±0.019	0.132±0.030***
THR	0.107±0.037	0.109±0.061	0.128±0.033	0.114±0.034	0.142±0.034	0.147±0.064
TYR	0.044±0.021	0.053±0.030*	0.074±0.008	0.084±0.015	0.077±0.030	0.086±0.025
PHE	0.044±0.011	0.051±0.022	0.065±0.011	0.101±0.020***	0.069±0.014	0.087±0.014***
MET	0.014±0.005	0.018±0.007	0.024±0.003	0.037±0.005****	0.028±0.008	0.034±0.008
GLN	0.360±0.216	0.249±0.215	0.651±0.052	0.484±0.056****	0.683±0.119	0.525±0.129**
GLU	0.156±0.142	0.060±0.035	0.052±0.020	0.032±0.017	0.033±0.008	0.032±0.009
ALA	0.239±0.116	0.150±0.108	0.375±0.097	0.211±0.033***	0.337±0.112	0.257±0.072
GLY	0.149±0.020	0.107±0.054	0.309±0.114	0.210±0.055**	0.243±0.056	0.200±0.085*
SER	0.101±0.029	0.076±0.031	0.120±0.015	0.108±0.024	0.126±0.033	0.127±0.049
ARG	0.081±0.027	0.066±0.036	0.111±0.027	0.088±0.028	0.108±0.020	0.098±0.022
HIS	0.070±0.026	0.057±0.028	0.086±0.010	0.067±0.005***	0.093±0.012	0.074±0.008**

Significance of difference between pre- and postoperative patients. * $p<0.05$, ** $p<0.025$, ***$p<0.01$, **** $p<0.001$.

TABLE II.

PLASMA/MUSCLE AA GRADIENT BEFORE & AFTER TOTAL HIP REPLACEMENT

	5% D/W		3.5% AA		5% D/W & 3.5% AA	
	Before	After	Before	After	Before	After
LYS	10.557± 3.390	10.522± 8.608	5.583± 2.163	3.212± 1.309	5.556± 0.662	3.781± 1.815*
VAL	1.864± 0.420	3.040± 1.951	2.361± 2.192	1.427± 0.372	1.509± 0.121	2.115± 0.218***
LEU	2.314± 0.964	3.563± 2.236	1.445± 0.336	1.444± 0.391	1.633± 0.759	2.321±0.675
ILE	3.380± 1.673	3.902± 2.404	1.647± 0.618	1.970± 0.590	1.615± 0.629	2.482±0.597
THR	7.265± 2.485	11.234± 7.401	5.436± 1.194	7.975± 2.505	6.831± 0.599	9.174± 2.283
TYR	2.407± 1.400	3.320± 1.656	1.452± 0.549	2.016± 0.849	1.813± 0.573	2.506± 0.351
PHE	1.980± 1.149	3.650± 1.740	1.512± 0.520	1.723± 0.824	1.349± 0.431	2.385± 0.687
MET	3.910± 1.890	8.308± 5.985	1.908± 1.249	3.715± 1.550	1.587± 0.832	4.248± 2.245
GLN	48.911±28.811	45.183±26.102	24.310± 5.039	14.614± 4.907***	31.250± 6.707	18.818± 6.536**
GLU	30.972±18.169	35.645±23.512	98.120±78.346	127.170±71.845	138.251±48.522	127.655±46.272
ALA	7.688± 3.350	22.605±11.180	6.493± 1.663	8.817± 2.744	8.830± 1.528	12.204± 3.351
GLY	7.156± 2.162	18.344± 9.724	6.237± 2.163	11.802± 4.640**	7.276± 1.905	13.541± 3.490**
SER	12.181± 8.424	17.054± 9.205	8.450± 4.774	12.480± 6.25 *	8.088± 1.680	12.071± 3.803
ARG	9.238± 5.861	8,950± 4.289	6.928± 2.517	3.232± 1.406***	6.943± 0.874	4.691± 2.918
HIS	6.455± 3.495	10.633±11.536	3.446± 1.042	3.633± 1.522	4.004± 0.569	3.766± 1.096

Significance of difference between pre- and postoperative patients, * $p<0.05$, ** $p<0.025$, *** $p<0.01$,

TABLE III.

PROGRESS REPORT

II. GLUCOSE METABOLISM IN HUMAN INJURY, SEPSIS AND DEPLETION

Elwyn, DH, Kinney, JM, Gump, FE, Askanazi, J. and Jeevanadam, M.

1. Introduction

One of the original purposes of this study was to examine the utilization of glucose in improving nitrogen balance when given in increasing amounts to acutely catabolic patients from injury or sepsis and to depleted patients for comparison. Preliminary studies suggested that the critical factor relating energy to nitrogen balance was the energy balance rather than the energy intake which had been commonly reported in previous studies.

The concept of obtaining the total energy balance each day for a hospitalized surgical patient has always seemed impossible since the amount of time spent and energy utilized in physical activity could not be accurately known. A novel approach to this problem was developed by Dr. Elwyn during the course of studying acute surgical patients with nitrogen balance and gas exchange while receiving various levels of high carbohydrate total parenteral nutrition.

This approach involves the determination of an apparent positive carbohydrate balance which must represent the glucose calories spent in physical activity, once changes in glycogen stores have been considered. The details of this study and its possible implications for various types of patients are presented in the following pages.

One of the patients in this study developed marked hyperapnea while receiving the parenteral nutrition. It was noted that the minute ventilation of this patient varied with the carbohydrate load (which was varied according to a standard protocol), the CO_2 output and the clinical state of the patient. The clinical state of the patient was found to vary with the O_2 consumption and catecholamine output, both being elevated when the patient was febrile and showed signs of abdominal inflamation. The case report of this patient has been submitted for publication and is presented as part of this Progress Report.

The dramatic response of the above patient to total parenteral nutrition prompted a prospective study of patients before and after starting TPN, and the associated changes in ventilation and their pattern of breathing. Two studies are partially completed and are presented in the following Research Plan for the coming year.

We feel that we have identified enough patients with a "stress response" to high carbohydrate parenteral nutrition, to emphasize the potential seriousness for the patient with diminished pulmonary reserve (often unsuspected) or for the patient requiring mechanical ventilation - where

nutritional support is needed but additional ventilatory stimulus should be avoided at the time of weaning from the ventilator. This is discussed in more detail under the Research Plan.

An additional aspect of this "stress response" to carbohydrate loads, is the respiratory quotient which rise to 0.90 to 0.95 instead of going over 1.0 as would be expected whenever net lipogenesis is present. Approaches to examine lipogenesis in such patients are discussed under Lipid Metabolism

Indirect Calorimetry in the Study of the Composition of Weight Gain in Man

There has been a growing and effective use of total parenteral nutrition in the last decade. In adults, this is used mainly to offset loss of lean body mass during gastrointestinal failure or to rebuild tissue after prior losses. Achievement of positive nitrogen balance is a rough indication of the efficacy of repletion therapy. But, there is still too little data available to provide quantitative guidelines as to what are optimal levels of protein deposition, how these relate to concurrent fat accumulation and how dietary intake can be manipulated to achieve these goals. In order to provide the data necessary for such quantitative guidelines we need to develop practicable methods for measuring fat or total energy balance as well as N balance.

A variety of methods are available for estimating loss or gain of fat and protein, including body composition and anthropometric measurements. However, there is little doubt that, for short-term studies, the most accurate and reproducible results are obtained by measuring both nitrogen balance and energy balance with the use of indirect calorimetry (8,20). The measurement of four independent quantities are required for this procedure; 1) daily intake of fat, carbohydrate and protein, 2) daily N excretion, 3) daily 0_2 consumption, and 4) daily $C0_2$ production. Of these measurements the last two are the most tedious to perform and probably the least accurate. Ideally, 0_2 consumption and $C0_2$ production should be measured for the whole study period or a continuous log of the subjects' physical activity be kept together with measurement of rates of gas exchange during each type of activity (5). Such studies are time consuming with normal subjects. They are even more difficult with seriously ill patients, although, in the absence of any better alternative, such methods have been used in prior studies of the composition of weight loss conducted in this laboratory (14).

Estimates of resting energy expenditure can be made quite accurately and reproducibly in contrast to the difficulties in estimation of total energy expenditure. An important innovation for such studies was the development of a non-invasive procedure for measuring gas exchange, utilizing a rigid lucite head canopy rather than a mask or mouthpiece (12,17). This makes possible reliable measurement of gas exchange in severely ill patients for as much as 5-12 hours per day.

Under certain dietary conditions it appears possible to gain a reliable estimate of TEE indirectly, based on measurements of REE. This obviates the need for detailed logging of patient physical activity and the uncertainty of the estimates so obtained. On hypercaloric diets, consisting primarily of protein and carbohydrate, the non-protein respiratory quotient (RQ) will be greater than one. It is assumed that all non-protein gas exchange, as measured at rest, results only from two net processes: Either oxidation of carbohydrate to CO_2 and H_2O or conversion of carbohydrate to fat. The difference between carbohydrate intake and resting carbohydrate utilization represents the difference between TEE and REE since the body's ability to accumulate carbohydrate stores is limited.

This approach is applied, in the present investigation, to the study of depleted patients on alternating hyper-and hypo-caloric intravenous intakes containing amino acids and glucose as the only sources of calories. In this paper, an assessment is made of the reliability and internal consistency of the methodology, and the relation between REE, AEE and TEE.

METHODS

Experimental Protocol - Ten depleted surgical patients were admitted to the study with weight losses ranging from 13 to 48% of body weight. All subjects required total parenteral nutrition on medical groujnds, which required a central venous catheter. They were given intravenous diets, containing glucose and amino acids as calorie sources, which were adequate in all respects except in calories. Three different caloric intakes were given, each for 4-day periods. These were based on individual patient's REE measured during a control period prior to the onset of TPN. They were nominally 0.5, 1.25, and 1.75 times the measured REE. Nitrogen intake (Aminosyn, 10% from Abbott Laboratories) was constant throughout the study, for each subject, and was approximately 6.2 mg N per kcal of REE. Studies ranged in duration from 2 to 13 4-day periods per subject. Subjects could be started on any one of the diets; the order of rotation was usually from low to medium to high and then back to low and so on. Daily measurements were made of body weight and nitrogen, energy and water balance. This protocol was approved by the Columbia University Institutional Review Board. The details of the studies were explained to each patient and written consents were obtained.

Balance Measurements - All intake, whether oral or infused, was measured by difference in weights of full and emptied containers. The amounts of each constitutent (H_2O, N, etc.) were calculated from the composition obtained from the manufacturer's specifications or by direct analysis in this laboratory according to established procedures (1). Energy contents of diets were calculated from published values, and in the case of oral intake corrected for digestibility (16). Urine, feces, and drainages were collected and analyzed for N, H_2O, Na^+ and K^+ content. In addition, urea was determined in urine and drainage, creatinine was determined in uring and glucose was determined in those urine samples in which qualitative tests (Ketodiastix^R, Ames Co., Elkhart, Ind.) were positive. A manual, micro-kjeldahl procedure was used for digesting samples for total N determination. Subsequent stages in total N determination and analyses of urea, creatinine, Na^+ and K^+ were

carried out with single channel automated analyzers according to the manufacturers procedures (Auto Analyzer, Technicon Company, Tarrytown, NY). Glucose was determined by an automated glucose oxidase procedure (Glucose Analyzer, Beckman Instruments, Inc., Fullerton, CA). Blood urea N was measured by the clinical chemistry laboratory as part of the SMA6 determination.

Gas Exchange - Oxygen consumption and CO_2 production were measured using a rigid lucite head canopy developed in this laboratory (12,17). This permits frequent measurements of relatively long duration, 3 to 5 periods per day of 40 to 60 minutes each, with minimal discomfort to the patient.

CALCULATIONS

1. Tissue Composition of Weight Loss - When the non-protein RQ was 1.00 or less, the amounts of protein, carbohydrate and fat oxidized and the amount of water produced by oxidation were calculated using standard procedures (19), with the addition that urea in drainage fluids was added to urinary N in calculating protein oxidation. N balance was corrected for variations in blood urea nitrogen.

2. Lipogenesis - When the non-protein RQ was greater than 1, calorimetric factors were derived as follows, based on the assumptions:

   a. That non-protein O_2 consumption and CO_2 production were derived solely from carbohydrate oxidation and lipogenesis.

   b. That the lipid produced consists of triglycerides containing equimolar amounts of palmitic, stearic and oleic acids represented by the compound palmitylstearyloleyltriglyceride ($C_{55}H_{105}O_6$) (PSOG).

   c. That established pathways for conversion of glucose to glycerol, and to fatty acids via pyruvate and acetyl-CoA, are the only ones involved.

The overall stoichiometry for the conversion of glucose to PSOG is shown in Equation 1, together with the mass and energy equivalents (17). The RQ for this stoichiometric reaction is 26/3 = 8.67.

$$13.5\ C_6H_{12}O_6 + 30_2 \rightarrow C_{55}H_{104}O_6 + 26CO_2 + 29H_2O \qquad 1.$$

(2,432 g) (67.2 l) (861.5 g) (582.4 l) (522.3 g)

(9,121 kcal) (8,184 kcal) $\Delta E = -937$ kcal

(3.75 kcal/g) (9.5 kcal/g) $= -0.39$ kcal/g glucose

The amount of O_2 consumed in fat synthesis is as follows:

$$RQ_{np} = \frac{CO_{2np}}{O_{2np}} = \frac{CO_{2ox} \quad CO_{2fs}}{O_{2np}} \qquad 2.$$

$$RQ = 1 = \frac{CO_{2ox}}{O_{2np}}, \text{ therefore } CO_{2ox} = O_{2ox} = O_{2np} - O_{2fs} \qquad 3.$$

$$RQ_{fs} = 8.67 = \frac{CO_{2fs}}{O_{2fs}}, \text{ therefore } CO = 8.67.O_{2fs} \qquad 4.$$

Multiplying the first and third terms of equation 2 by O_{2np} and substituting for the CO_2 terms from equation 3 and 4 gives:

$$RQ_{np} .O_{2np} = O_{2np} - O_{2fs} = 8.67.O_{2fs} \qquad 5.$$

Rearrangement of equation 5 gives:

$$O_{2fs} = \frac{O_{2np}(RQ_{np} - 1)}{7.67} \qquad 6.$$

Where the subscripts np, ox and fs refer respectively to total non-protein oxidation, carbohydrate oxidation and fat synthesis from carbohydrate; and O_2, CO_2 and RQ refer to the quantities of O_2 consumption, CO_2 production or respiratory quotient for each process.

The calorimetric factors relating O_{2fs} to other parameters of fat synthesis are derived from Equation 1 and are shown in Table 1.

3. Estimation of Total Energy Expenditure - When patients on hypercaloric glucose diets are studied according to the above procedures, in most instances there is an apparent accumulation of carbohydrate of the order of 100 g per day. Since total glycogen stores are of the order of 400 g (10,11), an apparent positive carbohydrate balance of many hundreds of thousands of grams cannot be real. Rather, this positive balance represents the expenditure due to physical activity and constitutes the difference between measured resting energy expenditure and total energy expenditure. If it is assumed that the effect of physical activity is to increase the rate of glucose oxidation and not to change the net rate of lipogenesis, then the apparent carbohydrate balance (appropriately corrected for any real glycogen accumulation) is a direct measure of AEE.

The equations relating energy intake (EI), energy expenditure, total energy balance (TEB) and the energy equivalents of fat balance (EEFB) and N balance (EENB) are:

$$REE = 3.75\ Glu_{ox} + Glu_{fs} + 25\ N_{out} \qquad 7.$$

TABLE 1

Calorimetric factors for fat synthesis. Factors for various parameters related to 1 liter of O_2 used in fat synthesis.

Starch consumption	32.6g/1 O_2
Glucose consumption	36.2g/1 O_2
Energy production	13.95 kcal/1 O_2
CO_2 production	8.67 1/1 O_2
Fat production	12.8g/1 O_2
Water production	7.78g/1 O_2

$$AEE = 3.75\ (Glu_{in} - Glu_{ox} - Glu_{fs} \quad 8.$$

$$TEE = REE + AEE = 3.75\ Glu_{in} - 3.36\ Glu_{fs} + 25\ N_{out} \quad 9.$$

$$EI = 3.75\ Glu_{in} = 25\ N_{in} \quad 10.$$

$$EB = EI - TEE = 3.36\ Glu_{fs} + 25\ (N_{in} - N_{out} \quad 11.$$

Since carbohydrate balance is assumed to be zero, then:

$$TEB = EEFB + EENB \quad 12.$$

Also:

$$EENB = 25\ (N_{in} - N_{out}) \quad 13.$$

By derivation from Equation 1:

$$EEB = 8{,}184\ kcal/2{,}432\ g\ glucose = 3.36\ Glu_{fs} \quad 14.$$

Thus, Equations 11 and 12 are seen to be identical.

Where:

Glu_{in} = grams glucose intake.

Glu_{ox}, Glu_{fs} = grams glucose oxidized or converted to fat at rest.

N_{in}, N_{out} = grams N intake or excreted.

The numerical coefficients represent kcal/g taken from reference 20 and derived from Equation 1.

Then total energy balance is equal to the energy equivalent of resting fat balance and of N balance (Equation 12) both of which are directly measured since, under the assumptions used, fat balance as measured at rest (Equation 14) is unchanged by physical activity and therefore, is a direct measure of daily fat balance.

These relations apply only when the non-protein RQ is above one. With hypocaloric intake, it was assumed that the effect of physical activity would be to increase fat oxidation with no change in the rate of glucose oxidation. This increase in fat oxidation cannot be measured directly by the methods used here. However, in our experimental design, each hypocaloric period was adjacent to one or two hypercaloric periods. On the assumption that physical activity is not appreciably affected by dietary intake, both REE and fat balance, as measured in hypocaloric periods, can be corrected using values of AEE obtained during adjacent hypercaloric periods.

4. Insensible Water Loss - This was taken to be equal to the algebraic sum of estimated balances of water, fat and carbohydrate and protein less the increase in body weight.

RESULTS

Activity and Total Energy Expenditure - One major problem in estimating AEE is to distinguish between that part of apparent carbohydrate accumulation representing actual glycogen storage, and that part representing AEE. Two approaches to resolving this problem have been used in this study. In the first approach, total apparent carbohydrate accumulation over the entire study period was divided by the number of days in positive energy balance and multiplied by the appropriate factor (4.17 kcal per g glycogen) to give the mean daily AEE (Table 2). This probably overestimates AEE, since all patients but one were on 5% dextrose at the start and on either low, medium or high intake at the end, and therefore, should have accumulated some glycogen during the study periods which ranged from 8 to 24 days of positive balance (Table 2). This overestimation of AEE was approximately 10%. In the second approach it was assumed that by the third day on each diet a new steady state had been reached and that there was no further change in glycogen content. Mean values of apparent carbohydrate balance for days 3 and 4 of each period were taken to represent only AEE. These were averaged for all periods of positive energy balance for each patient, converted to kcal, and are reported in Table 2. The mean value of AEE for all patients was approximately 7 kcal per kg body weight per day and was the same for both approaches (Table 2). Regression of the results of the two approaches gives a line that does not differ significantly from a line through the origin with slope of 1 (Fig. 1). Since use of the total carbohydrate accumulation overestimated AEE by 10%, the good agreement between the two approaches suggests that the second approach also overestimated AEE by about 10% and that a new steady state was not quite obtained by the third day of each diet period. Random errors, evidenced by differences in individual values of AEE measured by the two methods, averaged 1.7 kcal per kg or approximately 25% of the measured AEE.

The amount of AEE correlated well with the clinical evaluation of the patients' physical behavior. A value of 22 kcal per kg was observed for a septic, hypercatabolic male who was physically active both in and out of bed. A value of zero was obtained for a severely ill hypocatabolic patient, completely bedridden, who barely moved in bed. For most patients neither the observed physical behavior nor the calculated AEE changed much during the period of study. This is illustrated for patient JG in Fig. 2, which shows the apparent carbohydrate accumulation as a function of caloric intake over a 24-day period. As to be expected, there was loss of carbohydrate with low intakes (negative energy balance) and a gain with medium and high intakes (positive energy balance). The amount of accumulation during positive balance was essentially independent of the caloric intake, in agreement with the concept that this accumulation represents the amount of

TABLE 2.

Apparent carbohydrate accumulation and daily activity energy expenditure, resting energy expenditure, and total energy expenditure for 10 depleted patients during total parenteral nutrition.

Patient	Number of days in positive energy balance	Total apparent accumulation of carbohydrate as glycogen	REE	AEE calculated from Total accumulation	AEE calculated from day 3 & 4 of each period	AEE as % of REE	TEE	Predicted REE	TEE as % of Predicted REE
		g/kg	kcal/kg/day			%	kcal/kg/day		%
JG	16	53.0	29.1	13.6	11.2	39	40.3	22.1	182
EL	8	-3.4	22.8	-1.4	0.8	4	23.6	25.9	91
LC	12	12.4	27.0	4.3	0.0	0	27.0	30.8	88
EM	8	12.6	31.1	6.5	4.8	15	35.9	29.4	122
LW	8	8.7	24.6	4.5	4.1	17	28.8	19.8	145
MS	8	2.8	25.6	1.4	1.0	4	26.6	20.2	132
MD	16	22.0	21.1	5.8	5.9	28	27.0	17.5	154
RG	12	32.9	29.9	11.3	12.3	41	42.2	24.3	174
LG	12	65.1	36.3	22.4	24.7	68	61.0	23.6	258
SC	24	27.2	30.7	4.6	6.0	20	36.7	35.7	103
MEAN		23.3	27.8	7.3	7.1	26	34.9		140
SD			4.5	6.7	7.4				

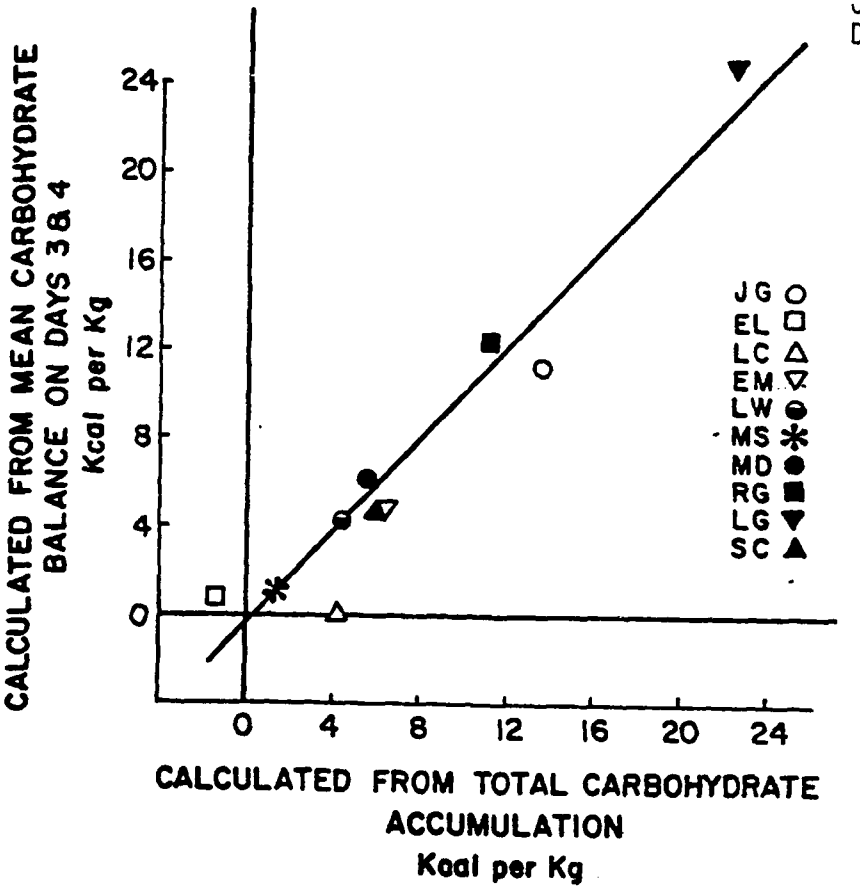


Figure 1

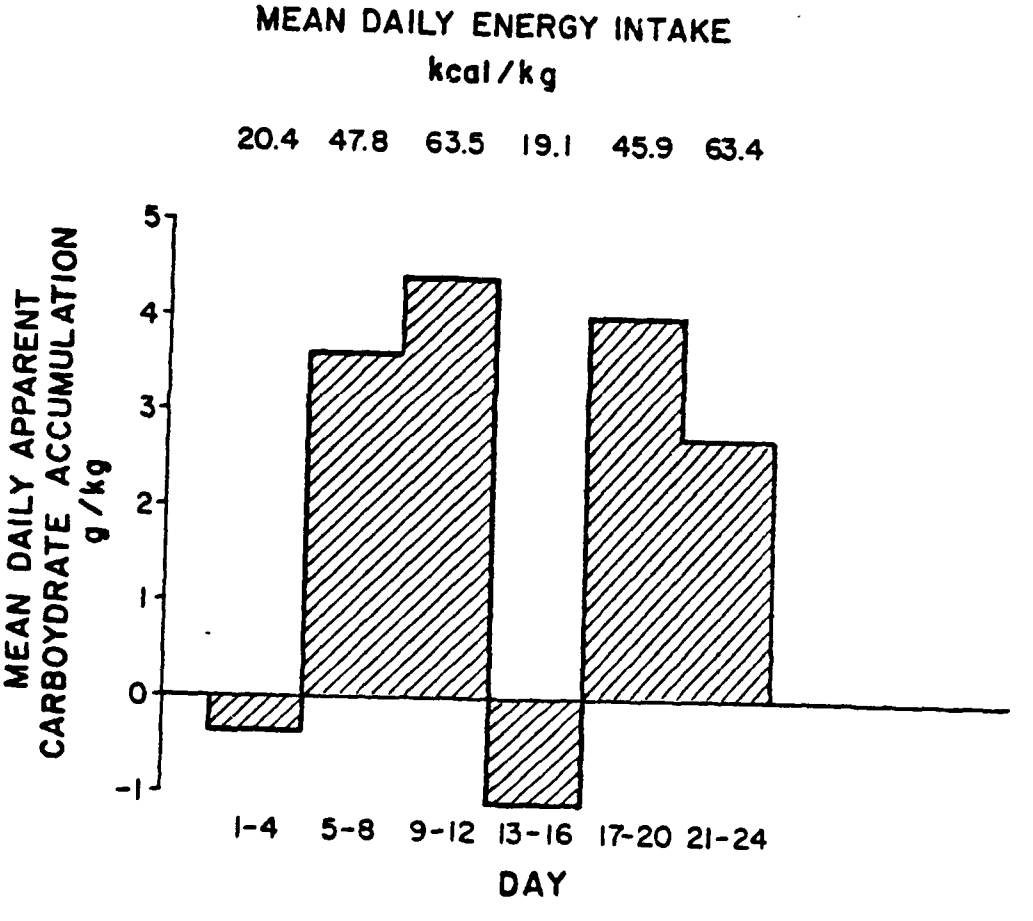


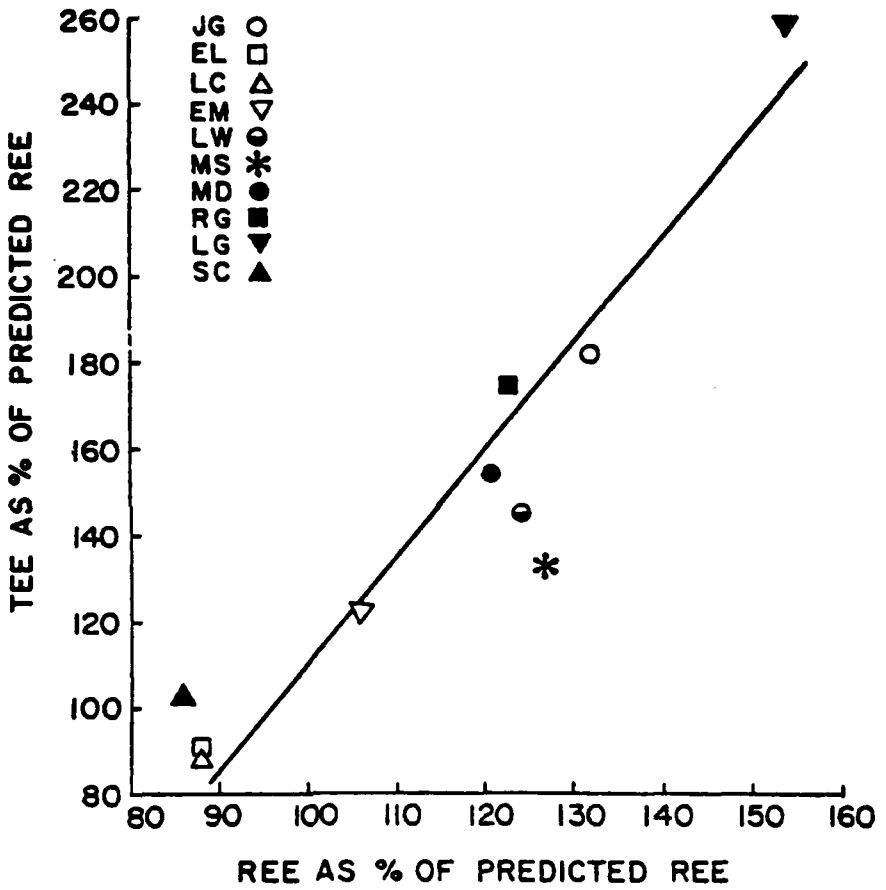
Figure 2

physical activity. Also, there was little indication of change in the rate of accumulation with time. By contrast, patient SC showed marked changes in both physical activity and AEE. He was a young man, severely injured, with considerable brain damage, who lost one-half of his body weight during six months of hospitalization. At the start of the study he appeared to be quadriplegic and could not talk. At the end of 24 days of total parenteral nutrition he could move all limbs, sit up in bed or in a chair, hold a telephone and carry on an intelligent conversation. His calculated AEE averaged 1.6 kcal/gk for the first 12 days and 10.4 for the last 12 days.

The AEE averaged 26% of the REE but was much more variable than the REE, in both absolute and relative terms (Table 2). As a result, the total energy expenditure (the sum of AEE + REE) was quite variable, with a standard deviation equal to 30% of the mean value (Table 2). Of some practical interest is the relation of TEE to predicted REE. Predicted normal REE (BMR + 10%) is a function of height, weight, age and sex. It is readily obtained, and can be used as a guide to repletion therapy in the ordinary clinical setting. The ratio of TEE to predicted REE for 10 patients averaged 141% but ranged from 88 to 258%. If all of these patients were fed at a rate 50% above their predicted REE they would be, on the average, just in positive energy balance. However, as individuals, three would be grossly overfed and four would be in negative energy balance.

This indicates that at least three factors should be used in estimating calorie requirements: The predicted normal REE, an estimate of the extent of hypermetabolism, and an estimate of the energy equivalent of physical activity. The extent of hypermetabolism is obtained by measuring REE, and physical activity is represented by AEE. In the present study there is a very good correlation between the two. Regression of TEE against measured REE gives the equation TEE = -42 kcal/kg + 2.8 REE, $r = 0.89$. This may be contrasted to the poor correlation between TEE and predicted normal REE ($r = 0.07$). An equally good or better correlation is obtained by regressing TEE and REE expressed as the ratios to predicted REE (Fig. 3). This gives the equation: TEE = 139 + 2.5 REE, $r = 0.93$. This suggest that it may be possible, in this type of patient, to estimate TEE fairly accurately from REE and predicted REE without the necessity of measuring AEE separately.

Insensible Water Loss - For nine of the patients, individual mean values of insensible water loss, as measured over the entire study period (8-24 days), averaged 22 ± 3.7 g per hour per m^2. This is in excellent agreement with the value of 23 ± 7.7 g per hour per m^2 previously obtained by direct measurements of 20 afebrile, hospitalized patients (9), and with values from normal subjects (13). In the present study, values for insensible water loss were obtained by difference and, therefore, include any errors or artifacts in measurements and assumptions. The good agreement with measured values serves as a heck on our balance methodology and indicates that any errors and artifacts are small.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TEE AS % OF PREDICTED REE
REE AS % OF PREDICTED REE

Figure 3

DISCUSSION

In the traditional method for calculating fat, protein and carbohydrate gain or loss, the first step is to estimate the amount of protein oxidized. The associated amounts of O_2 consumption and CO_2 production are computed from the amount of nitrogen excreted and then substracted from the measured amounts of O_2 consumption and CO_2 production to give the non-protein RQ. If the non-protein RQ lies between 0.707 (the RQ for fat) and 1.00 (the RQ for carbohydrate), the proportions of fat and carbohydrate oxidized can be estimated. A non-protein RQ greater than 1 indicates a combination of carbohydrate oxidation and lipogenesis. This requires additional calorimetric factors in order to calculate the amount of lipogenesis. There was considerable interest in this problem in the period up to World War II (2,3,7,15), and at least two different sets of calorimetric factors were derived to deal with lipogenesis. However, at that time, the metabolic pathways for conversion of carbohydrate to fat were not known and the stoichiometric equations which were used differed somewhat from those now known to be correct. We have been unable to discover any subsequent publication or use of these factors based on the correct metabolic pathways. Since we have used the traditional method of calculation, we have included a derivation of these factors. (Table 1, Equation 1).

An alternate method of calculation was first presented by Consolazio, Johnson and Pecora (5), and has been used in studies of weight gain in normal and obese subjects (18), and extensively in the study of weight gain in domestic animals (4). In this method, the derived parameters for energy expenditure, carbohydrate oxidation, fat oxidation, protein oxidation are each separately expressed as functions of the measured variables O_2 consumption, CO_2 production and N excretion. A major advantage of this procedure, in addition to simpler arithmetic, is that it applies equally well whether the RQ is less than or greater than 1. This method gives essentially the same results as the traditional procedure.

A unique property of the presently proposed procedure is that total energy expenditure can be calculated from highly reliable measurements of resting gas exchange, dietary intake, and N excretion, and does not require logging the time spent in various types of physical activity. This rests on two assumptions: a) that over the period of measurement there is no significant, unestimated gain or loss of carbohydrate stores, and b) that on hypercaloric diets, with non-protein RQ > 1, the effect of physical activity is to increase the rate of oxidation of carbohydrate without markedly affecting the rate of either lipogenesis or oxidation of lipids.

Overall, the proposed method for measuring AEE and TEE appears to be internally consistent, to rest on reasonable assumptions and to have an estimated error in the range of 25% of AEE which corresponds to 5% of TEE. Whatever the size of the errors, it almost certainly provides a better estimate of daily energy balance than can be derived from REE alone. For use with severely ill patients, it would appear to be more accurate and much less tedious than methods based on logging of daily activities. A limitation of this method is that it can be used only with diets in which net lipogenesis

is occurring, that is, hypercaloric diets with the bulk of calories coming from carbohydrate. However, this is the standard diet used in this country for intravenous feeding of patients who require adequate energy intake and who have nonfunctional gastrointestinal tracts. It is, therefore, applicable to many of the problems of total parenteral nutrition which relate to diet optimization. If it can be used to standardize other approaches to estimating AEE, it could be applied indirectly to diets in which there is no net lipogenesis.

REFERENCES

1. Official Methods of Analysis, 9th ed. Washington Association of Official Agriculture Chemists, 1963

2. Benedict FG, Lee RC: Lipogenesis in the animal body with special reference to the physiology of the goose. Washington, DC, Carnegie Institute, Publ. 489, 1937

3. Bleibtrau M: Fettmast und respiratorische Quotient. Pfluger's Arch. gesam. Physiol 85:345, 1901

4. Brouwer E: Report of sub-committee on constants and factors, in Blaxter KL (ed): Energy Metabolism. European Association of Animal Production Publication #11. New York, Academic Press, 1965, p. 441

5. Consalazio CF, Johnson RE, Pecora E: Physiological Measurements of Metabolic Function in Man. New York, McGraw-Hill, 1963

6. Elwyn DH, Gump FE, Munro HN, Iles M, Kinney JM: Changes in nitrogen balance of depleted patients with increasing infusions of glucose. (in preparation).

7. Forbes EB, Swift RW: Associative dynamic effects of protein, carbohydrate and fat. Am J Clin Nutr 27:453, 1944

8. Garrow JS: Energy Balance and Obesity in Man. Amsterdam, Elsevier, North Holland, Biomedical Press, 1978

9. Gump FE, Kinney JM, Long CL, Gelber R: Measurement of water balance - a guide to surgical care. Surgery 64:154-156, 1968

10. Hultman E, Bergstrom J, Roch-Norlund AE: Glycogen storage in human skeletal muscle, in Pernow B, Saltin B (eds.): Muscle Metabolism During Exercise. New York, Plenum Press, 1971 p 273

11. Hultman E, Nilsson LH: Liver glycogen in man. Effect of different diets and muscular exercise, in Pernow B, Saltin B (eds.): Muscle Metabolism During Exercise. New York, Plenum Press, 1971, p 143

12. Kinney JM, Morgan AB, Dominguez FJ, Gildner KJ: A method for continuous measurement of gas exchange and expired radioactivity in acutely ill patients. Metabolism 13:205, 1964

13. Kuno Y: Human Perspiration. Springfield, IL, Charles C. Thomas, 1956

14. Long CL, Kopp K, Kinney JM: Energy demands during ambulation in surgical convalescence. Surg Forum 20:93, 1969

15. Lusk G: Animal calorimetry: An investigation into the causes of the specific dynamic action of foodstuffs. J Biol Chem 20:555, 1915

16. Merril AL, Watt BK: Energy Value of Foods: Agriculture Handbook No. 74. Washinton, DC, U.S. Government Printing Office, 1955

17. Spencer JL, Zikria AB, Kinney JM, Broell JR, Michailoff TM, Lee AB: A system for the continuous measurement of gas exchange and respiratory functions. J Appl Physiol 33:523, 1972

18. Strong JA, Shirling D, Passmore R: Some effects of overfeeding for four days in man. Br J Nutr 21:909-919, 1967

19. Swift RW, French CF: Energy Metabolism and Nutrition. Washington, DC, Scarecrow Press, 1954

20. Yang MO, Wang J, Pierson RM, Van Itallie TB: Estimation of composition of weight loss in man: A comparison of methods. J Appl Physiol 43:331-338, 1977

PROGRESS REPORT

III. REGIONAL METABOLISM IN INJURY, SEPSIS AND DEPLETION NUTRITIONAL CHARACTERIZATION OF PATIENTS FOR STUDY

Gump, F.E., Elwyn, D.H. and Kinney, J.M.

I. Introduction

The metabolic response to trauma has routinely been investigated in the post-absorptive or fasting state. Information is needed regarding the effect of modern nutritional support on post-traumatic catabolism in order to gain the maximum benefit and minimize the metabolic penalties which may be associated with these new feeding techniques.

A detailed regional study of interorgan movements and regional metabolism of major substrates and hormones in trauma, sepsis and depletion was presented in our application to the U.S. Army Research and Development Command last year. These studies were to be performed on patients who would be placed in one of the above three categories according to generally accepted clinical criteria. However, introductory studies convinced us that some more objective indication of the patient's nutritional status should be used in characterizing the patients for study, considering the extensive work and expense which would be invested in each patient study. Therefore, the past nine months have been spent in developing the data for a diagram where the relation between the daily nitrogen balance and the daily calorie balance can be used to characterize a given patient better than the conventional approaches to patient selection.

We now believe we are in a much stronger position from which to undertake the regional study, which is presented in the Research Plan which follows this Progress Report.

2. Background

In normal adults, nitrogen balance is a function of both nitrogen and calorie intake but responds differently to changes in these two dietary components (1,2,3). Well-nourished individuals maintained at zero nitrogen and calorie balance will retain additional protein if the nitrogen intake is raised and lose protein if it is lowered. These effects last for about a week, after which the subject returns to zero nitrogen balance at a new level of body protein content (4). Feeding of excess energy as fat or carbohydrate at adequate levels of nitrogen intake causes retention of approximately 2 mg N per kcal., an effect which has been documented to last 15 days or longer (1,2,3). When N intake is limiting this N retention does not occur (3,5). Keys et al (6) found the weight gain due to overfeeding normal men to consist of approximately 63% fat and 37% lean tissue. This corresponds to nitrogen retention of 1.9 mg N per Kcal. which suggests that N retention under these conditions is related, in some way, to disposition and maintenance of fat. At marginal protein and energy, increasing

energy intake cause N balance to increase by roughly 1 to 2 mg N per kcal. depending in part, upon protein quality (7).

In a study which we have just completed, the relationship of calorie to N balance was explored in a group of ten surgical patients whose clinical condition made them candidates for total parenteral nutrition, having lost at least 20% or more of normal body weight. Energy intake has been varied in each patient with a constant N intake averaging 173 mg per kg. This intake of N is roughly twice that required to maintain zero N balance in normal adults on oral diets (8).

3. Methods

Patient Selection - Ten patients were admitted to the study. All were depleted; eight were severely depleted as indicated by a loss of 20% or more from the patient's normal or desirable weight (Table 1). Six patients were septic as defined by clinical observation and a resting energy expenditure more than 20% above predicted. None of the subjects had evidence of a prior history of diabetes, chronic liver disease or metastatic cancer. All patients required total parenteral nutrition on medical grounds. The details of the experiments, including risks, were explained to each patient, usually in the presence of members of his or her family and written consent was obtained. In one instance, where initial neurologic dysfunction restricted communication with the patient, written consent was obtained from next of kin. Particular emphasis was placed on the potential risk to the patients of providing a calorie intake during part of the time which left them in negative energy balance. The reasons for this are outlined in the Results section and considered further in the Discussion. The protocol of this study has been approved by the Columbia University Institutional Review Board.

Protocol - During the first day, patients were maintained on intravenous 5% dextrose. Their REE was measured and used as a basis for calculating subsequent dietary intake. Three intravenous diets, with low, medium or high calorie intake, were administered for 4 days at a time in sequence. All diets were infused continuously and at a constant rate over each 24 hour period. Nitrogen intake was maintained constant for each patient at a nominal level of 6.2 mg N per kcal. of REE. Nominal calorie intakes were set at 0.5, 1.25 and 1.75 times the REE as measured on the first day. The first diet given varied between any one of the three. The sequence of diet rotation was usually from L to M to H to L. Length of studies ranged from two to thirteen 4-day periods. Non-protein calories were provided as glucose. Vitamins and minerals were usually added to the infusion and where indicated individual adjustments were made. One mg of vitamin B_{12} was injected monthly. Vitamin K (Aquamephyten) was given as required. Essential fatty acids were administered by a daily massage with 1 tablespoon of corn oil. Iron was given intramuscularly as indicated. Zinc, copper and iodine, at 1 to 5 times the recommended intravenous doses (9), were given orally. Apart from trace elements and water, there was no oral intake. Amino acids were given as 10 percent Aminosyn (Abbott Laboratories, N. Chicago, IL).

The overall stoichiometry for the conversion of glucose to PSOG is shown in the following equation, together with the mass and energy equivalents (11). The RQ for this stoichiometric reaction is 26/3 = 8.67.

$$35.5\ C_6H_{12}O_6 + 3O_2 \longrightarrow C_{55}H_{104}O_6 + 26CO_2 + 29H_2O$$

(2,432 g) (67.2 l) (861.5 g) (582.4 l) (522.3 g)

(9,121 kcal) (8,184 kcal) $\Delta E = -937$ kcal

(3.75 kcal/g) (9.5 kcal/g) = -0.39 kcal/g glucose

Calorimetric factors for fat synthesis based on these assumptions and related to 1 liter of O_2 are shown in Table 4.

DISCUSSION

Quantitative Effects of Caloric Intake on N Balance - The effect of energy intake or energy balance on N balance was assessed by regression. A straight line was fitted to each patient's data. A composite line was calculated by averaging the individual slopes and intercepts, each weighted by the inverse of its variance. The adequacy of the fit was tested by comparing it to the fit of a quadratic polynomial and no significant departure from linearity was found.

Values of N balance for the last two days of each diet period are plotted against energy intake in Fig. 1. Each point represents the mean of the last two days on all the low, medium or high periods for any one patient. The data may be described by a straight line with slope of 1.4 ± 0.17 mg N per kcal and intercept -24.3 mg N per kg. When N balance is plotted against estimated total energy balance, the data may be described by a straight line with slope of 1.7 ± 0.19 mg N per kcal and intercept 19.8 ± 2.9 mg N per kg body weight. Zero N balance occurred at -11.7 kcal per kg energy balance. However, this is misleading since the N balance data was not corrected for integumental and methodological losses. Calloway et al (15) have estimated such losses at 0.5 g N per day per individual for sedentary males, which is approximately 9 mg N per kg per day. If this correction is made to all values in the present study, zero N balance occurred at an energy balance of -5.9 kcal per kg. At zero energy balance the mean value for N balance, corrected for integumental losses, was 9.9 mg N per kg with a standard error of 2.9. The energy intake for zero energy balance averaged 32 kcal per kg.

Effects of Caloric Intake on Resting Energy Expenditure - The effect of energy intake on REE was not linear over the range of intakes studied. Therefore, separate straight lines were fitted to the points with low to medium intake, and those with medium to high intake. A weighted average of these two sets of regression lines was computed in the same way as for the N balance data.

Considering days 3 and 4 on each diet, increasing energy balance had, at best, only a small effect on REE during hypocaloric intakes. When energy balance was positive there was an increase of 0.19 ± 0.03 kcal in REE

Balance Measurements - All intake, whether oral or infused, was measured by difference in weights of full and emptied containers. The amounts of each constitutent (H_2O, N, etc.) were calculated from the composition obtained from the manufacturer's specifications or by direct analysis in this laboratory according to established procedures (10). Energy contents of diets were calculated from published values, and in the case of oral intake corrected for digestibility (11). Urine, feces, and drainages were collected and analyzed for N,H_2O, Na+ and K+ content. In addition, urea was determined in urine and drainage, creatinine was determined in urine, and glucose was determined in those urine samples in which qualitative tests (Ketodiastix^R, Ames Co., Elkhart, Ind.) were positive. A manual, microKjeldahl procedure was used for digesting samples for total N determination. Subsequent stages in total N determination and analyses of urea, creatinine, Na+ and K+ were carried out with single channel automated analyzers according to the manufacturers procedures (Auto Analyzer, Technicon Company, Tarrytown, N.Y.). Glucose was determined by an automated glucose oxidase procedure (Glucose Analyzer, Beckman Instruments, Inc., Fullerton, CA.). Blood urea N was measured by the clinical chemistry laboratory as part of the SMA 6 determination.

Gas Exchange - Oxygen consumption and CO_2 production were measured, with the patients lying at rest, using a rigid lucite head canopy developed in this laboratory (12,13). This permits frequent measurements of relatively long duration, 3 to 5 periods per day of 40 to 60 minutes each, evenly spaced throughout the 24 hour period, with minimal discomfort to the patient.

CALCULATIONS

1. Tissue Composition of Weight Loss - When the non-protein RQ was 1.00 or less, the amounts of protein, carbohydrate and fat oxidized, and the amount of water produced by oxidation were calculated using standard procedures (14), with the addition that urea in drainage fluids was added to urinary N in calculating protein oxidation. N balance was corrected for variations in blood urea nitrogen.

2. Lipogenesis - When the non-protein RQ was greater than 1, calorimetric factors were derived as follows, based on the assumptions:

a. that non-protein O_2 consumption and CO_2 production were derived solely from carbohydrate oxidation and lipogenesis.

b. that the lipid produced consists of triglycerides containing equimolar amounts of palmitic, stearic, and oleic acids represented by the compound palmitylstearyloleytriglyceride ($C_{55}H_{105}O_6$) (PSOG).

c. that established pathways for conversion of glucose to glycerol and to fatty acids via pyruvate and acetyl-CoA are the only ones involved.

for each kcal increase in energy intake. Put another way, of each 5 kcal of increased intake, 4 went to storage (positive energy balance) and 1 went to increase the resting energy expenditure. That this was a consistent effect is indicated by the narrow confidence limits.

Quantitative Interrelations Between Glucose Intake and N Balance in Depleted Patients - Two aspects of the quantitative relations between glucose intake and N balance are of particular interest. The first is the slope of the line relating N balance to energy intake. Is this a more or less constant value, or does it change with varying dietary intake or physiologic or pathological state?

The value of 1.7 mg N per kcal of energy balance for depleted patients in the present study is similar to values found for normal subjects. In young men on daily intakes of 89 mg N of egg white protein per kg of body weight, Calloway (7) found improvement in N balance of 1.9 and 1.1 mg N per kcal in going from 34 to 40 and from 40 to 46 kcal per kg respectively (energy balance was zero at an intake of 40 kcal per kg). In a number of studies (16-18) as summarized by Calloway (7), the range of N intake was from 43 to 117 mg per kg, of energy intake from 38 to 57 kcal per kg, and of increase in N balance from 0.7 to 1.2 mg N per kcal. On a higher N intake (213 mg N per kg), by increasing caloric intake from 44 to 55 kcal per kg, Munro and Wikramanayake (19) found N balance increased by 2.9 mg N per kcal. Inoue (5) studied young men with energy intakes of 35-60 kcal per kg and a constant protein intake (10 g N per day) of variable biological value. The increase in N balance (about 1.80 mg N per kcal) was almost independent of the biological value of the protein fed, but the energy intake for zero N balance ranged from 40 to 53 kcal per kg depending on the protein source. Studies by Bozetti (20) of both depleted and postoperative patients with a wide range of N intake, also show a response of N balance to increasing energy intake, which appears quantitatively similar to that in normal subjects. In the above studies, energy intakes were within the range, 0.4 to 2 times energy requirements, used in the present study. When energy intakes ranged from 0.0 to 0.4 times requirements, changes of N retention with energy intake were roughly 8 mg N per kcal in normal men (1), much greater than with more adequate calorie consumption; a value of 8 mg N per kcal was also observed in this laboratory with postoperative patients with energy intakes in this range (21). Large variations in N retention with calorie intake have also been shown in pigs at extreme values of N energy intake (26).

In experiments on overfeeding physically healthy men for many months, Keys et al (6) found the mean composition of the weight gain to be 63% fat and 37% lean body mass (LBM). In the experiment of Calloway (7) cited above, tissue changes were calculated to be 34% LBM and 66% fat in the hypocaloric range and 28% LBM and 72% fat in the hypercaloric range. Using a similar calculation with our data, assuming LBM equal to 32 times N (22), the composition of tissue balance in in the present study was found to be 35% LBM and 65% fat. This general agreement suggests that the increase in N balance resulting from increasing energy intake may be mainly used for support of the increased fat stored. That is to say, muscle hypertrophy may be necessary to support the added weight of fat; more blood vessels

may be needed to support both fat and muscle; and so forth (6). Thus, the function of LBM increments associated with gain of fat may not be the same as that of LBM lost due to fasting or hypercatabolism, even though the tissue composition of the LBM may be similar in each case. The tissue composition of weight loss in hypercatabolic states or in early stages of starvation was found to be approximately two parts LBM to one part fat (23). We may consider LBM as divided into two functional compartments: That which is obligatorily associated with fat, comprising one-half the weight of fat, and that which is not associated with fat. Since weight loss of surgical patients comprises twice as much LBM as fat, only one quarter of this loss is related to fat loss; the other three fourths is unrelated. Therefore, since increasing energy intake increases deposition of only that part of LBM associated with fat, restoration of the major part of LBM losses will not be affected by changes in energy intake, but only, as discussed below, by changes in N intake.

The other aspect of interaction of diet and N retention of interest, is the extent to which protein intake influences N retention in depleted patients. Studies with rats (24), dogs (25), or human subjects (20,24, 26,27,28) indicate that protein-calorie depleted adults will increase N balance with increased N intake, independent of any changes in energy intake. These increases are not transient (as with normal adults) but can be maintained for weeks in the dog or months in man. To our knowledge, it is not possible from previous human studies to completely and quantitatively separate, from each other, the effects due to degree of depletion, rate of N intake, rate of energy intake and rate of energy expenditure. These relations are illustrated schematically in Fig. 2 in which N balance is plotted against energy intake. For the normal adult under steady state conditions, this line should pass through zero N balance at an energy intake required for zero energy balance. In depleted patients it should be displaced upward. However, this displacement can result either from a reduction in energy expenditure causing a shift of zero energy balance and the line to the left, or from a greater affinity for N at zero energy balance, causing an upward shift in the line. Similarly, a downward shift with hypercatabolic patients may result from either increased energy requirements, or decreased N balance at zero energy balance. In either case, as illustrated in the lower part of Fig. 2, both factors may be involved. The magnitude of any upward shift in the line may, itself, be a function of a number of factors, which include degree of depletion of the patient, the amount, or preferably biological value, of the protein supplied, and the composition of the non-protein portion of the diet.

The present study was designed to separate out the effects of energy and N intake, of energy expenditure, and of depletion on N balance. Nitrogen intake was kept constant. Energy expenditure was estimated, permitting an estimation of N balance at zero energy balance. The value found of 10 mg N per kg body weight per day constitutes the difference between normal adults, who should have zero N balance at zero energy balance, and this particular class of depleted patients (mean weight loss 27%) at this particular N intake (173 ± 42 mg N per kg per day). If this finding is combined with those of Rudman et al and Bozetti, it is possible to construct a grid, as

Table 1. Age, sex, body weight, energy expenditure and diagnosis of patients studied.

Patient	Age	Sex	Initial body wt (kg)	Percent wt loss from normal or desirable	Initial body surface area (m^2)	Mean REE entire study (kcal)	REE as percent of predicted	Diagnosis
JG	58	M	64.4	21	1.78	1866	129	Sepsis-fistulae
EL	64	F	38.2	25	1.32	881	86	Gastric outlet obstruction
LC	47	F	34.8	42	1.29	922	87	Bile duct stricture
EM	40	F	38.6	37	1.37	1194	104	Pelvic abscess
LW	66	F	69.0	15	1.70	1552	124	Small bowel obstruction
MS	68	F	60.7	13	1.69	1541	126	Pancreatitis
MD	52	F	87.0	21	1.88	1838	121	Dehydration-ileostomy
RG	47	M	58.9	22	1.64	1658	121	Pancreatic pseudocyst
LG	59	M	60.0	26	1.76	2031	152	Colon infarction
SC	21	M	34.4	48	1.35	1082	84	Severe depletion secondary to Neurological dysfunction
MEAN				27			113	

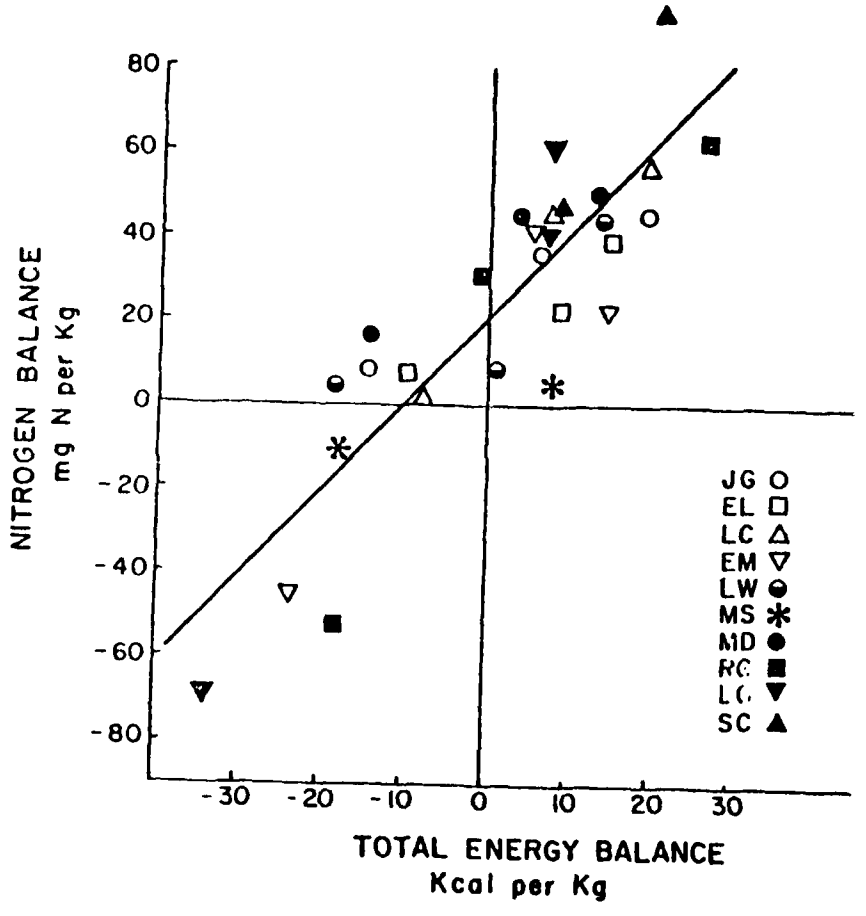
NITROGEN BALANCE
mg N per Kg
80
60
40
20
0
-20
-40
-60
-80
-30
-20
-10
0
10
20
30
TOTAL ENERGY BALANCE
Kcal per Kg
JG
EL
LC
EM
LW
MS
MD
RC
LC
SC

FIG 7

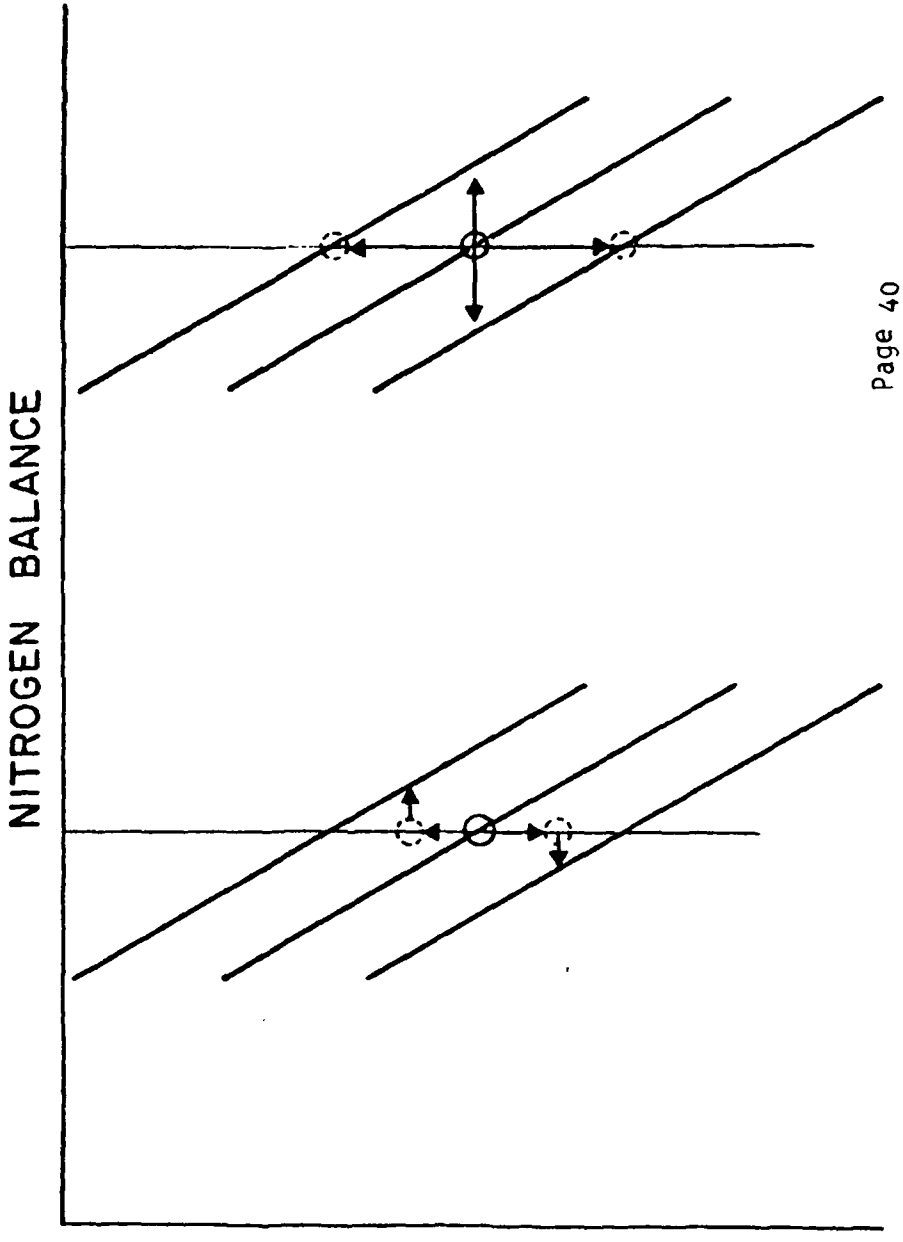
NITROGEN BALANCE
ENERGY INTAKE

FIG 8

EFFECTS OF ENERGY BALANCE
ON NITROGEN BALANCE AT
DIFFERENT LEVELS OF NITROGEN INTAKE

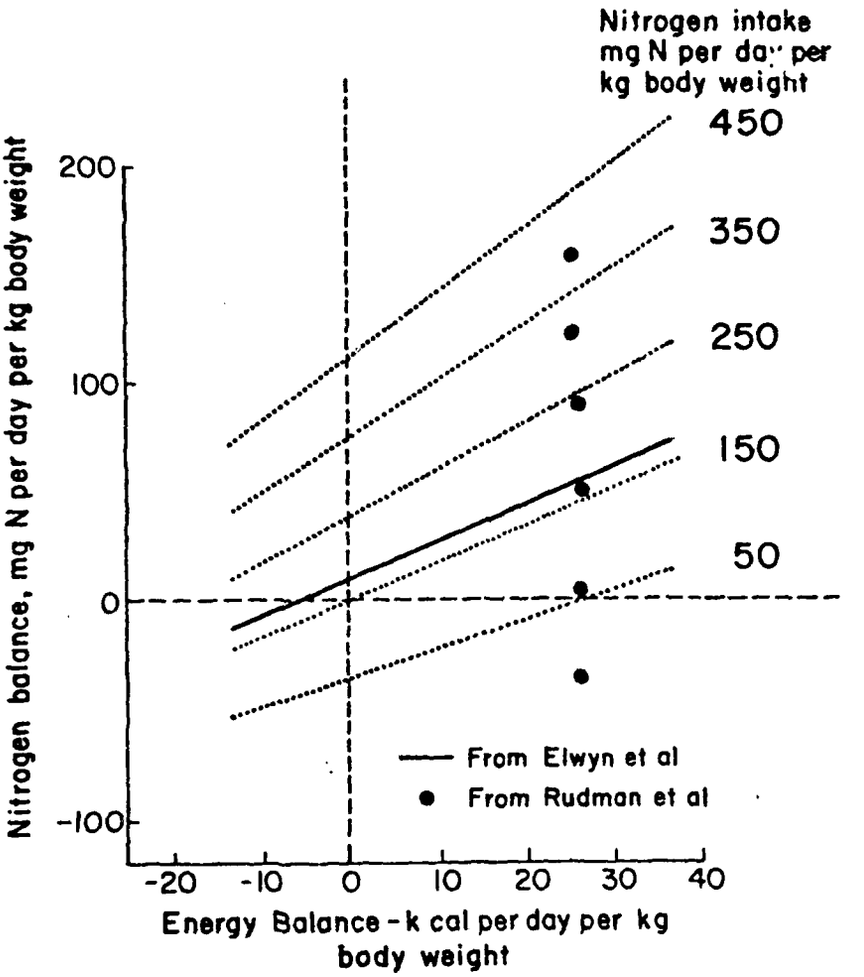

FIG 9

shown in Fig. 3, relating N balance to energy balance at different rates of N intake. This is consistent with the concept, discussed by Calloway and Spector (1) and Munro (3), that within minimal and maximal limits of energy and protein intakes there is a region where changes in either energy or nitrogen intake will effect changes in N balance. With the present information we can tentatively attach numerical values to the grid as they apply to severely depleted adult patients on completely adequate total parenteral nutrition, using crystalline amino acids as the source of nitrogen and glucose as the source of non-protein calories. The slope of 1.7 mg N per kcal at an N intake of 170 mg N per kg is taken from the present study. Since, in normals, this slope increases with increasing N intake, similar increases are shown in Fig. 3. (Further studies of depleted patients at higher levels of N intake are needed to verify this assumption). The efficiency of 50% for the increment in N retention per increment of N intake at high energy intakes is derived from Rudman et al (27) and Bozetti (20). Assignment of 150 mg N per day per kg to the intake line passing through the origin is made from the data of this study, showing +10 mg N per kg at zero energy balance, with an intake of approximately 170 mg N per kg, modified by the 50% efficiency figure. Although, this grid rests on very limited experimental evidence, and the actual numbers can only be considered as approximations, it can, nevertheless, serve as a tentative guide to the quantities of nitrogen and energy best suited for repletion of severely depleted patients. Further information is needed for the effects of high N intakes at zero energy balance.

Effects of Energy Expenditure on Nitrogen Balance - The measured values for REE varied quite widely, from 84 to 152%, when compared to predicted values (Table 1). Nevertheless, there was no separation between hyper- and hypocatobolic patients when N balance was plotted against energy balance (Fig. 1). This suggests that there was no difference in breakdown of lean body mass for these hyper- as compared to the hypocatobolic patients, but only an increased energy requirement. This does not mean that under other circumstances there may not be increased breakdown of LBM associated with hypercatabolism, it does suggest that such an association is not always obligatory.

Implications for Patient Management - It would seem almost axiomatic from a nutritional view that patients unable to be fed via the gastrointestinal tract should be provided with a balanced intravenous diet adequate in protein, energy and other essential nutrients. However, most surgical patients are maintained on 5% glucose together with adequate fluid and electrolytes. In some instances, this regimen is continued for long periods and may lead to serious nutritional depletion. This is indicated by the ready availability of patients for the present study, who had lost 13 to 48% of their body weight, largely or entirely during their hospital stay. The technical difficulties in providing an adequate diet constitute a part of the reason for this practice, particularly those connected with insertion and maintenance of a central venous catheter. With the growing use of total parenteral nutrition, catheter complications have been greatly reduced (30,31). However, until education and experience have overcome these difficulties, there will be a continued use of diets with less than adequate energy intake. Therefore, we need more complete

understanding of the nutritional effects of intravenous diets in the hypocaloric range.

The present study indicates that in severely depleted patients, a moderate intake, averaging 65 g amino acids per individual per day, provides a very modest increment in lean body mass when energy intakes are in the range of 0.5 to 2 times requirements. In this range, it seems likely that varying energy intake affects mainly fat storage and that fraction of lean body mass (muscle, blood vessels, etc.) required as its supporting structures; while increasing N intake affects only lean body mass unrelated to fat deposition. If this is true then it should be possible to manipulate an intravenous, or oral, diet so as to restore fat and LBM independently of each other. High rates of restoration of LBM appear to require high levels of N intake and cannot be attained by increasing energy intake even to very high levels. This suggests that there is no single calorie to nitrogen ratio most suitable for treating all depleted patients, but rather that for each patient the goals in restoring LBM and fat should be assessed separately and the energy and N intakes adjusted accordingly. Further work is needed to provide confirmation of this hypothesis, in particular exact information is needed on the quantitative effects on N balance of varying energy intake at high levels of N intake.

Characterization of Patients for Regional Studies

This approach to the characterization of surgical patients who may be considered for total parenteral nutrition requires further study for confirmation of certain assumptions mentioned above. However, it is planned that the regional studies described in the following research plan, will make use of this approach to patient evaluation in two ways: Initially, each patient will have four days of nitrogen and resting calorie balance determined before and again after starting total parenteral nutrition. The position of the patient on the calorie-nitrogen grid will then be studied retrospectively to determine if more precise characterization is possible than by conventional clinical criteria which we have used in the past. If this proves to be the case, we will then continue the studies utilizing such evaluation in a prospective manner to obtain groups of surgical patients which are more uniform in metabolic behavior, for comparison between groups.

References

Tables and Figures

Table I Study patients - Age - Dx - Wt. Loss.

Fig. 1 - Nitrogen balance as a function of total energy balance (formerly Fig. 7).

Fig. 2 - Schematic representation of the relations between N balance and energy intake (formerly Fig. 8).

Fig. 3 - Idealized schema of quantitative relations between N balance and energy balance at different levels of N intake in severely depleted patients on intravenous glucose and amino acids. See discussion for details.

REFERENCES

1. Calloway DH, Spector H: Nitrogen balance as related to caloric and protein intake in active young men. Am J Clin Nutr 2:405, 1955

2. Munro HN: Carbohydrate and fat as factors in protein utilization and metabolism. Physiol Rev 31:449, 1951

3. Munro HN: General aspects of the regulation of protein metabolism by diet and hormones, in Munro HN and Allison JB (eds): Mammalian Protein Metabolism, Vol 1. New York, Academic Press, 1964, p 381

4. Martin CJ, Robison R: The minimum nitrogen expenditure of man and the biological value of various proteins for human nutrition. Biochem J 16:407, 1922

5. Inoue G: Protein-sparing effect of excess calories during adaptation to low protein intake, in Olson RE (ed): Protein and Calorie Malnutrition. New York, Academic Press, 1975, p 323

6. Keys A, Anderson JT, Brozek J: Weight gain from simple overeating. I. Character of tissue gained. Metabolism 4:427, 1955

7. Calloway DH: Nitrogen balance of men with marginal intakes of protein and energy. J Nutr 105:914, 1975

8. Recommended Dietary Allowances, 8th ed. Washington, National Academy of Science, 1974

9. Shils ME: Total parenteral nutrition, in Goodhart RS and Shils ME (eds): Modern Nutrition in Health and Disease. Philadelphia, Lea & Febiger, 1973, p 966

10. Official Methods of Analysis, 9th ed. Washington DC, Association of Official Agriculture Chemists, 1963

11. Merrill AL, Watt BK: Energy Value of Foods-Agriculture Handbook No. 74. Washington DC, US Govt Printing Office, March 1955

12. Kinney JM, Morgan AP, Dominguez FJ, Gildner KJ: A method for continuous measurement of gas exchange and expired radioactivity in acutely ill patients. Metabolism 13:205, 1964

13. Spencer JL, Zikria AB, Kinney JM, Broell JR, Michailoff TM, Lee AB: A system for the continuous measurement of gas exchange and respiratory functions. J Appl Physiol 33:523, 1972

14. Swift RW, French CF: Energy Metabolism and Nutrition. Washington DC, Scarecrow Press, 1954

15. Calloway DH, Odell ACF, Margen S: Sweat and miscellaneous losses in human balance studies. J Nutr 101:775, 1971

16. Anderson HL, Heindel MB, Linkswiler: Effect on nitrogen balance of adult man of varying source of nitrogen and level of calorie intake. J Nutr 99:82, 1969

17. Inoue G, Fujita Y, Niiyama Y: Studies on protein requirements of young men fed egg protein and rice protein with excess and maintenance energy intakes. J Nutr 103:1673, 1973

18. Scrimshaw NS, Taylor Y, Young VR: Lysine supplementation of wheat gluten at adequate and restricted energy intakes in young men. Am J Clin Nutr 26:965, 1973

19. Munro HN, Wikramanayake TW: Absence of a time factor in the relationship between level of energy intake and protein metabolism. J Nutr 52:99, 1954

20. Bozetti F: Parenteral nutrition in surgical patients. S G & O, 142:16, 1976

21. Elwyn DH, Gump FE, Iles M, Long CL, Kinney JM: Protein and energy sparing of glucose added in hypocaloric amounts to peripheral infusions of amino acids. Metabolism 27:325, 1978

22. Reifenstein EC, Jr, Albright F, Wells SI: The accumulation, interpretation and presentation of data pertaining to metabolic balances, notably those of calcium, phosphorus and nitrogen. J Clin Endocrinol 5:367, 1945

23. Kinney JM, Duke JH, Long CL, Gump FE: Tissue fuel and weight loss after injury. J Clin Pathol Suppl Royal Col Pathol 23:65, 1970

24. Benditt EP, Woolridge RL, Stepto R: The dynamics of protein metabolism. II. The relationship between the level of protein intake and the rate of protein utilization by protein depleted men and rats. J Lab Clin Med 33:269, 1948

25. Allison JB: Calories and protein nutrition. Ann NY Acad Sci 69:1009, 1958

26. Beattie J, Herbert PH, Bell DJ: Nitrogen balances during recovery from severe undernutrition. Brit J Nutr 1:202, 1947

27. Rudman D, Millikan WJ, Richardson TJ, Bixler TJ, II, Stackhouse WJ, McGarrity WC: Elemental balances during intravenous hyperalimentation. J Clin Invest 55:94, 1975

28. Wilmore DW: Energy requirements for maximum nitrogen retention, in Green HL, Holliday MA and Munro HN (eds): Clinical Nutrition Update: Amino Acids. Chicago, American Medical Association, 1977, p 47

29. Plough IC, Iber FL, Shipman ME, Chalmers TC: The effects of supplementary calories on nitrogen storage at high intakes of protein in patients with chronic liver disease. Am J Clin Nutr 4:224, 1956

30. Brown RS, Grenkoski: Total parenteral nutrition: A safe procedure in the small community hospital? Crit Care Med 5:241, 1977

31. Ryan JA, Abel RM, Abbott WM, Hopkins CC, McChesney T, Colley R, Phillips K, Fischer JE: Catheter complications in total parenteral nutrition. N E J M 29:757, 1974

PROGRESS REPORT

IV. STUDIES OF LIPID METABOLISM IN INJURY, SEPSIS AND DEPLETION

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Nordenstrom, J., Askanazi, J. and Gump F.E.

1. Introduction

Major injury and infection are known to induce increases in resting energy expenditure, nitrogen excretion and mobilization of fat from adipose tissue, together with varying degrees of carbohydrate intolerance. These conditions appear to modify the natural pattern of lipid metabolism which is associated with partial and total starvation alone.

The overall objective of this project is to examine the influence of injury and infection on selected steps in mobilization, plasma transport, peripheral uptake and oxidation of endogenous lipids. Correlation is being sought between the metabolism of endogenous lipids and the metabolic handling of an exogenous triglyceride emulsion given for parenteral nutrition.

2. Specific Aims

This project involves the utilization of special methods to examine selected steps in the lipid metabolism of acutely ill surgical patients and patients having undergone severe tissue depletion as the result of injury or sepsis. The particular steps in lipid metabolism and the methods selected for their study are listed below:

a. Mobilization of body fat stores - in vitro measurement of FFA and glycerol release in biopsies of adipose tissue.

b. Kinetics of circulating glycerol - labelled glycerol will be given intravenously to measure oxidation to CO_2, turnover in the bloodstream and contribution to glucose synthesis.

c. Kinetics of triglyceride breakdown - staged glycerol infusion will be used to determine glycerol turnover rate and hence glycerol release from adipose tissue (Methodology of Hirsch, et al.)

d. The rate of clearance, tissue sites of removal (in animals) and oxidation of a labelled exogenous triglyceride emulsion will be studied in relation to our knowledge of endogenous lipid metabolism.

e. The infleunce of nutritional intake on lipid metabolism during injury or infection is being studied during hypocaloric infusions by peripheral vein and by total parenteral infusion in a central vein using either carbohydrate alone or carbohydrate and lipid for the supply of non-protein calories.

3. Completed Studies

During the past year, the following studies have been completed, the material has been presented at scientific meetings and two of the studies have been accepted for publication during the coming months while the third study has been submitted for publication.

A. SOME METABOLIC EFFECTS OF FAT INFUSIONS IN DEPLETED SURGICAL PATIENTS

Elwyn, D.H., Kinney, J.M., Gump, F.E., Askanazi, J., Rosenbaum, S.H. and Carpentier, Y.A.

Intravenous lipid emulsions have been shown to be safe for clinical use since the pioneering work of Wretlind approximately 20 years ago. However, such preparations were not available when Dudrick and colleagues introduced hyperalimentation in the United States in the late sixties. Therefore, the conventional form of total parenteral nutrition in this country is built around glucose and amino acids without the administration of fat. Now that intravenous fat preparations are commercially available in this country, there is considerable controversy concerning when and how much of it should be used. Some authorities advise against the use of any but minimal fat administration (i.e., one or two bottles per week to prevent essential fatty acid deficiency) since it has been reported to cause little nitrogen retention in burn patients and may have some undesirable effects of blood clotting or pulmonary function. Other authorities feel that fat is similar to carbohydrate in nitrogen retention beyond the first few days of administration, has no significant toxic properties when given in modest amounts, and probably plays important synthetics roles which carbohydrate cannot. Therefore, a prospective study of surgical patients needing total parenteral nutrition was undertaken which involved careful measurement of daily calorie balance, to help in clarifying the proper role of lipid in the parenteral nutrition of such patients.

Patient Selection. Protein-calorie depleted patients who had undergone prior weight loss of 15 percent or more and who required total parenteral nutrition (TPN) on medical grounds were admitted to the study. Age, weight, and diagnosis of each patient is shown in Table 1.

The details of the experiments, including risks, were explained to each patient, usually in the presence of members of his or her family, and written consent was obtained. The protocol of this study has been approved by the Columbia University Institutional Review Board.

Protocol. During the first day, patients were maintained on intravenous five percent dextrose. Their REE* was measured and used as a basis for calculating subsequent dietary intake. They were then randomly assigned to one of two diets for one week, and received the other diet on the following week. In three instances studies were continued for a third week. Eight studies were performed on six patients, studies 2 and 8 were performed on one patient, studies 3 and 7 on another patient. Patients in Group I were started on diet I, with all nonprotein calories as glucose, and shifted on day 8 to Diet II, in

TABLE I Patients

Study Number	Age	Sex	Body Weight (kg) Before Study	Body Weight (kg) Estimated Normal	Wt. Loss Percent	BSA (M^2)	Resting Energy Expenditure (Cal/M^2 BSA) Before	Predicted	Measured & of Predicted	Diagnosis
1	66	F	57.3	64.1	11	1.63	650	737	88.2	GI Obstruction
2	71	M	59.5	65.9	10	1.76	643	742	86.7	Achalasia
3	47	M	58.4	77.3	24	1.75	692	828	83.5	Pancreatitis
4	47	M	56.8	77.3	27	1.73	708	822	86.1	Gastric Ulcer
5	66	F	52.9	57.3	8	1.48	744	764	97.4	Diverticulitis
6	67	F	38.8	52.3	26	1.35	591	747	79.0	Radiation Enteritis
Mean	60.7		54.9	65.7	17.7	1.62	671	737	86.8	
± SEM	± 4.4		± 3.2	± 4.2	± 3.6	± .07	± 22	± 17	± 2.5	

which one third of the glucose was replaced isocalorically by the fat emulsion Liposyn^{R}. Group II were given the diets in the reverse order. Diets were infused continuously over each 24 hour period except that fat emulsion, when given, was infused over a 6 to 8 hour period between 09:00 and 17:00. Nitrogen balance was measured daily throughout the study. Oxygen consumption and carbon dioxide production were measured three to five times daily with at least one measurement during the interval 09:00 to 17:00, when fat emulsion was infused, and one measurement during each of the other 8 hour periods, 17:00 to 01:00 and 01:00 to 09:00.

Diets. Nitrogen intake was maintained constant for each patient at a nominal level of 10 mg N, equivalent to 0.28 kcal, per kcal REE. Nonprotein calorie intake was nominally 1.07 kcal per kcal REE, all as glucose in Diet I, two thirds as glucose and one third as 10% Liposyn^{R} in Diet II. Vitamins and minerals were usually added to the infusion as shown in Table 2. Where indicated, individual adjustments were made. One mg of vitamin B_{12} was injected monthly. Vitamin K (Aquamephyton) was given as required.

Iron was given intramuscularly as indicated. Zinc, copper and iodine, at 1 to 5 times the recommended intravenous doses were given orally. Apart from trace elements and water, there was no oral intake. Amino acids were given as 10 percent Aminosyn^{R} (Abbott Laboratories, N. Chicago, Ill.).

Balance Measurements. All intake, whether oral or infused, was measured by difference in weights of full and emptied containers. The amounts of each constituent (H_2O, N, etc.) were calculated from the composition obtained from the manufacturer's specifications or by direct analysis in this laboratory according to established procedures. Energy contents of diets were calculated from published values. Urine, feces, and drainages were collected and analyzed for total nitrogen. In addition, urea was determined in urine and drainage, creatinine was determined in urine, and glucose was determined in those urine samples in which qualitative tests (Ketodiastix^{R}, Ames Co., Elkhart, Ind.) were positive. A manual, micro-Kjeldahl proceudre was used for digesting samples for total N determination. Subsequent stages in total N determination, and analyses of urea and creatinine, were carried out with single channel automated analyzers according to the manufacturers procedures (Auto Analyzer, Technicon Company, Tarrytown, N.Y.). Blood urea N was measured by an automated enzymatic procedure (BUN Analyzer, Beckman Instruments, Inc., Fullerton, Cal.).

Gas Exchange. Oxygen consumption and CO_2 production were measured, with the patients lying at rest, using a rigid lucite head canopy developed in this laboratory. This permits frequent measurements of relatively long duration, 3 to 5 periods per day of 40 to 60 minutes each, evenly spaced throughout the 24 hour period, with minimal discomfort to the patient.

CALCULATIONS

1. Tissue composition of weight change. When the nonprotein RQ was 1.00 or less, the amounts of protein, carbohydrate and fat oxidized, and the amount of water produced by oxidation were calculated using standard procedures, with the addition that urea in drainage fluids was added to urinary N in calculating protein oxidation. N balance was corrected for variations in blood urea nitrogen. When the nonprotein RQ was greater than 1.00 the procedures

TABLE II

BIOLOGICAL AND ENERGY DATA (Values ± SEM)

	GTO ($\mu M/min/M^2$)	GLY Conc ($\mu M/l$)	Glucose Conc (mg/100 ml)	Insulin Conc ($\mu U/ml$)	FFA Conc (mEq/l)	βOH B Conc ($\mu M/l$)	REE ($Kcal/M^2/day$)	Non-Protein RQ
Basal (D_5W)	87.5	124	80	5	.85	523	679	.76
	± 9.1	± 13	± 7		±.09	± 113	± 24	± .01
Diet I	70.7	83	90	11	.58	67	700	.86
	±12.6	± 10	± 5	± 6	± .14	± 1	± 42	± .02
Diet II	48.2	66	95	34	.29	77	715	1.02
	± 8.6	± 17	± 4	±10	± .13	± 8	± 15	± .03

were modified, as described elsewhere, to include conversion of glucose to fat.

2. Energy Balance. Resting energy expenditure was calculated by indirect calorimetry from gas exchange measurements on the resting patient. Activity energy expenditure was estimated from steady state values of the apparent resting carbohydrate balance by a procedure described elsewhere. An estimate of total energy expenditure is obtained by summing REE and AEE. Energy balance is obtained in turn by subtracting total energy expenditure from energy intake. This give an approximation of energy balance which is clower to the true value then would be obtained if AEE were ignored.

RESULTS

Diet Intake. Differences between two diets averaged less than 5% for protein and total calories. Fat, in diet II, averaged 30% of nonprotein calories although Liposyn^{R}, which contains free glycerol (10% of calories) provided 33%. Mean nitrogen intake for the six subjects, each averaged over the entire study period, was 266 mg per kg with an SEM of 12.

Energy Expenditure and Balance. Activity energy expenditure was approximated for the entire study. Resting energy expenditure showed no significant differences between Weeks I and II or diets I and II, and little difference between groups. As shown by the small standard deviations, there was not much individual variation within groups. There was no significant day-do-day variation in REE. Energy balance showed little difference between diets or groups. Dietary intake was originally chosen to be approximately equal to total energy expenditure. Mean energy balance for all patients was 2.0 kcal per kg with a standard error of 1.1.

Nitrogen Balance. There were no significant differences, for nitrogen balance by Student's paired t test, between the first and second week ($P > 0.5$). Furthermore, there were no significant changes from one day to the next within each week. To a first approx-mation there was no period of adaptation, with respect to N balance, in going from the prestudy diet (in most instances 5% dextrose) to either of the study diets, nor in going from one diet to the other.

The mean value for daily N balance, derived from the mean values for each of the six patients over the entire study, was 64 mg N per kg with a standard error of 10. Since both energy intake and nitrogen intake influence nitrogen balance, it is useful where possible to express the effect of N intake on N balance at a constant energy balance and if possible at zero energy balance. Since energy balance was close to zero in these patients, individual values of N balance were corrected by applying the factor of 1.7 mg N per kcal determined previously. In addition, a correction of approximately 10 mg N per kg may be made to cover integumental and methodologic losses. The corrected value for N balance, thus obtained at zero energy balance and at an N intake of 266 mg per kg, is equal to 50 mg per kg with a standard error of 10.

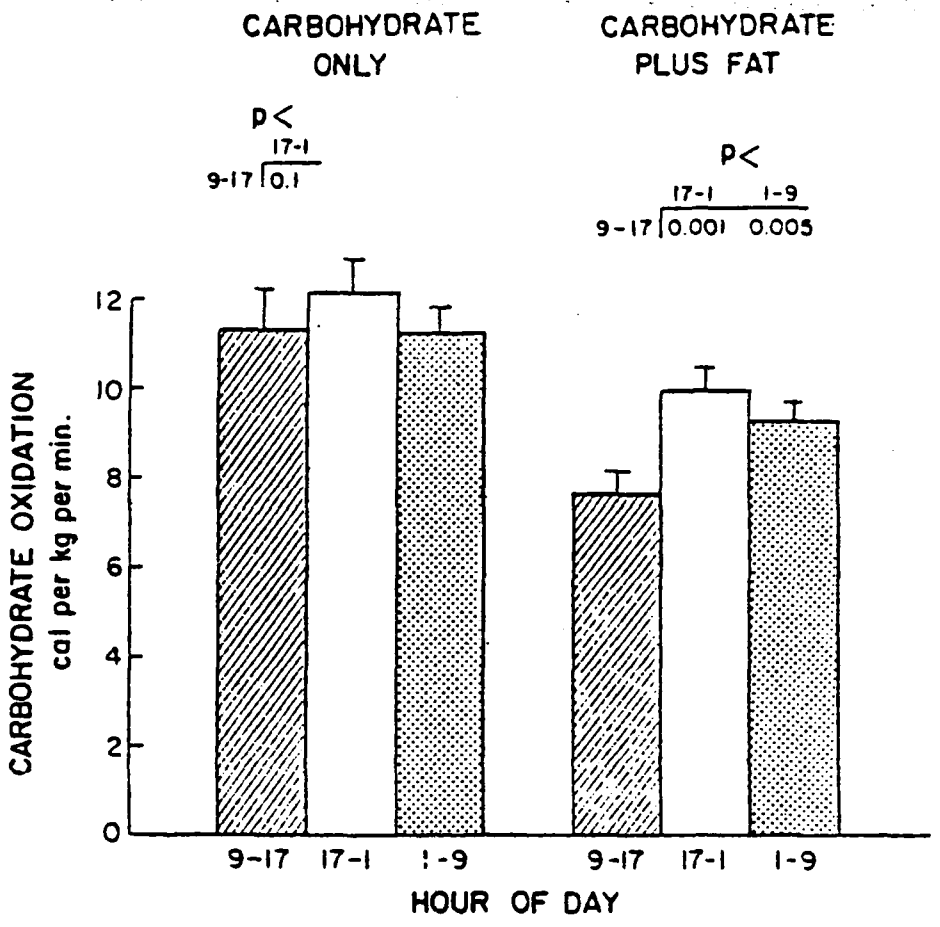
Respiratory Quotient. It took two to three days to complete changes in nonprotein RQ in going from either the fat or prestudy diet to the carbohydrate diet, or from the carbohydrate to the fat diet. These transient changes presumably reflect repletion or depletion of glycogen stores. After three days, the RQ was quite stable on either diet. The difference in nonprotein RQ between diets averaged 0.10 ($p < 0.001$).

Timing of Gas Exchange Measurements. A total of 392 measurements were made of gas exchange, 166 between the hours of 09:00 and 17:00, 131 between 17:00 and 01:00 and 95 between 01:00 and 09:00. Measurements during the day were fairly evenly distributed although they were more frequent from 10:00 to 12:00 and from 14:00 to 16:00 than at other times. Ninety percent of the evening measurements were made between 18:00 and 23:00. Ninety percent of the night and morning observations were between 06:00 and 08:00. No measurements were made between 23:00 and 05:00 when the patients were sleeping.

Diurnal Changes in Rates of Carbohydrate and Fat Oxidation and Resting Energy Expenditure. The rates of infusion of glucose and amino acids were kept constant over each 24 hour period when patients were on either diet. However, the fat emulsion was infused only during a six to eight hour period. This resulted in marked diurnal changes in RQ_2, and the rates of oxidation of carbohydrate (Fig 1) and fat (Fig 2). During fat infusion, fat oxidation was faster, while RQ and carbohydrate oxidation were lower than during the rest of the day. These differences were highly significant. By contrast, there was much less diurnal change in these parameters during administration of the carbohydrate diet. This suggests that the changes seen with the fat diet are the direct result of fat infusion and are not attributable to other possible factor which show diurnal variation.

The complete write-up of this study includes 5 tables, 5 figures and 23 references which will be supplied as reprints upon publication, but will be forwarded sooner upon request.

Implications for Clinical Management. The immediate effects of infusing the fat emulsion under the conditions of this study were to increase the rate of fatty acid oxidation and glycogen deposition while decreasing the rate of glucose oxidation with no change in the rate of resting energy expenditure. While these effects are consistent with metabolic data from studies in other conditions, this is the first demonstration of such data in depleted surgical patients. The amount of administered fat emulsion was undoubtedly effective in preventing an essential fatty acid deficiency. However, certain other observations are of importance when considering this type of surgical patient. The nitrogen retaining properties of intravenous lipid have been a source of much controversy, with certain claims that it is of no use for nitrogen retention in surgical patients. The results of this study indicate that in the depleted patient receiving total parenteral nutrition based on glucose and amino acids, the substitution of a fat emulsion for one third of the glucose calories proved as effective as the glucose alone in maintaining a positive nitrogen balance. The nitrogen conserving effect of intravenous lipid is apparently a function of the physiologic or pathologic state of the patient and the proportion of non-protein calories supplied as lipid. These studies, taken with certain other data in the literature, suggest that depleted patients can maintain a highly positive nitrogen balance for weeks and perhaps even months, while at an approximately zero energy balance. This is in marked


CARBOHYDRATE ONLY
CARBOHYDRATE PLUS FAT
p<
17-1
9-17 0.1
p<
17-1 1-9
9-17 0.001 0.005
CARBOHYDRATE OXIDATION cal per kg per min.
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2
4
6
8
10
12
9-17 17-1 1-9 9-17 17-1 1-9
HOUR OF DAY

FIG 1

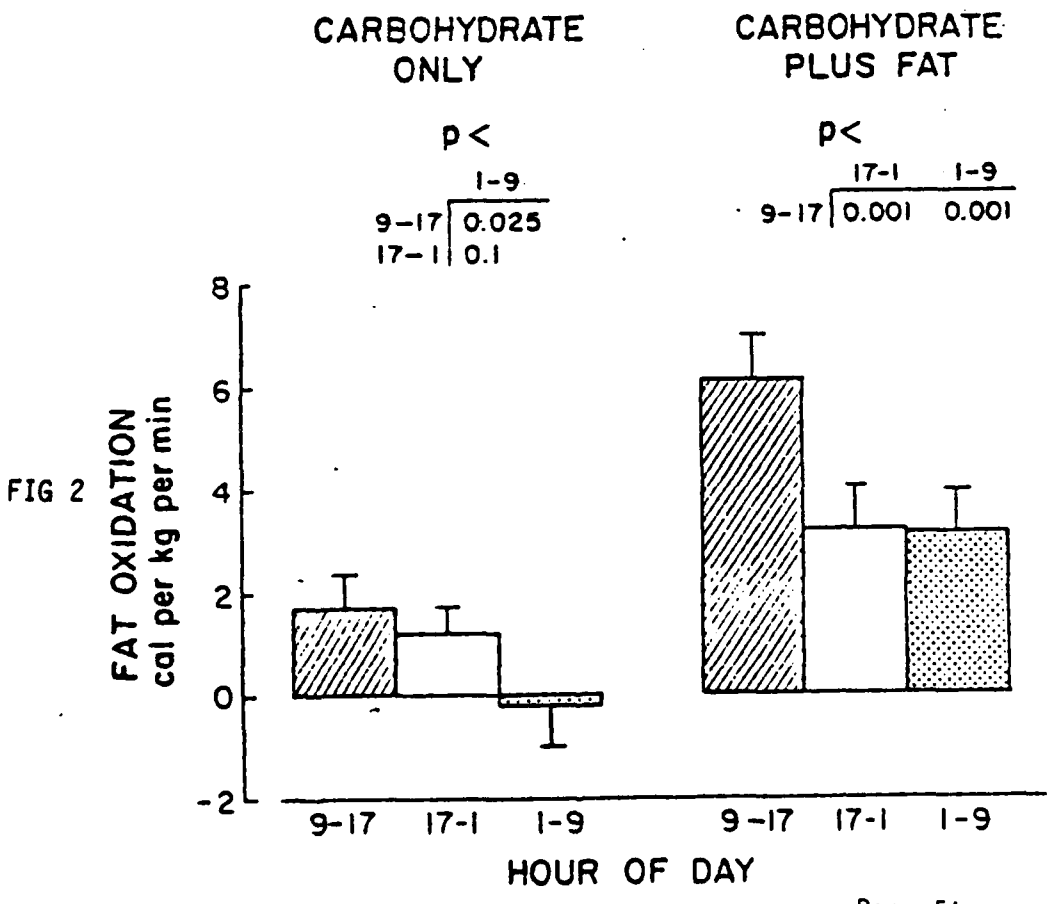

CARBOHYDRATE ONLY
CARBOHYDRATE PLUS FAT
p<
1-9
9-17 0.025
17-1 0.1
p<
17-1 1-9
9-17 0.001 0.001
FAT OXIDATION cal per kg per min
-2
0
2
4
6
8
9-17 17-1 1-9 9-17 17-1 1-9
HOUR OF DAY

FIG 2

contrast to the normal adult who at steady state would be expected to be at a zero nitrogen balance, if receiving a nitrogen intake above the minimum requirement. Thus, excessive calories are not required for restoration of lean body mass in depleted patients. In our opinion, this data supports our previous belief that energy intake and nitrogen intake should be varied independently, in contrast to maintaining some arbitrary calorie to nitrogen ratio. Calorie intakes should be based on estimates of total energy expenditure plus or minus an amount to restore or lose body fat. Nitrogen intakes should be based on what is felt to be the most desirable rate of restoration of lean body mass.

B. THE EFFECT OF OPERATIVE INJURY ON THE RATE OF MOBILIZATION

Carpentier, Y.A., Burr, R.E., Askanazi, J., Gump, F.E., Stinchfield, F.E., Kinney, J.M.

The mobilization of body fat consists of the hydrolysis of triglycerides (TG) to fatty acids (FFA) and glycerol (GLY) mediated by the action of hormone-sensitive lipase. This process takes place mainly in adipose tissue. The resulting FFA are partly released into the blood stream and partly reesterified within the adipocytes, while GLY leaves the cell almost entirely. Glycerol turnover (GLYTO) is thus an accurate index of TG hydrolysis. In vitro studies showed that hormone-sensitive lipase was activated by β-adrenergic stimulation and catecholamines and inhibited by insulin and possibly carbohydrates (1,2). Surgical stress is expected to increase TG breakdown while dextrose infusion - and the resulting insulin release - should inhibit peripheral lipolysis (3).

MATERIALS AND METHODS

Six patients were studied before and four days after total hip replacement. The patients were infused faily with 2.5 L. of D_5W during the postoperative course. No food, only water was allowed. CLYTO was measured using a method developed by Burr and Hirsch at Rockefeller University. After the withdrawal of basal blood samples, glycerol 10% in water was infused intravenously at three different rates (0.2, 0.5 and 1.0 mM/min), each during one hour. Three blood samples were taken during the last 15 minutes of each infusion rate. GLY concentration was determined in each sample. GLY clearance (ml/min) was obtained by the ratio: Δ infusion rate/Δ GLY concentration, and GLYTO (μM/min) by: basal GLY concentration x GLY clearance. The preoperative test was performed after an overnight fast. D_5W was replaced by saline 8 hours before the postoperative study.

RESULTS AND CONCLUSIONS

Biological data are summarized in the following table.

Preoperative GLYTO values were in the normal range as compared with results reported using isotopic glycerol (4). GLYTO was more than doubled after surgery, while GLY concentration was increased by only 43%. FFA concentration was not significantly increased. Postoperative glucose and insulin

BIOLOGICAL DATA OF 6 PATIENTS BEFORE AND AFTER SURGEY
(Values ± SEM)

	CLYTO $M/minM^2$	GLY Conc. µM/ml pl.	FFA Conc. mg/l pl.	GLUCOSE Conc. mg/100 ml	INSULIN Conc. µU/ml pl.	CATECHOLS Ur. Excretion (mean 3 days) $\mu g/day/M^2$	R.Q.	NITROGEN BALANCE g/day
Pre op.	58 ± 3	74 ± 6	.78 ± .10	99 ± 4	< 2	25 ± 5	.84 ± .01	-
Post op.	126 ± 6	106 ± 11	.95 ± .18	100 ± 4	< 2	58 ± 15	.77 ± .02	-7.9 ± .8
Changes (% pre op)	+125	+43	+22	+1	-	+132	-	-
p val.	<.001	<.05	NS	NS	NS	<.005	-	-

levels were not changed when measured 8 hours after stopping infusion of D5W. A positive correlation was found between postoperative GLYTO and the individual increase in urinary excretion of free catecholamines ($r = .79$). There was no correlation between GLY concentration and catecholamine excretion. Low postoperative R.Q. indicated a predominant oxidation of fat as energy substrate. These results suggest that plasma levels of GLY and/or FFA are not adequate to measure changes in fat mobilization in conditions such as operative trauma. Kinetic studies are then required.

REFERENCES

1. Steinberg D, Khoo JC: Hormone-sensitive lipase of adipose tissue. Fed Proc 36:1986-1990, 1977

2. Jungas RL, Ball EG: Studies on metabolism of adipose tissue. XII. The effects of insulin and epinephrine on free fatty acid and glycerol production in the presence and absence of glucose. Biochemistry 2:383-388, 1963

3. Blackburn GL, Flatt JP, Clowes GHA Jr, et al: Protein sparing therapy during periods of starvation with sepsis or trauma. Ann Surg 177: 588, 1973

4. Björntorp P, Bergman H, Varnauska E, et al: Lipid mobilization in relation to body composition in man. Metabolism 18:849-851, 1969

C. THE EFFECT OF CARBOHYDRATE INTAKE ON THE LIPOLYTIC RATE IN DEPLETED PATIENTS

Carpentier, Y.A., Askanazi, J., Elwyn, D.H.,
Gump, F.E., Nordenström, J., Kinney, J.M.

INTRODUCTION

The effects of various pharmacological and hormonal agents on triglyceride (TG) hydrolysis have mostly been studied by in vitro incubation of fat biopsies or determinations of plasma free fatty acid (FFA) and glycerol concentrations. Glucose and insulin have often been reported to exert an inhibitory effect on lipolysis. To our knowledge, no in vivo measurement of the effect of carbohydrate infusion - and the resulting insulin release - on the kinetics of lipolysis has been performed in man. Since glycerol is almost completely released from the adipose cell after TD hydrolysis in man, measurement of glycerol turnover is adequate for estimating TG breakdown. A close positive correlation has been found between the turnover and the plasma concentration of glycerol in normal and obese subjects. Previous work from our unit showed the absence of correlation between glycerol turnover and plasma concentration in severely injured and infected patients. Infusion of a parenteral nutrition (TPN) providing 1.75 x resting energy expenditure (REE) as glucose and amino acids was found to have no inhibitory effect on glycerol turnover in these traumatic patients.

The present study was designed in order to evaluate the effect of TPN with two different levels of carbohydrate intake-one below and one above the energy expenditure - on the kinetics of lipolysis in nutritionally depleted patients.

MATERIAL AND METHODS

Patient Selection - Six protein-calorie depleted patient who required total parenteral nutrition (TPN) on medical grounds were admitted to the study. Age, sex, weight, body surface area (BSA) resting energy expenditure (REE) and diagnosis of each patient are shown in Table 1. The average body weight was 17.7% below normal and the REE was 13.2% below predicted. No patient was in an immediate postoperative period. None had an active cancer or a disease susceptible to altering their metabolic response to carbohydrate infusion.

Informed Consent - The protocol of this study has been approved by the Columbia University Institutional Review Board. The details of the experiments, including risks and purposes, were explained to each patient, usually in the presence of members of his or her family. Written consent was obtained in each case.

Protocol - Except for water no oral intake was allowed during the study. During the two first days, 2.5 l of D_5W were infused daily into a peripheral vein. Basal REE values measured during this period were used for calculating subsequent dietary intake. The patients were then randomly assigned to one of two diets for one week, and received the other diet on the following

week. Three different measurements of glycerol turnover (GTO) and other biological determinations were performed. The first one was done before starting TPN, D_5W infusion having been replaced by saline six hours before the test. The second and third measurements were performed after four days on each TPN diet. TPN was not stopped during these tests. Gas exchange measurements were performed three to five times daily throughout the study.

Diets - Both TPN diets were administered 24 hours a day through a subclavian catheter. Amino acid intake (Aminosyn^R 7%, Abbott Laboratories, N. Chicago, Ill.) of 9 mg N per kcal basal REE was constant in both diets. The difference in calorie intake was due entirely to the carbohydrate load. Glucose intake was calculated so that total calorie intake would reach .75 x REE for diet I and 1.30 x REE for diet II. Vitamins and minerals were added to the infusion and individual adjustments were made when necessary. Zinc, copper and iodine were given orally. Iron was given intramuscularly. Essential fatty acids were administered by a daily massage with 1 tablespoon of corn oil.

Glycerol Turnover - The method, developed at Rockefeller University, has been reported in previous work and is summarized as follows: Two peripheral intravenous lines - one for infusion, one for drawing blood samples are inserted in both forelimbs under local anesthesia and are kept patent with a slow infusion of saline. Forty minutes after the lines are inserted, three blood samples are drawn at intervals of 10 minutes for determination of basal concentrations. A solution of 10% glycerol in water is then infused at three different rates (approximately .22, .55 and 1.10 mM/min) each during an hour. Three 3 ml blood samples are drawn during the last 15 minutes of each infusion setting. Glycerol concentration is determined on each sample by a semi-automated procedure using a centrifugal analyzer Centrifuchem^R (Union Carbide Corp, Rye, NY). The clearance of glycerol (ml/min) has been demonstrated to be constant within the range of concentrations encountered with this procedure and is obtained by the ratio: Δ infusion rate (mM/min)/Δ glycerol concentration (mM/ml). GTO is obtained by multiplying clearance x basal glycerol concentration.

Determination of Other Biological Parameters - Glucose concentration in the plasma was measured by an automated procedure (Glucose analyzer, Beckman Instruments, Inc., Fullerton, CA).

Insulin concentration in the plasma was assayed using Phadebas^R kits (Pharmacia, Uppsala, Sweden).

Plasma fatty acid concentrations were titrated according to Dole and Meinertz.

β-OH-butyrate concentrations were determined by semi-automated procedure using a centrifugal analyzer Centrifuchem^R (Union Carbide Corp, Rye, NY).

Urine drainages and feces samples were digested by a manual micro-kjeldahl procedure and total N output was measured with an automated analyzer (Auto Analyzer, Technicon Company, Tarrytown, N.Y.).

Blood urea nitrogen was measured by an automated enzymatic procedure (BUN analyzer, Beckman Instruments, Inc., Fullerton, CA). Nitrogen balance was corrected for changes in BUN.

Gas Exchange - Three to five daily determinations of oxygen consumption and CO_2 production were performed, using a non-invasive canopy system, as previously reported.

REE was calculated by indirect calorimetry from gas exchange measurements on the resting patient.

Values of non protein respiratory quotient (R.Q.) were obtained by including the daily nitrogen balance in the calculations.

RESULTS

Diet Intake - Amino acid intake for each patient was the same in both diets. The average for all patient was 8.95 + 1.29 mg N per kcal basal REE. The total calorie intake was 72.2% of basal REE with diet I and 127.9% of basal REE with diet II.

Glycerol Turnover and Concentrations - The results (Table II) suggest two-step decrease in glycerol turnover and concentrations in inverse correlation with the carbohydrate intake. However, while the 55% decrease in GTO and 53% decrease in glycerol concentration observed during Diet II were highly significant ($P < .001$), the 19% decrease in GTO and the 33% decrease in glycerol concentration observed during Diet I were respectively non-significant and borderline significant.

A very close correlation was observed in these patients between glycerol turnover and plasma concentration (Fig. I).

Determination of Other Biological Parameters - Plasma glucose concentrations showed a slight increase with both levels of TPN, that was significant only when Diet II was infused.

Insulin levels showed a significant two-step increase in correlation with carbohydrate intake.

Free fatty acid concentrations showed a two-step (32% and 66%) decrease, in inverse relation to the carbohydrate intake.

In contrast to FFA, β-OH butyrate concentrations showed a dramatic drop at the lower calorie intake and no further change with the highest glucose load.

Gas Exchange - Basal values of REE and the average of the values measured during the last three days on each TPN diet are shown in Table II.

REE showed a slight tendency to increase during TPN, but the rise was statistically significant only when Diet II was infused.

Non-protein R.Q. showed a significant two-step increase from .76 at the basal state to over 1.00 during Diet II.

DISCUSSION

Basal Values in Depleted Patients - The depleted patients studied in the present work showed a pattern typical of moderate starvation: Low REE and RQ values as well as insulin and glucose concentrations, increased glycerol turnover and concentration and high β-OH butyrate concentration.

Effect of Carbohydrate Intake in Triglyceride Breakdown - Several factors have been reported to influence TG hydrolysis in the adipose tissue, the most potent of which seem to be catecholamines, insulin and glucose. In unpublished studies performed in this laboratory on five depleted patients similar to those in this study, there was no effect of TPN on catecholamine excretion. This suggests that the main factor in this study influencing TG breakdown was the carbohydrate intake. The design of the present work does not permit separation of the direct effect of a glucose load on the various measured parameters from the influence of the resulting insulin response. Only carbohydrate loads providing a calorie intake in excess of REE proved to have a statistically significant inhibitory effect on TG breakdown in this group of depleted patients. However, although not reaching significance, a tendency to decreased glycerol turnover was observed during infusion of TPN with the lowest carbohydrate intake. These results are in agreement with in vitro measurements of decreased lipolytic rates induced by glucose and insulin. They contrast with our study showing that in severely injured patients, TPN providing 1.75 x REE did not change the rate of glycerol turnover; it should be pointed out that high catecholamine outputs were measured in these hypermetabolic patients.

Relationship Between Turnover and Plasma Concentration of Glycerol - A very close positive correlation was observed between GTO and glycerol concentration in our depleted patients. This is in agreement with other observations on normal and obese subjects, but in contrast to our measurements performed in traumatized patients.

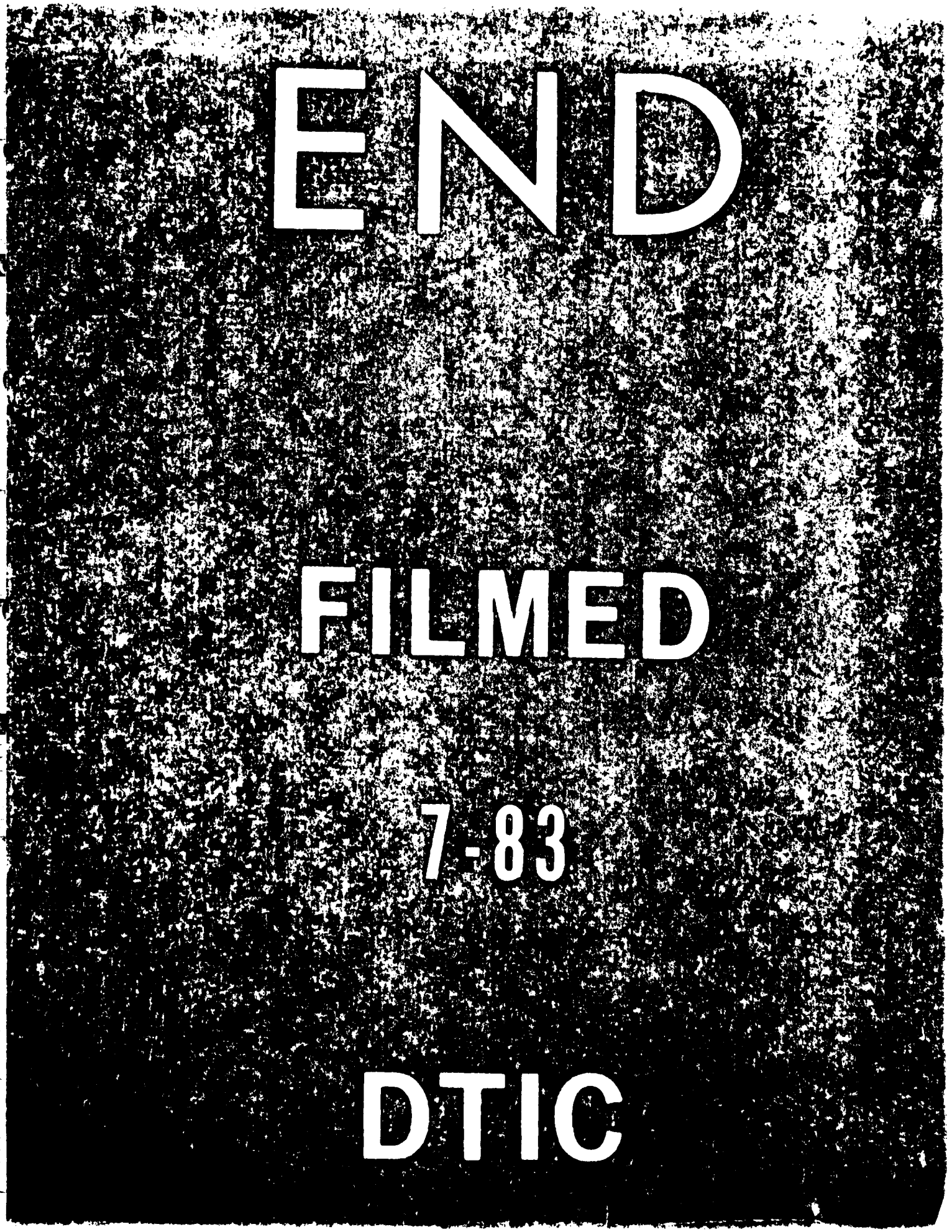
Influence of Carbohydrate Intake on Other Parameters - The lower TPN diet, although having a moderate and non-significant effect on TG hydrolysis, did induced significant 32% decrease in FFA plasma levels. If the assumption is made that FFA turnover is correlated with plasma concentration in depleted patients, this would indicate that the lower glucose load - or/and the resulting insulin response - could promote FFA recycling within the adipose tissue or the liver. The lowest levels observed during high TPN intake could result from decreased TG breakdown together with increased FFA reesterification.

By contrast to the two-step decrease in FFA concentration, a dramatic- and possibly maximal-drop in β-OH-butryate levels was observed already during the lower carbohydrate intake. The increased peripheral utilization of ketones induced by insulin cannot totally explain this fall in plasma level. A marked inhibition in hepatic ketogenesis likely due to insulin should be responsible for this phenomenon.

The two-step increase in the values of non-protein R.Q. is inversely proportional to the changes in FFA plasma levels. This indicates that fat was still a significant energy substrate during the lower carbohydrate intake. Non-protein R.Q. values rose over 1.0 during the higher TPN intake, indicating that not only could carbohydrate account for the whole energy requirement but

that the glucose excess was converted to fat. This is in contrast to observations that administration of TPN well above REE could not achieve R.Q. values of 1.0 in traumatized patients.

In conclusion, the present study is to our knowledge, the first one in which in vivo kinetic measurements were performed to estimate the effect of carbohydrate intake on the kinetics of TG breakdown in depleted patients. Glycerol turnover was closely correlated with glycerol concentration, as seen in normal subjects. Carbohydrate infusion - and the insulin response - induced an inhibition in fat mobilization that was proportional to calorie intake. Endogenous fat was still a significant energy substrate when glucose load was below energy requirements but no longer when calorie intake exceeded REE.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