A NEW CONCEPT AND ITS APPLICATION FOR PROTEIN SPARING THERAPIES DURING SEMI-STARVATION

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ABSTRACT

The preservation of the body cell mass is a major goal of nutritional therapy. Attempts to assure this preservation during acute phases of trauma, burn or sepsis have relied on treatments which appear to maintain the clinical picture of the "fed state" either orally or parenterally ("hyperalimentation").

Recognizing the importance of rapid fat mobilization and the favorable effects of starvation ketosis during periods of severe negative caloric balance, the efficiency of a limited, carbohydrate-free alimentation was tested. Based on a metabolic fuel regulatory system that integrates carbohydrate, amino acid and fat metabolism 0.8 to 1.0 g/kg/day of protein was administered orally or intravenously. This regime does not appreciably impede fat mobilization as does comparable intake of carbohydrate. The ingested or infused amino acids need therefore not be expended primarily to support energy production, but can replenish the amino acid pools which sustain protein synthesis. Losses in lean body mass were prevented in obese patients undergoing prolonged periods of acute weight reduction and considerably reduced or even suppress losses in patients supported only by peripheral intravenous infusions.

These investigations, hopefully, will provide improvements in nutritional therapies designed at reducing protein catabolism during periods of negative caloric balance.

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I. INTRODUCTION

Preservation of the body cell mass is a major goal of nutritional therapy. Protein loss is a major contributor to the morbidity and mortality in many disease states involving infection, sepsis and injury. This protein loss is generally associated with inadequate caloric and protein intake. While particular success has been achieved through parenteral and elemental diet nutritional therapies in reducing the prolonged negative nitrogen balance, this has required the mass action of the food intake to counter-act the strong catabolic trend associated with these severe disease states.

Recognizing the importance of adequate fat mobilization and the favorable effects of starvation ketosis during periods of negative caloric balance, we have undertaken to determine the protein sparing effects of protein administered free of carbohydrate. The rationale for this approach is the expectation that administered protein will replace the protein usually catabolized during starvation and thereby maintain protein balance and thus preserve the body cell mass. This would be possible if the introduction of a limited amount of protein did not significantly raise the peripheral insulin levels to avoid reducing the mobilization of fat and the development of starvation ketosis so as to allow endogenous fat stores to meet almost all the energy requirements.

Since muscle protein synthesis is relatively more sensitive to insulin than adipose tissue it is possible to achieve insulin levels sufficiently high to exert an anabolic effect on muscle while remaining low enough not to interfere with fat mobilization.

II. LITERATURE REVIEW

Body cell mass may be defined as the energy-exchanging, work-performing moiety of body tissue (Moore, 1963). Body cell mass is approximately 35-45% of body weight in the normal adult male and 30-40% of body weight in the normal adult female. The two major components of body cell mass are skeletal muscle and visceral parenchyma. Visceral parenchyma functions more or less constantly day and night in oxidizing substrates, exchanging energy and using oxygen even while the body as a whole is at rest. Skeletal muscle mass can rest between exertions, varies widely in size according to physical condition, shrinks markedly in disease or disuse and is essential only for the motility of skeleton and the diaphragm. Visceral components usually do not decrease in size during early phases of wasting diseases.

Severe protein loss, primarily from the skeletal muscle portion of the body cell mass, during infection, sepsis and injury is a major contributor to morbidity and mortality. Studley (1936) observed a mortality figure of 30% with patients with ulcer, coli-colitis, severe burns, acute renal failure and peritonitis. In those patients with an acute 30% weight loss there was a near 100% mortality. Taylor and Keyes (1958) noted changes in physical fitness when protein loss exceeded 150 grams (24 grams nitrogen). The mechanism for protein wasting is not known; although it is often associated with inadequate caloric intake.

Since Cuthbertson's (1931) original observation of an increased

urinary nitrogen excretion in groups of patients with long bone fractures, the importance of protein catabolism in the post-traumatic state has been extensively studied. It is widely held that the increment in nitrogen excretion after injury reflects the mobilization of amino acids to meet increased demands for metabolic fuels. Stephen and Randall (1969) has stated that protein becomes the primary fuel during the period following injury. Moore (1953) using serial measurements of body composition concluded that the tissue loss during surgical convalescence is approximately half fat and half lean body mass by weight.

The total cellular protein of the body cell mass (which would not include connective tissue or bone matrix) is about eight kilograms, the greatest proportion of which (7kg or 87%) is present in the form of skeletal muscle. The nitrogen content of this protein mass is approximately 1,000 grams. In addition to this large amount of cellular protein, there are three other large masses of protein in the body: the soluble protein mass (mostly enzymes and plasma proteins); the extra cellular connective tissue protein; and the skeletal protein.

Nitrogen balance refers to the difference between intake and excretion of nitrogen. Under normal conditions, and provided that the diet supplies sufficient calories and at least 0.5 grams of protein per kilogram/day, the nitrogen excretion equals the intake. More than 90% of this excretion is in the urine, with most of the remaining lost in sweat or as fecal nitrogen. Urea is the major form of excreted nitrogen, representing about 80% of the total, while ammonia, uric acid, creatinine, amino acids and some peptides make up the rest. Nitrogen

balance indicates the overall predominance of synthesis or degradation of protein since there is no storage of inactive nitrogen compounds in the body. It is a common fallacy to assume that total plasma protein, or its concentration is directly related to the overall nitrogen balance or to the "protein reserves" in the body. Indeed, the lean tissue mass may gain or lose large amounts of protein without much alteration in the concentration of plasma proteins or plasma albumin. However, protein can be synthesized in one site and broken down in another simultaneously. After trauma or hemorrhage there is extensive protein synthesis in the wound as well as synthesis of hemoglobin and albumin, whereas body muscular tissue as a whole is breaking down.

Extracellular Supporting Tissues

Outside the body cell mass are supportive fluids, including the plasma, lymph, and interstitial and transcellular fluids, and solids, including tendon, dermis, collagen, elastin, and fascia. In prolonged starvation, all of these substances begin to slowly lose their integrity as the rate of protein breakdown rises above the rate of synthesis. Plasma proteins tend to maintain normal concentrations for some time after large amounts of protein are lost from the body cell mass; also, the synthesis of hemoglobin and albumin continues while muscle tissue generally is breaking down. Eventually, however, the onset of edema signals the loss of protein osmotic balance in the extracellular fluids.

Body Fat

The body's store of fat, contained mostly in adipose tissue, is an anhydrous accumulation of neutral triglycerides. The reciprocal of lean body mass is the fixed amount of fat-free water in the body. This important concept provides a means of calculating the body-fat mass by measuring total body-water content (usually by techniques using deuterium oxide or tritiated water).

Of all the elements in body composition, the fat mass is the most variable in human subjects who might be regarded as normal. Very short obese females may show as much as 50% of their body weight as fat, while trained athletes show a fat content of 10% of body weight. Thus, differences appear not only in the body's fat content but also in total body water. At puberty girls gain fat

relative to weight and surface area, while boys gain skeletal muscle, and thus water, relative to weight and surface area.

The importance of body fat in starvation is its contribution to the body's energy fuel--it is by far the most abundant source of energy (e.g. 8 cal/g adipose tissue versus 1 cal/g of muscle tissue). Thus it is a major factor in the conservation of body protein.

Skeleton

The skeleton is a special component of extracellular tissues which are dry, heavy, dense, fat-free, and potassium-free. Approximately 10% of the fat-free body weight is skeleton. Theoretically, total starvation could lead in time to the depletion of bone substance and breakdown of the structure; actually, death from starvation occurs before this ultimate dissolution can take place. Therefore the major skeletal effect is not a change in the bony structure but rather its failure to develop further. In children, growth stops as soon as protein-building energy declines (Young and Scrimshaw, 1971), and even though a child's normal growth rate may be restored, after a long period of food deprivation, he will not reach normal size for his age.

It should be noted that under conditions of partial starvation, nutritional deficiencies and imbalances may induce specific skeletal changes, as in rickets and osteomalacia, or exacerbate other bone-disease processes.

Metabolic Fuels and Energy Stores

Specific Classes

Glucose and free fatty acids (FFA) are the major metabolic fuels supplied by the blood to peripheral tissue for energy production. Fat is the most abundant and efficient fuel in terms of energy available per gram of tissue in man. Each gram of adipose tissue yields approximately 8 kcal, which is close to the 9.4 kcal obtained by the oxidation of a gram of triglycerides (Renaud, 1955). Triglycerides are hydrolyzed to glycerol and FFA. Glycerol is primarily converted to glucose in the liver (gluconeogenesis) and accounts for only a small fraction of the energy stored as fat requirement; whereas FFA are a major fuel. Important metabolites of FFA oxidation in the liver are ketone bodies, which become a substitute source of energy for the brain during starvation (Cahill, 1971; Owen et al., 1967; Blackburn and Flatt, 1973).

Glycogen the storage form of glucose is present in relatively small amounts in man (200-300g). It is stored in muscle and liver, and is used for short-term requirements, as in glucose shortages occurring between meals and in brief periods of unusual stress.

Protein, when oxidized, yields 4 kcal/g. Studley (1936) observed a mortality rate of 30% among patients who lost more than 20% of their weight during illness--a loss primarily of skeletal muscle. Approximately half of the amount of protein lost during starvation is by direct oxidation of amino acids, while the other half is used for the production of glucose in the liver, via gluconeogenesis, a

process which assumes great significance when the supply of exogenous carbohydrate is restricted. This is important to supply glucose for the brain early in starvation. The body's metabolic adaptation to starvation minimizes this consumption of protein, as indicated above, by providing ketone bodies (KB) as substitute sources of energy for the brain (Owen et al., 1967).

Substrate Cycling

The common pathway for the conversion of foodstuffs to energy is the tricarboxylic acid (TCA, or Krebs) cycle. In starvation, the liver's energy expenditure is primarily for gluconeogenesis (Krebs, 1966; Flatt, 1972; Owen and Reichard, 1971; Blackburn and Flatt, 1973), and is largely met through the partial oxidation of FFA to KB. This diminishes the need for the TCA cycle providing energy for liver function. The oxidation of free fatty acids to CO_2 and water cannot proceed normally, thus FFA is converted to ketone bodies which enter the blood stream and become available to other energy requiring tissues primarily brain and muscle. Liver cannot oxidize ketones since it does not possess the enzymes necessary for ketone oxidation. In brain and muscle, the oxidation of ketone bodies and free fatty acids respectively to carbon dioxide via the TCA cycle allows the energy requirements of these tissues to be met. These processes therefore are largely responsible for the reduced requirements for glucose during the body's adaption to starvation.

Some glucose is essential for providing energy to certain tissues, primarily those without mitochondria and therefore no TCA

cycle. In this connection two cycles for conserving glucose are of interest:

The Cori cycle, named for the two biochemists who first described the phenomenon, is a process by which some of the glucose is converted to lactate rather than completely oxidized to carbon dioxide and reconverted to glucose by the liver. Glycolytic tissues such as the renal medulla, erythrocytes, bone marrow, peripheral nerve, and to a small degree muscle, metabolize glucose to lactate and pyruvate. These substances, when carried to the liver and kidneys, are remade into glucose. About 36g (20%) of the glucose utilized daily is broken down by glycolysis and reconverted into glucose (Cahill and Aoki, 1970).

The alanine cycle, first described by Felig and Cahill (1970), also recycles glucose. In this instance, alanine produced by transamination of pyruvate in the tissues is converted to glucose in the liver and then re-oxidized to pyruvate. In this manner the alanine cycle serves to transport nitrogen to the liver, where it is converted to urea.

Starvation

The physiological state of starvation has been the subject of many clinical and experimental studies since the beginning of the Century. Probably best known is the studies by Benedict in 1915 when he fasted a volunteer subject for 31 days. Certainly the most comprehensive study was carried out in the 1940's by Ansel Keyes (1950) and his collaborators of the University of Minnesota, and in

the past decade significant understanding as to the mechanisms involved in starvation have been provided by Dr. Cahill and his many collaborators.

In starvation the metabolism gradually shifts toward a proportionally greater use of FFA, which usually can be supplied in liberal amounts from the fat stored in adipose tissue. Ketone bodies begin to appear in the circulation in the first few days and gradually build up to concentrations of 40 to 50 mg/100 ml after 10 days of starvation (Owen et al., 1967; Madison et al., 1964). The metabolic shift enables blood glucose levels to remain constant beyond the third day, but glucose utilization keeps decreasing, as shown by the decline of nitrogen excretion and hence of gluconeogenesis.

With FFA levels in the range of 1.1 to 1.3 mM the respiratory quotient (RQ) approaches 0.7, indicating that fat has become the major fuel for energy, and net protein catabolism can now be reduced (Issekutz et al., 1967). By the third day of total starvation, insulin, glucose, and FFA reach concentrations of 20 uU/ml, 65 mg/100 ml, and 1.2 mEq/liter, respectively (Madison et al., 1964).

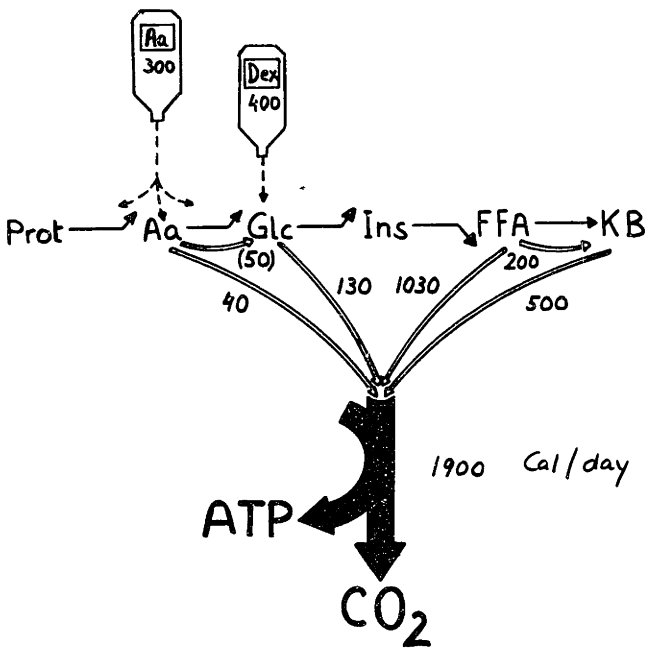
Figure 1 describes energy metabolism in man during prolonged starvation as calculated from the studies of Owen et al (1969). The numbers shown near the double-lined arrows express the daily calorie fluxes--shifts in the source of energy fuel to meet the requirements of a given state of energy expenditure. It is of interest to note that protein catabolism provides only 5% (90 in

FIGURE 1: Utilization of Metabolic Fuels in Prolonged Starvation

The double lined arrows describe the utilization of substrates from energy production with numbers describing the fluxes quantitatively in terms of calories per day, as calculated from the data Owen et al., (1969) from patients having undergone 5 to 6 weeks of complete starvation. The single line arrows indicate the type of effect produced by increased protein catabolism during sepsis and trauma.

"I. V. bottles" show the potential effect of isotonic solutions administered.

The table indicates the relationship of serum FFA, RQ and contribution to caloric requirement. (Issenkutz, 1967)



[FFA]	RQ	% Cal from fat
0.5	0.81 - 0.84	~60
1.1 - 1.3	0.7 - 0.72	>90

1,900 kcal) of the total calorie requirement, while ketone bodies supply the peripheral tissue with some 500 kcal, or 25% of the body's total calorie expenditure. The remainder is covered by oxidation of FFA.

Hormone Action

The elucidation of the metabolic response to starvation includes alterations in glucagon, catecholamines, glucocorticoids, aldosterone and many other hormone activities. Throughout intermediate metabolism, two categories of hormones are seen to play opposing roles. Epinephrine causes glycogenolysis in liver and muscle, leading to hyperglycemia and depletion of carbohydrate reserves. The reverse process is stimulated by insulin. Cortisone induces the formation of key glyconeogenic enzymes, and suppresses that of glycolytic enzymes, whereas insulin has the opposite effect. Similarly glucagon stimulates hepatic glycogenolysis, gluconeogenesis and hyperglycemia. Protein breakdown for glyconeogenic purposes is stimulated by cortisone, spinephrine and glucagon whereas insulin enhances the transport of amino acids into cells and hence their incorporation into protein. In adipose tissue, an increase in lipolysis is promoted by epinephrine, whereas insulin causes synthesis of triglycerides from carbohydrate.

Insulin, clearly is the key anabolic hormone and can be considered as playing the major role in the interaction of metabolic fuel utilization. These "other" hormones are involved in the subtle modulation of the physiological response to starvation.

The Role of Ketogenesis

The high efficiency of protein- and glucose-sparing in the body during prolonged starvation is made possible by adaptive mechanisms for using ketones as a major source of energy fuel. The rate of ketone production was recently determined to be 130 g per day by the third day of total starvation (Owen and Reichard, 1971). Even the brain, which normally derives 100% of its energy from glucose, can substitute ketones to the extent of 70% of its energy requirement (Owen et al., 1967; Wieland et al., 1968).

In addition to sparing the use of glucose and protein in peripheral tissues, ketone bodies enable the liver to reduce its demands for amino acids to be used in gluconeogenesis, thus further slowing the breakdown of muscle protein. Normally the liver's energy requirement is met principally through amino-acid oxidation; in starvation, its energy can come almost entirely from FFA oxidation. Moreover, after about 6 weeks of starvation the kidney cortex becomes a major site for the synthesis of glucose (Owen et al., 1969).

Ketogenesis thus represents a vital mechanism in the physiologic adaptation to starvation, facilitating the use of endogenous fat stores by tissues which ordinarily cannot utilize FFA as such. When the body's triglyceride reserves are used to supply most of its energy requirements, nitrogen loss can be reduced to only 4 to 5 g per day, late in starvation.

In a patient with a strongly negative caloric balance, therefore, the presence of ketone bodies should be regarded as beneficial. It

is important to note that this condition does not induce the pathologic ketosis that occurs in decompensated diabetes. Ketone bodies stimulate insulin secretion, (Madison et al., 1964), and since insulin strongly inhibits ketogenesis (Wieland et al., 1968), a feedback control is established which prevents ketosis from reaching pathological levels. Failure to recognize this physiological role of ketogenesis in the starved patient accounts for the common medical practice of supplying at least 100 g of glucose per day to minimize ketosis (Randall, 1969). Unfortunately, making glucose available tends to derange the metabolic pathways which enable the body to conserve its protein under conditions of partial starvation.

The Effect of Disease on Adaptation to Starvation

Stress due to trauma, sepsis or infectious disease increases the catabolism of protein. The caloric need of a subject can be determined by measuring his oxygen consumption plus carbon dioxide and nitrogen output. On the basis of these multiple measurements in patients with sepsis and trauma it has been shown that their metabolic expenditures were about 20 to 40 percent above basal requirements compared to normal men with the same weight and surface area (Duke et al., 1970). (A burn patient's energy requirements are much higher due to extensive evaporative fluid loss from the burn surface area.) It is not unusual that the increased energy requirements cannot be met by oral feedings, for example when there is disruption in the function of the GI tract. Duke (1970) has measured the resting metabolic expenditure (RME) by analysis of oxygen consumption and CO_2 production. His results show that the RME was not significantly different among normal pre-operative

or early post-operative and septic patients. There was a rise in RME in trauma and burn groups. The mean nitrogen excretion in pre-operative, early post-operative and septic groups was 5.9, 5.2 and 7.2 g of N/m^2. Later the post-absorptive group's mean excretion fell to 3.1 g of N/m^2/day while the trauma group was 8.3 g of N/m^2/day and the burn group was 7.7 g of N/m^2/day. These high rates of protein loss represent about 16% of the RME, a loss three times that observed in prolonged starvation. Moreover, the RQ values derived in these studies, 0.84, was consistent with the expenditure of 50-100 more grams of endogenous carbohydrate (assuming 100-150g from intravenous feeding) than were expended among patients in a similar but not pathological state of starvation. Duke's (1970) report indicates that severe injury impairs those mechanisms which minimize protein breakdown and the nitrogen excretion, so that these values remain high even when fat stores might be adequate for the energy demands of the body.

The prevailing doctrine in the management of patients is to avoid the manifestations typical of the starvation state, particularly as regards ketosis (Randall, 1969). In view of the importance of ketone body metabolism in preserving body protein, this doctrine must be challenged whenever it is not possible to provide a patient with sufficient nutrition to reach or at least approach caloric balance. The current use of 5% dextrose and water as the sole source of exogenous calories appears to fall short of the optimal treatment possible by parenteral feedings by isotonic infusates. It is generally held that the intake of 100 g of glucose per day will achieve as much protein sparing as can be obtained by peripheral carbohydrate infusion

(Gamble, 1946). This amount of glucose is recommended also because it is sufficient to prevent ketosis. Additions of amino acids to isotonic glucose have been unsuccessful in improving nitrogen balance appreciably (Werner et al., 1947). Positive nitrogen balance was achieved by parenteral administration of amino acids only when combined with concentrated hyperosmolar glucose solutions providing more than the daily caloric requirements (Dudrick et al., 1970).

Many studies have been made on the nitrogen balance during starvation and semi-starvation. During total starvation of obese patients, the rate of daily nitrogen excretion decreases gradually as the adaptation to starvation improves (Owen et al., 1969). When 100 g of glucose were ingested daily by a group of normal, healthy volunteers, (Consolazio et al., 1968), nitrogen losses remained below those seen in total starvation. Thus, the intake of 100 g of glucose per day exerts a protein sparing effect. In a similar study, but with a daily intake of 40 g of protein plus 80 g of carbohydrate, the protein sparing was not further improved (Johnson et al., 1971). The ingested amino acids were obviously used to support the production of energy.

It is interesting to note further in these two studies that adaptation to starvation which would be shown by a gradual reduction in nitrogen excretion, appeared to be prevented. When obese patients undergoing weight reduction were allowed 55 g of protein per day without any carbohydrate (Apfelbaum, 1969), the loss of nitrogen was much more efficiently reduced. After a period of adaptation to starvation the nitrogen balance actually became slightly positive. The ingestion of protein compensates for the degradation of amino acids and the nitrogen

balance will be close to zero (Bolinger et al., 1966; Apfelbaum et al., 1969).

The elucidation of the metabolic response to injury has to include an explanation of how injury to a leg can, for example, lead to a gluconeogenic response by catabolism of muscle protein in other parts of the body. Decreased insulin, increased adrenal glucocorticoids and increased levels of catecholamines have all been implicated, but no explanation to date has been adequate. It is possible that all may play a minor role, and that some other "hormone", perhaps directly released in the injured area may be the initiating signal.

Hinton (1971) has shown a significant alternation in the catabolic response to injury in burn patients with an infusion of insulin and glucose. The treatment reduced the urea loss from 25 to 11 mg per kilogram per body weight/percent burn per day. While this represents a considerable improvement, the effect of other catabolic stimuli are still obvious in these patients and secretion of catabolic hormones, glucocorticoids, glucagon and catecholamines must remain high, as evidenced by the diminished sensitivity to insulin. Wilmore (personal communication) has evaluated glucagon and insulin levels following thermal injury. In 10 burn patients, despite continuous IV glucose (200 to 400 g of dextrose per day), the glucagon levels averaged 340 picograms/ml in patients who died compared with 127 picograms/ml in 7 survivors and 57 picograms/ml in fasting normals. The insulin values varied widely, but the insulin to glucagon ratio

in 5 patients was less than 2, compared with 3.1 in fasting normals. This data would suggest that the hormone signal in the liver is similar in some ways to that seen in the fasting state but would include a higher rate of gluconeogenesis.

The real nature of the catabolic response to injury remains to be elucidated, but it is now apparent that the protein breakdown and resultant gluconeogenesis is a significant part of the physiological response to meet the energy requirements. It can be well argued that efforts to alter the metabolic response in routine and early convalescence is not worthwhile in the present state of the art. However, in prolonged post-traumatic starvation and sepsis the consequences of severe protein depletion are grave. This condition can leave the patient exhausted, cachetic and unable to tolerate any additional trauma and sepsis. It is in this situation that efforts must be made to alter the metabolic response and to reduce protein breakdown and resultant gluconeogenesis. It is important to understand why the body catabolizes protein and by what mechanism it is controlled. The nature and the rate of mobilization of amino acids needs further definition to be able to evolve treatment directed toward reversing this degradative process. If protein catabolism is to be reversed more efficiently, it is essential to answer two questions: 1) why does the body under certain conditions catabolize protein in greater amounts; and 2) which of the mechanisms involved is susceptible to protein sparing therapy? As the first line of approach to these problems it is useful to consider the interplay of the major metabolic fuels.

Rationale

Metabolic Regulatory System

The proposed structure of the metabolic fuel regulatory system (Flatt and Blackburn, 1973) is very simple yet it includes enough interactions to explain how the integration of carbohydrate, fat and amino acid metabolism takes place. Figure 2 shows the concentrations of circulating insulin (Ins), glucose (Gluc), free fatty acids (FFA), ketone bodies (KB) and the various amino acids (AA). The plasma concentration of each of these substances is determined by its rates of release into and removal from the circulation.

The pathways for oxidative degradation of the various fuels lead to terminal reactions which these pathways have in common. The metabolic pathways thus serve as a metabolism funnel. In this integration of metabolic oxidations, the total energy generated equals the energy expended. The interactions between insulin and the metabolic fuels include three major feedback loops corresponding to the major nutrient substrates:

Insulin plays a major role in regulating energy metabolism by increasing the rate of glucose utilization and controlling the rate of FFA release from adipose tissue. Figure 2 shows the relationships between plasma concentrations of insulin, glucose, and

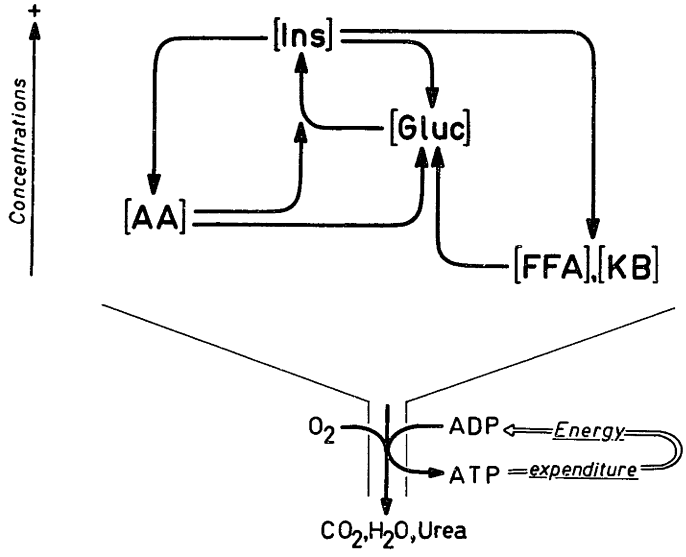


FIGURE 2: The Metabolic Fuel Regulatory System

The arrows describe how the metabolic fuels and insulin influence each others concentration and extracellular fluids.

The metabolic pathways for the oxidative degradation of the various fuels lead to a common terminal reaction and thus have the effect of a metabolic funnel. In this manner the oxidation of all metabolic fuels is integrated so that the total energy generated is equal to the energy expenditure.

FFA, the direction of the arrows indicating whether the concentration of the substance acted upon is depressed or elevated. Thus insulin acts to depress blood-glucose concentrations by facilitating peripheral glucose uptake, particularly in muscle and adipose tissue. With respect to FFA, the chart reflects the inverse relationship between FFA and insulin (Owen et al., 1969), leaving out of consideration the agents which stimulate FFA release from adipose tissue and from this standpoint insulin decreased plasma FFA by reducing the rate of FFA mobilization (Cahill, 1971). FFA can serve as a substrate for energy production in most tissues, where it is oxidized at rates which increase with FFA concentrations in the plasma (Issekutz et al., 1967), thus diminishing the need for glucose as a fuel for energy. Because of this glucose-sparing effect, FFA tends to elevate blood glucose concentrations (Randle et al., 1963). Finally, as the blood glucose rises, the pancreas releases more insulin, so that blood glucose helps to regulate blood insulin.

Blood levels of insulin, glucose and FFA fluctuate primarily in response to nutrient intake, adjusting themselves to each other through the effects of the negative-feedback loops shown in Figure 2.

Insulin-Glucose Feedback Loop (Flatt and Blackburn, 1973)

Insulin facilitates peripheral uptake of glucose, (Levine et al., 1950), in particular by muscle, (Morgan et al., 1961), and adipose tissue, (Crofford and Renold, 1965), where it also stimulates the conversion of glucose to glycogen, (Larner et al., 1959), or triglyceride, (Winegrad and Renold, 1958), respectively. Thus, insulin lowers blood glucose levels, (Levine et al., 1950), as illustrated graphically in Figure 1 by the arrow which suggests that insulin "pushes" glucose levels down. The release of insulin by the pancreas is stimulated by increasing concentrations of blood glucose (Yalow and Berson, 1960). Thus, glucose concentration "push" blood insulin levels up, as suggested by another arrow in the figure. Since glucose has a positive effect on insulin concentration, and insulin, a negative effect on glucose concentration, the interactions between glucose and insulin create a negative feedback loop of the simplest possible design. The existence of a negative feedback loop in a regulatory system has a stabilizing effect, but it requires in addition a set point if the system is to avoid drifting away from a particular operating range.

A steady state condition is established when the inflows of glucose and of insulin are equal to their outflows, and when their concentrations thus remain constant. The most common steady state condition is that encountered in the post-absorptive state. Under these conditions glucose levels in man are adjusted so as to remain within a relatively narrow range of 70 to 90 mg%. Insulin levels are

subject to greater variations as illustrated by the fasting hyperinsulinemia in obesity, (Karam et al., 1963; Rabinowitz, 1970). Insulin secretion seems to occur to the extent required to achieve a fasting blood glucose concentration which is near the physiological norm. This norm apparently constitutes the primary set point in the glucose-insulin feedback loop.

Insulin-Fatty Acid-Glucose Loop (Flatt and Blackburn, 1973)

Insulin decreases the release of free fatty acid from adipose tissue, (Jungas and Ball, 1963), by reducing cyclic AMP mediated activation of a hormone sensitive lipase, (Rizack, 1964; Butcher, 1966), as well as by increasing glycerol-phosphate availability for FFA reesterification, (Cahill et al., 1960). Ketone body levels tend to fluctuate in parallel with free fatty acid levels, (Hanson et al., 1965; Wieland, 1968). Insulin has in addition an antiketogenic action on the liver, (Foster, 1968), so that ketogenesis can be suppressed even in a situation where FFA levels are high, (Bieberdorf, 1970). The effects of insulin on FFA and ketone body production thus tends to lower the levels of these substrates in the blood as shown by an arrow in Figure 1.

Although FFA at very high levels, (Seyffert, 1967),and perhaps more significantly the ketone bodies, (Madison, 1964), appear to be able to directly stimulate insulin secretion, the major feedback effect on insulin secretion is considered here to occur indirectly under normal physiological conditions. Free fatty acids and ketone bodies can be used as substrates for energy production by most tissues,

where their utilization is to a large extent proportional to their concentration in the circulation, (Issekutz et al., 1967; Bates et al., 1968). Since the total oxidation of substrate is limited by the energy expenditure, elevation of FFA and KB levels will reduce the consumption of glucose for energy production in the body, (Randle et al., 1963; Nestel et al., 1964; Gomes et al., 1972). The extent to which glucose can be spared is demonstrated during prolonged starvation, when glucose oxidation is reduced to some 50 grams per day, (Owen et al., 1969; Paul and Bortz, 1969). In reducing glucose outflow, FFA and KB tend to elevate the levels of glucose, and they are thus able to indirectly promote insulin secretion, (Gomes et al., 1972). This effect is attenuated, however, because it operates through an intermediate, glucose, subject to diverse influences. Furthermore this feedback effect can function only to the extent required to reduce blood glucose, but not necessarily FFA levels, (Flatt, 1972), to near normal values. Since, on the other hand, insulin levels directly affect FFA release from adipose tissue, (Jungas and Ball, 1963; Butcher et al., 1966; Cahill, 1971), the feedback regulation between insulin and FFA plus KB is asymmetrical in nature.

The organism's ability to regulate carbohydrate metabolism is commonly determined by performing glucose tolerance tests. When the removal of a glucose load proceeds more slowly than under standardized post-absorptive conditions, this is taken to indicate an "impairment in carbohydrate tolerance". However, to the extent that this is due to elevation of FFA levels, a reduced rate of glucose removal is

due to a physiologically normal phenomenon, of crucial importance in sparing glucose when carbohydrate intake is restricted, (Randle et al., 1963; Cahill, 1971). Under these conditions a decrease in the rate of glucose removal from the circulation should perhaps not be regarded so much as an "impairment", but rather as an indication of the organism's ability to adapt to carbohydrate or caloric deprivation. The qualification implied by the term "impairment" could then be reserved for situations where a reduced ability to utilize exogenous glucose is due to causes different, or manifest beyond, an elevation of FFA levels.

Insulin-Amino Acid-Glucose Loop (Flatt and Blackburn, 1973)

Insulin enhances the uptake of amino acids by peripheral tissues, (Cahill, 1971; Lotspeich, 1969; Manchester, 1970), where it promotes the conversion of amino acids into proteins, (Wool et al., 1968), while its effects on different amino acids is variable, (Pozefsky et al., 1969), the predominant action of insulin is to lower plasma amino acid concentration (arrow pointing downwards in Figure 2). As in the case of glucose, the action of insulin includes stimulation of transport, stimulation of synthesis, resulting in the deposition of glycogen, fat or protein, and inhibition of the rate of breakdown of these substances, (Cahill et al., 1972).

Glucogenic amino acids are converted to glucose in the liver and to a limited extent in the kidney, (Owen et al., 1969). Amino acids thus increase glucose formation. This tends to elevate blood glucose levels (arrow in Figure 2), which in turn stimulates insulin release.

This indirect effect of amino acids on insulin levels is similar to that of the FFA in the feedback loop involving fat metabolism: in one case this is due to increasing glucose formation while in the other case it is achieved by reducing glucose utilization. Furthermore, just as ketone bodies can stimulate insulin secretion directly, (Madison et al., 1964), certain amino acids can directly increase the release of insulin, (Floyd et al., 1966 & 1970). This stimulation operates most effectively when prevailing glucose concentrations are relatively elevated, (Mudler et al., 1971). The arrow describing the direct effect of amino acids on insulin levels is therefore shown in a manner suggesting that this action is subordinated to, and reinforces the effect of glucose in driving insulin levels up.

The Metabolic Fuel Regulatory System

In order to maintain a constant body composition, food consumption must be adjusted to energy expenditure in terms of total calories, and the oxidation of carbohydrates, fats and amino acids must be adjusted to the proportion of these nutrients in the food ingested. The efficiency with which this goal is achieved is illustrated by the fact that a relatively stable body composition is maintained during long periods in the life of an organism, even though the proportions of different nutrients ingested varies widely. Certain minimum requirements for amino acids, vitamins, and minerals must of course be satisfied without which various forms of malnutrition would develop, but this is not pertinent to the discussion of fuel metabolism

presented here. Maintenance of a constant lean body mass is sought by the organism even when the intake of calories does not match the caloric expenditure. Thus excesses or deficits in the caloric balance are translated largely into changes in the amounts of fat stored in adipose tissue. The nitrogen balance remains close to zero even during periods during which intakes of protein and calories are high, (Cahill, 1971). This shows that the degradation of amino acids can vary over a considerable range in order to match protein intake.

It warrants an important conclusion, namely that amino acid oxidation can be rapid in spite of the prevalence of anabolic conditions associated with high insulin levels. Indeed administration of large doses of insulin can adversely affect the nitrogen balance, by increasing the need for gluconeogenesis from protein (Kiech & Luck, 1928; Ingle et al., 1944).

On the other hand, during periods of deprivation, where low insulin levels prevail, the consumption of metabolic fuels shifts so as to minimize amino acid degradation and to preserve body protein masses with remarkable efficiency, (Owen et al., 1967; Cahill and Aoki, 1970).

In order to function under such variable conditions, a considerable degree of integration between amino acid, carbohydrate and fat metabolism must be possible. To the extent that the metabolic fuel regulatory system described in Figure 2 gives a correct description of the major physiological regulatory interactions in the system,

it appears that this integration is achieved primarily by the use of a component common to the three feedback loops involved in the control of carbohydrate, lipid and amino acid metabolism. This common component is insulin, and its importance in the control of all aspects of energy metabolism has been increasingly recognized, (Cahill, 1971).

The structure of the regulatory system is such that glucose, more than the other metabolic fuels is able to influence insulin levels. This is enhanced by the ability of the β-cells in the pancreas to perceive, and to react to changes in glucose concentration, (Portel and Bagdade, 1970). Furthermore the maintenance of normal fasting blood glucose levels appears to provide the fundamental set point in the metabolic fuel regulatory system. Carbohydrates consitute more than half or sometimes most of the nutrients ingested, and glucose is qualitatively and quantitatively one of the most important substances for energy production in peripheral tissues. Fluxes through the glucose pool must therefore be able to vary widely, but with glucose levels remaining within a relatively narrow concentration range, to avoid hypoglycemia or glucosuria, and this in spite of a rather limited capacity for storage of carbohydrate, (Cahill and Aoki, 1970). Thus it is not surprising that glucose should play a predominant role in the regulation of insulin levels.

In the regulatory system discussed here no explicit provision is made for hormones or other agents which directly enhance catabolism of glycogen, fat, or protein. Some of these are short lived

"emergency" signals, which have priority over fuel economy. The others are considered to be inversely related to the concentration of insulin. Thus, for example, FFA release will increase when insulin levels become low, (Cahill, 1971). Whether the release is due to a relative lack of insulin or to relatively high levels of lipolytic signals does not need to be specified in the simplified model developed here.

The important point, is that as a result of its basic structure, the regulatory system allows for one of the metabolic fuels to influence the metabolism of the others. The qualitative nature of these repercussions can be easily understood by the use of the scheme proposed.

III. MATERIALS AND METHODS

Fourteen obese volunteers were admitted to the Clinical Research Center at Massachusetts Institute of Technology to determine the metabolic effects of starvation and starvation modified by protein sparing diet (Appendix I). Each subject was informed of the potential risks involved in both starvation and protein sparing diet before admission to the Center. During the pre-starvation period all subjects were screened regarding pulmonary, renal hepatic and endocrine abnormalities.

During the pre-starvation (4 or more days), subjects were fed on isocaloric diets containing 0.5 to 1.0 grams/kilogram/day of protein.

Daily intake during starvation consisted of 1 multiple vitamin capsule, 17 mEq Na Cl (sugar-free tablets), at least 1500 cc of water, 25 mEq of potassium carbonate, 300 mg of Ferrous sulphate, and a trace mineral capsule. Eleven obese volunteers received in addition twice daily a portion of meat providing 0.65 to 1.0 grams/protein/kilogram/body weight/day.

Table I. Nitrogen and Caloric Composition of Oral Protein-Sparing Diet

	Weight (cooked)		Approximate Calories	Nitrogen	(n)
	ounces	grams		grams	
Beefsteak	6	170	290	8.7 ± 0.3*	(23)**
Veal	10	285	440	8.5 ± 0.7	(5)
Chicken	6 1/2	185	330	9.3 ± 0.4	(4)
Shrimp	8	235	260	8.7 ± 0.4	(6)

* Numbers are mean ± standard deviation of mean.
**(n) = number of samples assayed.

TABLE III

Composition of Freamine

Amino Acid Essential	% w/w	A/E FreAmine	Egg	Rose
Isoleucine	6.94	150	129	110
Leucine	9.06	195	172	170
Lysine Acetate	9.06 (Hcl)	157	125	130
Methonine	5.29 (Hcl)	114	108	170
Phenylalanine	5.65	122	195	170
Theonine	4.00	86	99	80
Tryptophan	1.53	33	31	40
Valine	6.59	142	141	130

Calculated Parameters

		Human Milk	Egg	Cow's Milk (Casein)
E/T	3.3	3.1	3.2	3.2
Total Nitrogen %w/w	14.7	15.6	15.9	15.7
αNH_2-Nitrogen %w/w	12.4			
% αNH_2-Nitrogen	84			
% Essential Amino Acid	47	50	51	52

TABLE II

Composition of Freamine® (McGaw Labs)

Amino acid	g/100 mL	g in 2.5 L of isotonic (3%) solution	Amino acid min. requirement for 70 kg man	
			Adult Std	Child 10-12 y Std
Isoleucine	0.21	5.25	0.7	2.0
Leucine	0.27	6.75	0.8	3.4
Lysine	0.27	6.75	0.6	4.1
Methonine	0.16	4.00	1.0	1.9
Phenylalanine	0.17	4.25	1.0	1.9
Theonine	0.12	3.00	0.4	2.4
Tryptophan	0.05	1.25	0.2	0.3
Valine	0.20	5.00	1.0	2.3
All essential	1.45	36.3	5.7	18.3
Alanine	0.21	5.25		
Argininine	0.11	2.75		
Histidine	0.08	2.00		
Proline	0.33	8.25		
Serine	0.18	4.50		
Glycine	0.63	15.75		
Cysteine	0.01	0.25		
All amino acids	3.0	75.0		
g amino acid N	0.45	11.3		
Protein equivalent	2.8	70.0	30.0	49.0
Calories	120	300		

Nineteen surgical patients requiring intravenous fluid replacement received the following parenteral solutions: 1) (5% dextrose solution) D5W to deliver 100 grams of dextrose per 24 hours, 2) 3% crystalline L-amino acid solutions containing essential and nonessential amino acids delivered 90 grams of amino acids per 24 hours (Table II and III), 3) solutions containing 3% L-amino acids and 5% dextrose to deliver 70 grams of amino acids plus 100 grams of dextrose per 24 hours. Each solution was administered two or more days, alternating the type of infusates in the same patient. A cross over routine was used so that the average infusion period for each solution was approximately equal in time from the moment of initiation of parenteral therapy. These studies were performed on the Harvard Surgical Service at New England Deaconess Hospital and Boston City Hospital.

Blood and Urine Collections

Blood samples, for metabolic fuel "substrate profile" which includes glucose, insulin, free fatty acids (FFA), ketones (KB) and amino acids (AA) were obtained from the antecubital vein at 7:00 A.M. on arising from bed as follows: pre-starved state, on the day after an overnight fast (day 0), on each Monday and Friday in the period of study.

In the surgical patient blood samples were taken on the 48th hour and 96th hour, or at both times and at the end of each study period. The parenteral solutions were infused at a constant rate. 1/8 of the daily fluid requirements was usually delivered in the 3 hours prior to blood sampling (Appendix A).

Urine was collected and refrigerated in plastic containers for a 24 hour period beginning immediately after the 7:00 A.M. blood specimen was obtained. The volumes were measured and aliquots were frozen at -20°C until analysis of total nitrogen, urea nitrogen, and creatinine were completed. Methods used for determining these urine substances are reported in Appendix

Glucose, lactate, pyruvate, B-hydroxybutyrate and acetoacetate were determined enzymatically after deproteinization with perchloric acid using the procedures adapted from Bergmeyer (1965)(Appendix A). Free fatty acids were titrated assymetrically Dole (1960)(Appendix D). Insulin was measured by the radioimmunoassay of Soldner (1965)(Appendix E) and urinary nitrogen by the microKjeldahl method (Appendix B).

Statistical analysis of variants was used to determine the significance between the various studies. Data average reported as ($\bar{x}$) and standard deviation (S.D.). Significance for the difference between the groups means were determined by a calculation of the least significant difference as described by Snedecor (1967). (Appendix F)

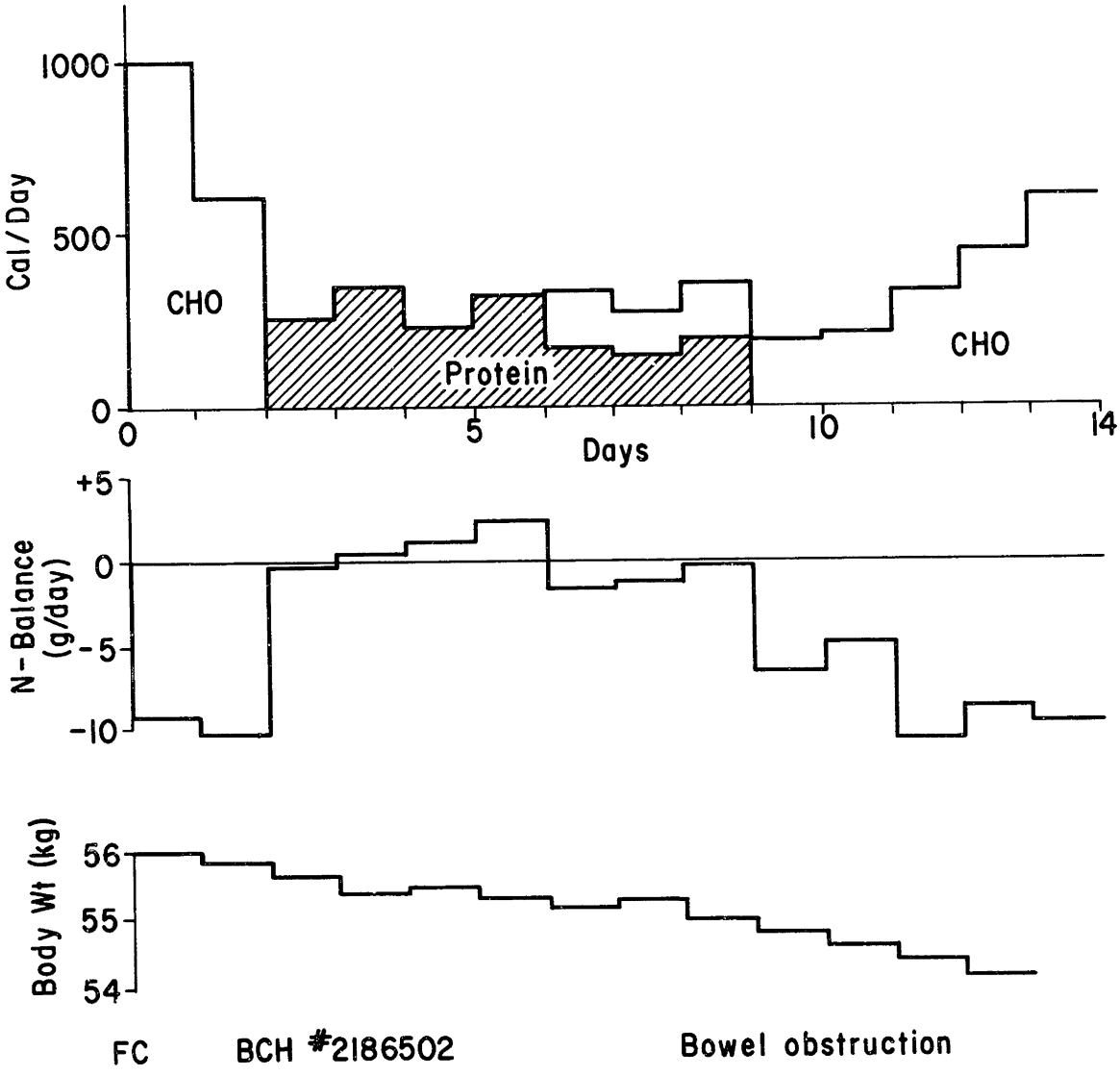
The experimental protocol are illustrated in Figure 1 and 2. Daily urinary nitrogen excretion was determined on 24 hour urine collections. The "ΔNu" corresponds to the urinary nitrogen loss when on total fast or on dextrose infusions, or to the difference between the grams of nitrogen administered in the form of lean beef protein or infused amino acids and the urinary nitrogen excretion. The overall nitrogen balance was calculated by adding 1 1/2 grams of nitrogen to the urinary nitrogen loss to account for fecal and cutaneous losses (Bergstrom et al., 1972).

FIGURE 3: Metabolic Study Protocol in a 70 Year Old Woman

The woman was in a state of semi-starvation.

Improved nitrogen balance resulted from the administration of amino acids alone.

Ins	44	31	34	34	37	42	46
Gluc	7.9	4.9	4.7	8.0	8.6	7.9	8.6
FFA	0.3	1.1	1.3	0.4	0.3	0.2	0.3
KB	0.2	0.7	1.7	0.4	0.2	0.2	0.1



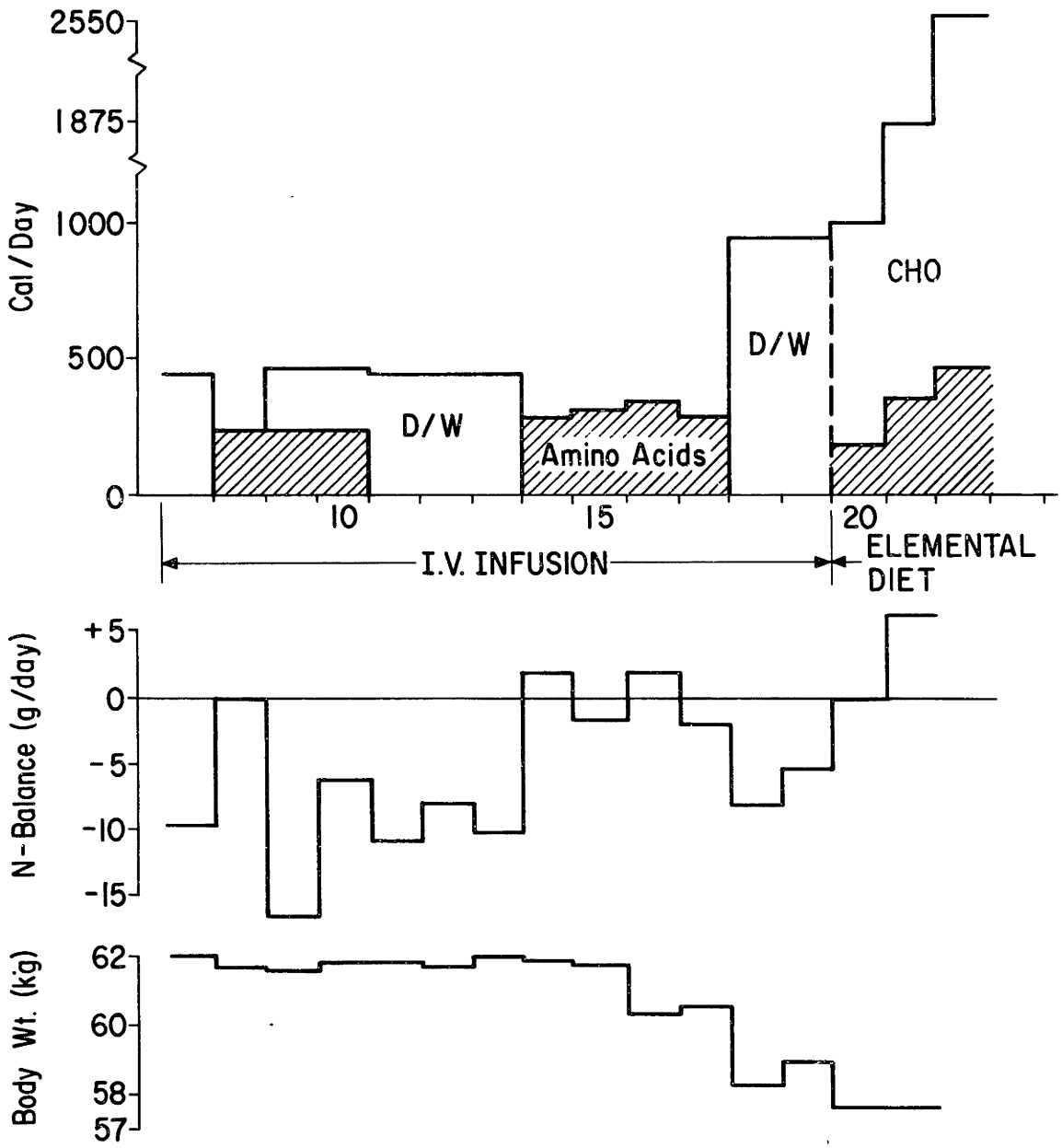
FC BCH #2186502 Bowel obstruction

FIGURE 4: Metabolic Study Protocol

Crossover infusion of dextrose and water (D/W) and 3% Amino Acids (A/A)

52 year old male demonstrated immediate improvement in nitrogen balance when an amino acid infusion was used instead of the routinely used 5% dextrose in water.

Ins	25	20	27	30	26	25	24	30	μU/mℓ
Gluc	7.2	4.8	5.1	5.8	6.7	5.0	5.0	7.5	mM
FFA	0.5	0.8	0.6	0.5	0.4	0.9	1.1	0.2	mM
KB	0.2	1.4	0.2	0.1	0.1	0.4	0.8	0.0	mM



CW BCH #218558 Reactive hepatitis + subhepatic abscess

RESULTS

Oral Protein Sparing Diet

Clinical: the obese volunteers tolerated both diet programs (total starvation and the protein supplement to starvation) without adverse effect. No general or specific organ dysfunctions were noted as monitored by patient symptoms, physical exam or routine blood chemistry tests. These data are contained in Appendix I .

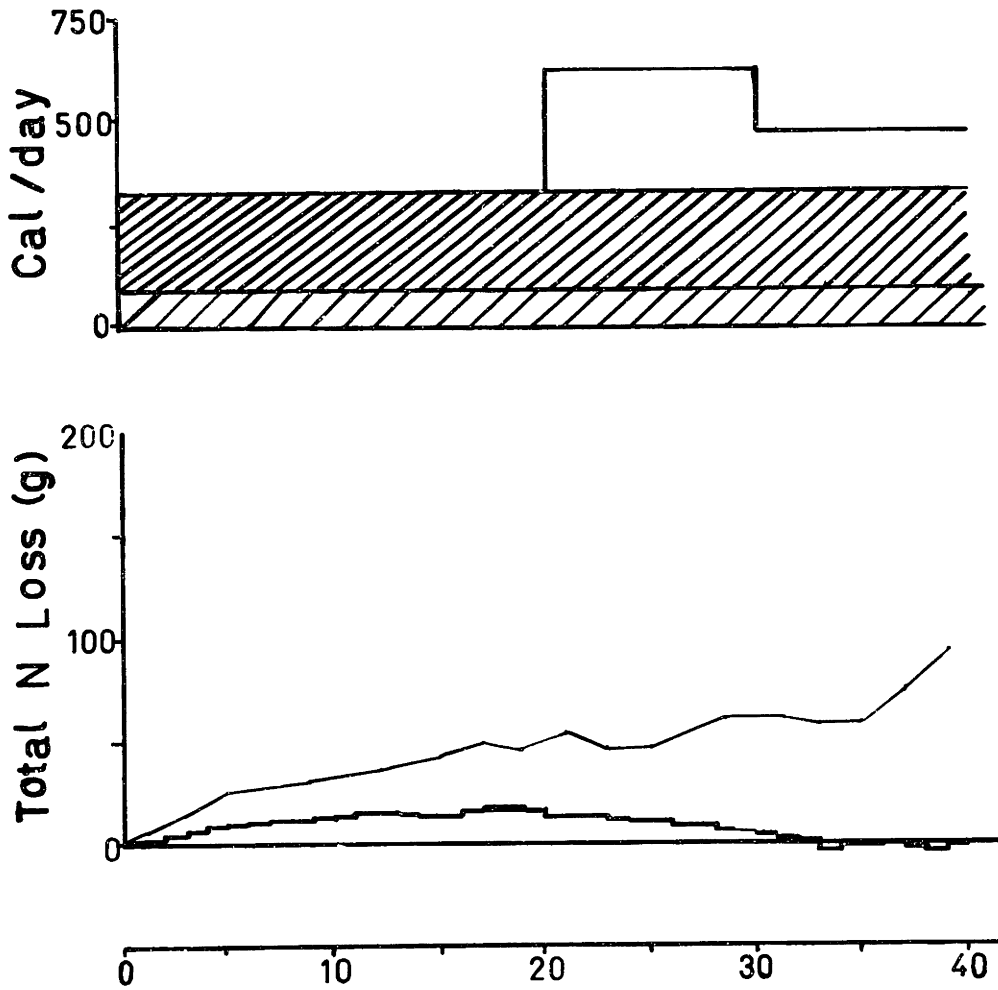
Figure 5 shows the metabolic balance of a 22 year old woman who is 40% overweight. In this 40 day study, the patient received 55 g of protein per day (0.75 g/kg) in the form of lean meat as her only caloric intake during the first 20 days. As in starvation there was a drop in the level of insulin and glucose and a rise in the free fatty acid and ketone body levels. After 20 days a small amount of glucose, 40 g twice a day, was administered. There was no appreciable effect on the nitrogen balance in this adaptive phase of starvation as the amount of carbohydrate did not significantly impair the contribution of calories from fat mobilization. Thus no increase demand to meet the energy requirements of the body were made for the direct oxidation of amino acids to increased gluconeogenesis.

However there was a decrease in ketone body levels which was associated with the return of hunger. As measured by accumulated nitrogen imbalance the loss of lean body mass was negligible during the study, although the patient kept losing approximately 1/3 of a

FIGURE 5: Forty day metabolic study

A 22 year old female (40% overweight) eating 3 ounces of lean meat twice a day containing approximately 10 g of fat. Loss of nitrogen (ΔNu) is negligible. An addition of carbohydrate twice or once a day lowered ketone body level but did not significantly change nitrogen balance.

Ins	20	8	9	8	8	14	15	µU/ml
Gluc	4.7	2.7	3.3	2.4	2.9	3.0	3.4	mM
FFA	0.6	0.7	0.9	1.1	1.1	0.9	0.6	mM
Ketones	0.4	2.6	3.5	4.1	2.0	1.4	1.4	mM
Lact	2.4	1.1	1.8	0.6	0.3	0.3	0.4	mM



pound of weight per day. The patient tolerated this program well, while experiencing little hunger, except when eating some carbohydrate, and she was allowed to go to work daily as a school teacher. She found this program much more acceptable than total starvation.

Figure 6 shows the metabolic balance of a 29 year old male maintenance man who was 70% overweight. In a 40 day study, the patient received 55 g of protein per day (0.45 g/kg) in the form of lean meat as his only caloric intake during the first twenty days. While the accumulated nitrogen loss was less than in total starvation, nitrogen balance was not achieved until the 17th to 20th day of this diet.

Figure 7 shows accumulated nitrogen loss in patients in various states of starvation and semi-starvation. J.T. and J.C. and M.R. underwent 20 or more days of total starvation. The accumulated nitrogen loss was approximately 150 g after 20 days. M.S. and J.F. underwent 20 days of diet consisting of 55 g of protein, 10 g of fat (170 g of lean meat) per day. Significant decreases in the accumulated nitrogen balance was observed. The administration of carbohydrate at two levels of 40 and 80 grams do not appreciably alter the nitrogen balance at the end of twenty days. To further explore this result D.P. was put on a diet of 40 g of carbohydrate plus 55 g of protein and 10 g of fat: later in this study the carbohydrate was omitted. In this case it was found that accumulated nitrogen loss during the first few days was moderate to severe and, therefore, did not show the same propensity for the decrease in nitrogen excretion as compared to lean

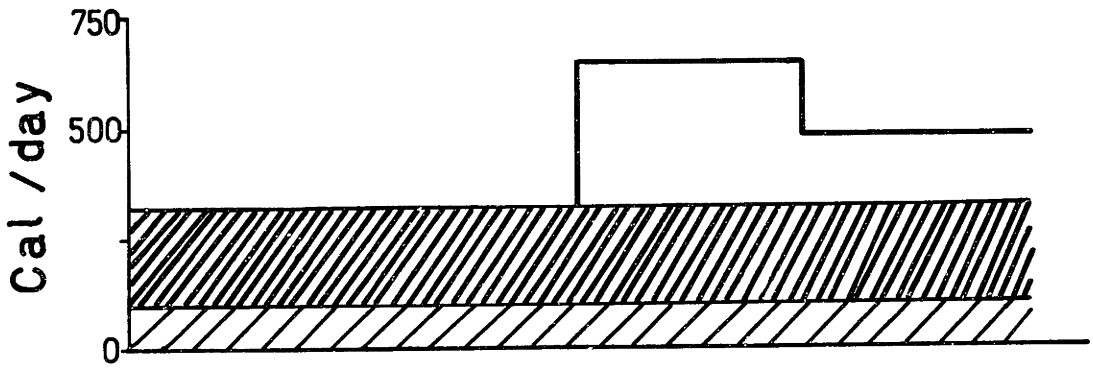
FIGURE 6: Metabolic Study

A 29 year old male (70% overweight) eating 55g of protein per day (0.45g/kg/day).

Nitrogen balance is not obtained until the 17th to 20th day of the diet regimen.

The administration of 40g of carbohydrate twice a day did not significantly effect protein sparing but did produce hunger associated with a decrease in circulating ketone bodies.

Ins	17	9	8	8	13	16	14	µU/ml
Gluc	4.7	3.0	3.7	2.3	4.3	3.7	2.1	mM
FFA	1.0	0.9	0.7	0.8	0.8	0.7	0.5	mM
KB	0.6	2.0	3.1	2.9	1.4	1.0	1.6	mM
Lact	0.8	1.1	1.5	2.1	1.4	0.7	0.7	mM



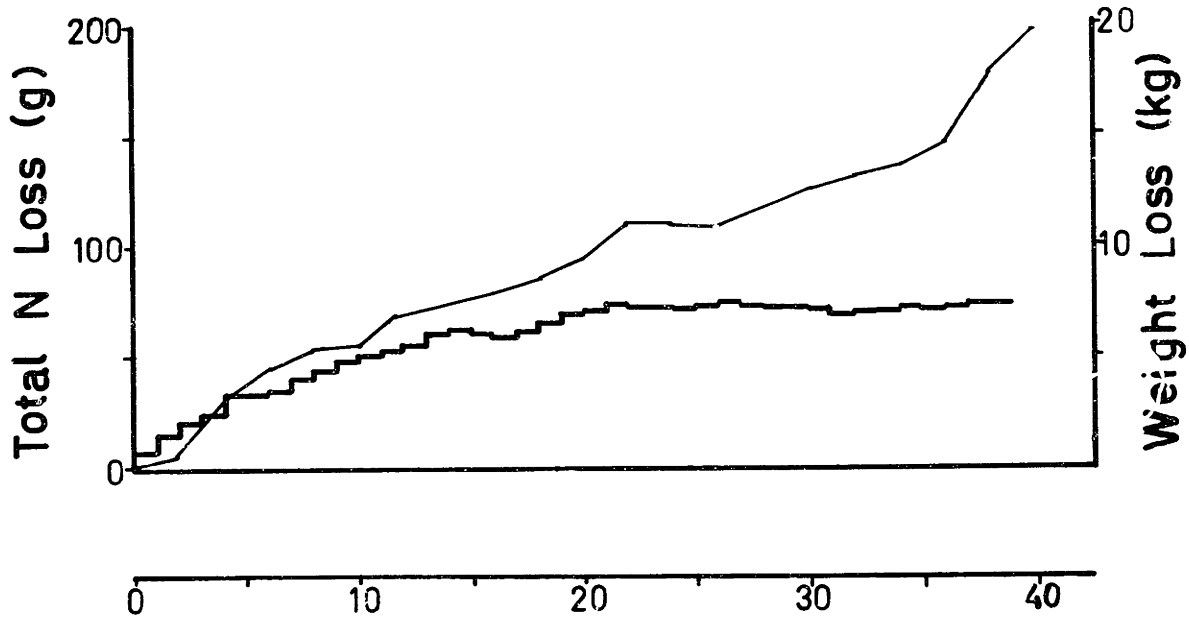
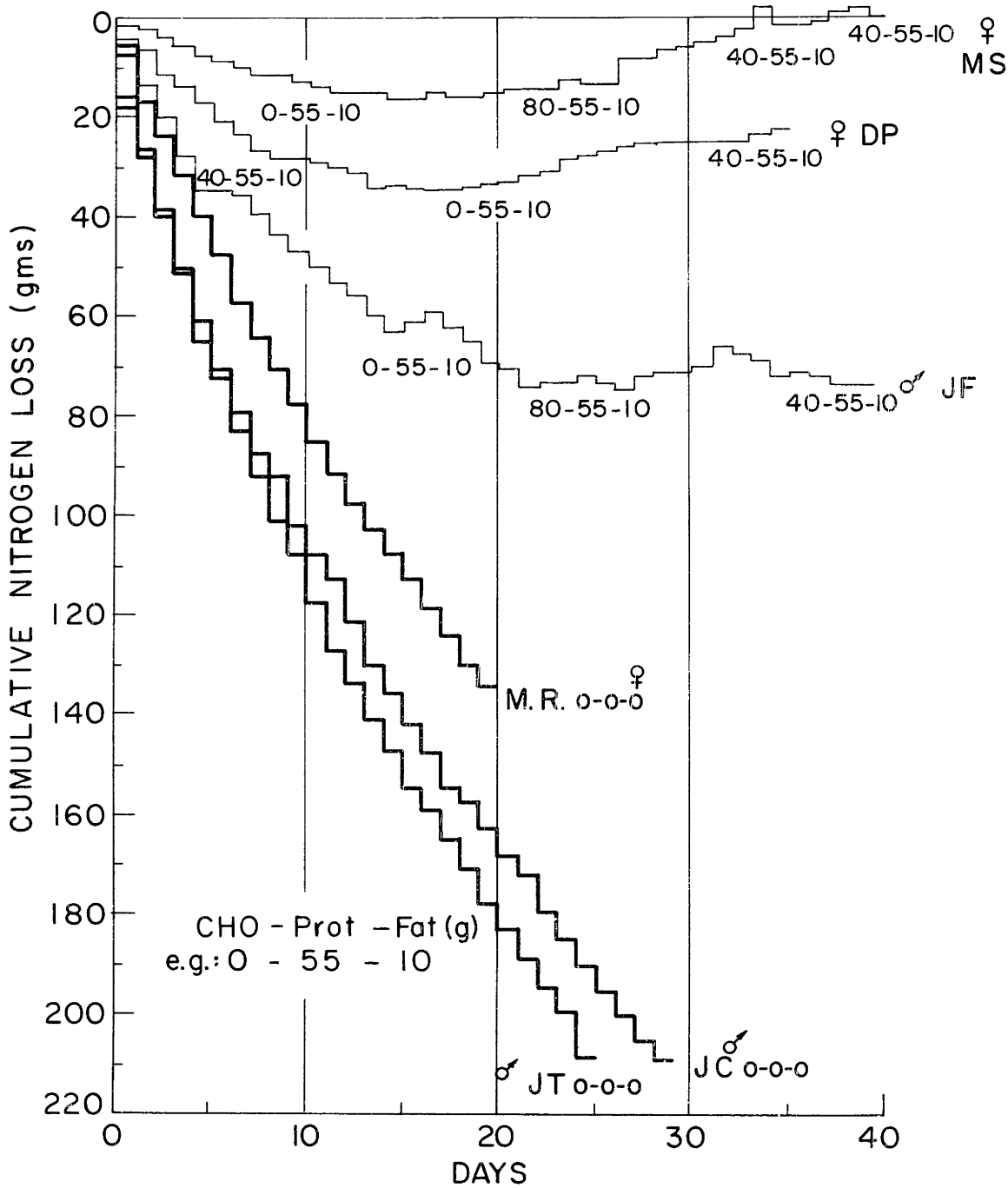


FIGURE 7: Cumulated Nitrogen Balance

Cumulated Nitrogen balance (ΔNu) on subjects during starvation and semi-starvation receiving small amounts of carbohydrate and/or protein.

Significant protein sparing occured. ($p < 0.01$)


CUMULATIVE NITROGEN LOSS (gms)
0
20
40
60
80
100
120
140
160
180
200
220
0
10
20
30
40
DAYS
40-55-10 ♀ MS
40-55-10
80-55-10
0-55-10
♀ DP
40-55-10
0-55-10
40-55-10
0-55-10
80-55-10
40-55-10 ♂ JF
M. R. o-o-o ♀
CHO - Prot - Fat (g)
e.g.: O - 55 - 10
♂ JT o-o-o
♂ JC o-o-o

meat diets alone, due to an inability to adapt to the starvation state. Thus, the small amount of carbohydrate (20 to 40 grams administered after a period of semi-starvation would not appear to be adequate enough to alter the fat contribution to the energy requirements of the subject. A slight improvement in the total urinary urea nitrogen excretion seen in this period may reflect a decrease in the rate of gluconeogenesis. It may be explained on the basis of insulin release being significant enough to have an effect on the liver or on muscle but not significant to alter the peripheral release of FFA from adipose tissue.

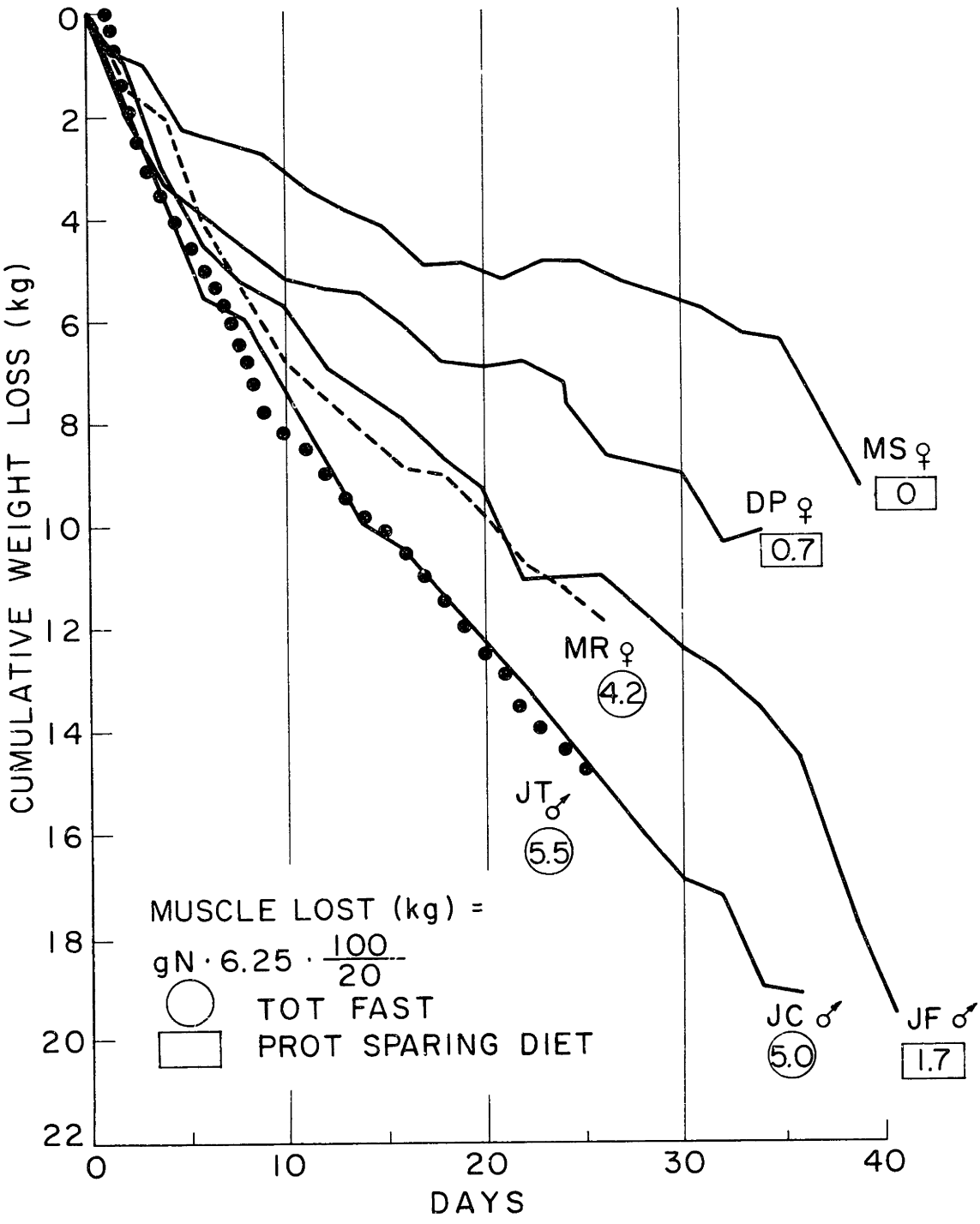
Figure 8 shows accumulated weight loss seen in the patients shown in Figure 7. Muscle loss as calculated from nitrogen data during periods of total starvation compared to protein sparing diets are significantly different.

Three patients undergoing total starvation and eleven patients on the protein sparing regimen are shown in Figure 9. The changes in lean body mass were calculated from the loss of nitrogen, which was converted to protein and in turn to the equivalent mass of muscle tissue. Fat loss is shown as the difference between weight loss and the loss of lean body mass. The fact that EKG, chest x ray, hematocrit, hemoglobin, albumin, total plasma protein, creatinine, transaminase, bilirubin and other blood parameters did not change suggest no significant alterations occured in organ function. These data together with no evidence of dehydration or formation of edema by physical examination, presence of oliguria or polyuria suggest no appreciable fluid shift.

FIGURE 8: Weight Loss

Comparison of weight loss between controlled starvation and starvation modified by 55g of protein supplement per day.

Cumulated Nitrogen loss is converted to equivalent muscle loss.


CUMULATIVE WEIGHT LOSS (kg)
0
2
4
6
8
10
12
14
16
18
20
22
0
10
20
30
40
DAYS
MS ♀
0
DP ♀
0.7
MR ♀
4.2
JT ♂
5.5
JC ♂
5.0
JF ♂
1.7
MUSCLE LOST (kg) =
gN · 6.25 · 100/20
TOT FAST
PROT SPARING DIET

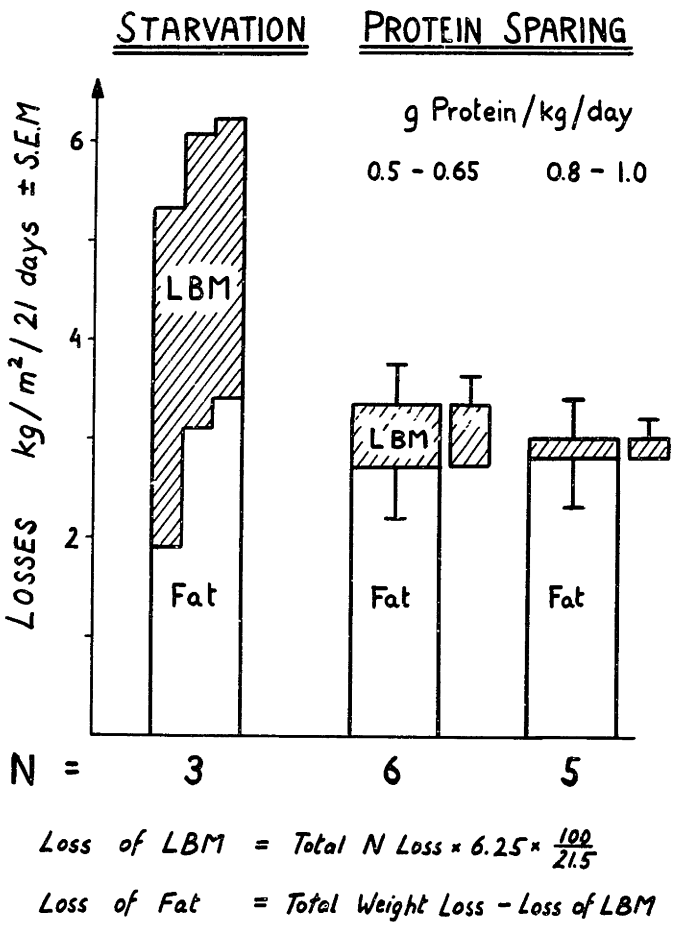


FIGURE 9: Weight Losses

The relationship of the loss of lean body mass and the loss of body fat during 21 days of starvation or protein sparing therapy.

No significant shifts of body water were observed or included in the calculations.

In starvation the loss of lean body mass was similar in the three patients studied whereas the degree of fat loss was related to the individual's caloric expenditure. The administration of 0.5 to 0.65 g/kg/day did not alter the amount of fat loss but significantly reduced the loss of lean body mass. When 0.8 to 1.0 g/protein per day were given, fat loss remained unchanged but the loss of lean body mass became negligible.

Figure 10 shows the relationship of "substrate profile" in starvation compared to the protein sparing regimen. In the patients receiving these limited amounts of protein, blood glucose and insulin levels decreased to an extent comparable to the change observed in total starvation after 21 days. Free fatty acids and ketone body levels increased though not to the degree observed in total starvation.

Figure 11 shows the comparison with the uric acid, cholesterol and triglyceride levels in total starvation for 21 days and on a protein sparing diet for periods up to 140 days. Only a slight increase in uric acid was observed, which was significantly less than that observed in total starvation. This may be due to a decrease in lean body mass breakdown or to a reduced competition by ketone bodies for renal excretion. No significant change in cholesterol was observed. The most significant drop in triglycerides was observed in those patients who had elevated triglycerides at the beginning of the study.

Four patients who showed a positive nitrogen balance while receiving only 300 to 900 calories per day in the form of lean meat are

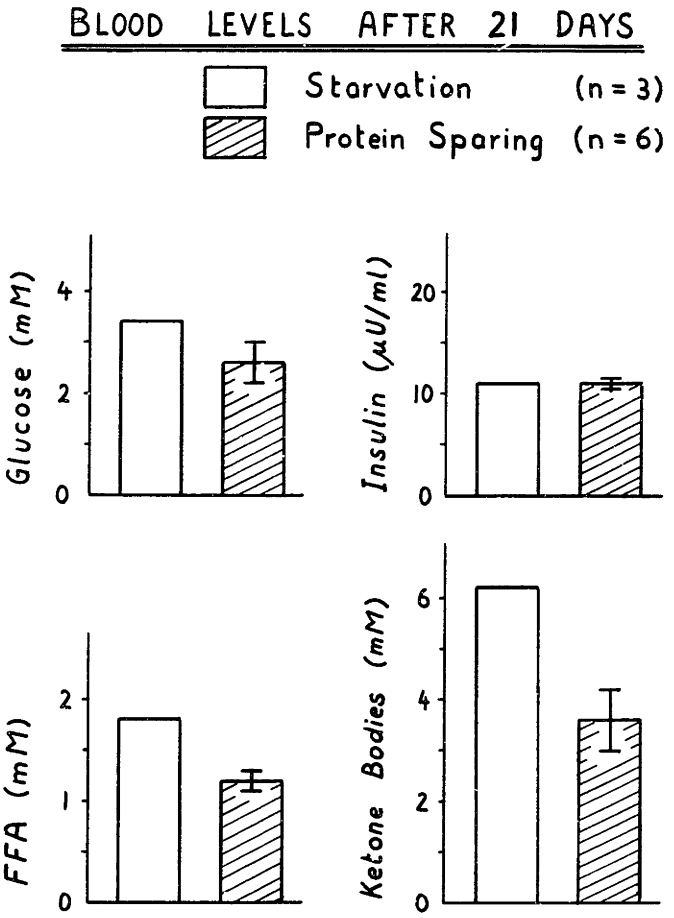


FIGURE 10: Comparison of the Metabolic Fuel "Substrate Profiles" of Starvation and Protein Sparing

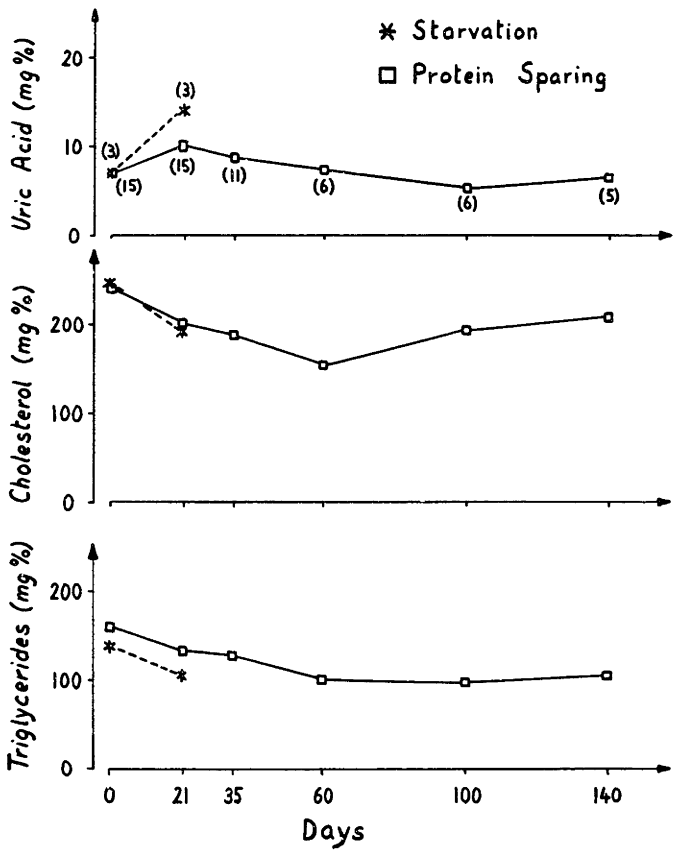


FIGURE 11: Uric Acid, Cholesterol, and Triglyceride Levels on Patients on a Protein Sparing Regimen for up to 140 days Compared with 21 Days of Starvation

presented in Table IV. A number of other patients show positive nitrogen balance when the study is carried beyond 21 days. It is certainly remarkable that fat mobilization should be able to provide enough metabolic fuels to prevent increased muscle tissue breakdown while the caloric balance is so negative. The emphasis on increased physical activity may be an important parameter in attaining positive nitrogen balance.

TABLE IV

Patients With Positive Nitrogen Balance
After 21 Days of Protein Sparing Regimen

	Age	Sex	B.W. kg	Condition	g Prot /kg/day	Cal /d	B.W. kg/21 days	LBM kg/21 days
L.R.	69	F	90	Depleted Hypothyroid Hypercholes-teremia	0.6	330	-7.0	+0.5
E.S.	47	F	151	Diabetes Hypertension	1.0	910	-13.0	+5.1
K.D.	20	F	125		0.8	550	-4.2	+0.8
C.J.	31	M	165		0.8	730	-10.1	+1.4

Diet delivered as lean meat in two equal meals.

Intravenous Protein Sparing Therapy

Clinical: Table V represents a clinical description of 10 patients receiving two to three liters of parenteral infusion per day for 10 to 14 days.

Table VI represents an additional 9 patients receiving a similar therapy but were generally more severely ill and demonstrated a higher protein catabolism. No adverse effects could be attributed to the intravenous therapy in any patient; no infections cellulitis, thrombosis . pyrogenic responses occurred. Except in one patient, (C.W.) with reactive hepatitis, serum enzymes (SGOT and LDH), bilirubin and ammonia levels remained within normal limits.

The first ten patients represented moderate protein catabolism, averaging a daily nitrogen loss of 7.1 g $\pm$ 3.5 g on administration of dextrose in water. This is equivalent to loss of 44 g of protein per day (6.25 g of protein/gN), which, if this protein was mobilized from muscle tissue, represents a loss of about 0.5 pounds per day of muscle tissue.

Infusion of an amino acid solution, instead of the routinely used 5% glucose infusate, resulted in a reduction in the nitrogen loss from-8.5 to -1.0 gN/day. The infusion of a mixture of glucose and amino acids resulted in some improvement in the nitrogen balance but not to the degree observed from an amino acid infusion alone.

In a subsequent study of 9 patients (Table VI) the infusion of amino acids instead of glucose resulted in a reduction in nitrogen loss

TABLE V

PATIENTS

	Weight (kg)	Age	Sex	
JS	58.6	56	M	Obstructing cancer of colon
LM	56.3	52	F	Pelvic abscess
FC	61.2	69	F	Small bowel obstruction
CW	54.5	52	M	Reactive hepatitis, subhepatic abscess
IC	76.2	28	M	Stab wound of liver
JY	89.5	64	M	Gunshot to abdomen with peritonitis
BH	64.3	37	F	Subheptatic abscess with perforated duodenal ulcer
RW	54.5	47	M	Emphysema, chronic lung disease, ileus
LS	60.0	42	M	Pancreatitis and pseudocyst
SW	32.0	56	F	Subtotal gastrectomy and dumping syndrome

TABLE VI

PATIENTS

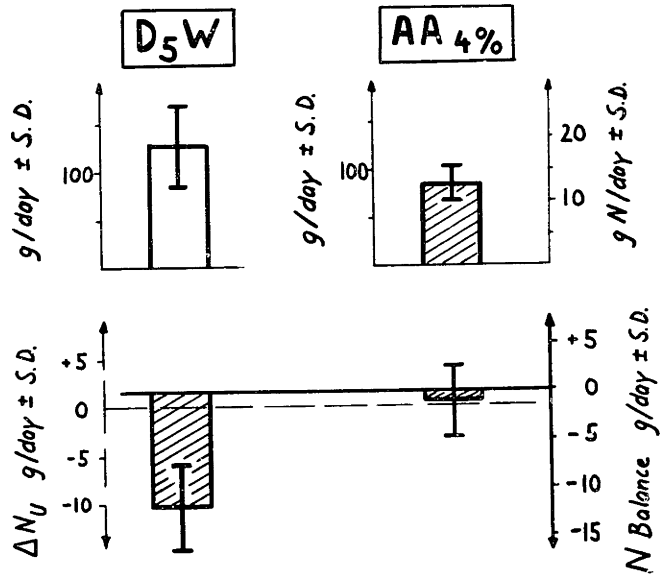
	Weight (kg)	Age	Sex	
K.A.		84	F	40% Burn
J.B.	54.1	54	M	Pancreatitis
R.B.	106.4	46	M	Multiple trauma
W.C.	63.6	26	M	Pancreatitis
A.D.	55.9	36	F	Cholongitis
T.G.	55.9	73	M	CA of sigmoid colon
W.M.	61.4	40	M	Perforated duodenal ulcer, subphrenic abcess, pelvic abcess
J.P.	109.1	25	F	Subphrenic abcess
A.V.	61.8	74	F	Entero cutaneous fistula

FIGURE 12: Peripheral Protein Sparing Therapies

Nitrogen balance data on a second group of patients receiving intravenous peripheral protein sparing therapies.

Urinary nitrogen measurements (Δ Nu) have been corrected for fecal and skin nitrogen losses.

PERIPHERAL PROTEIN SPARING THERAPIES



Therapy	D_5W	AA
# Patients	6	9
# Days	17	55
N Balance g/day	-11.8	-1.0
S. D.	±4.5	±3.7
S.E.M	±1.1	±0.5

$P < 0.001$

from -11.8 to -1.0 g/day, as shown in Figure 12.

The metabolic study of a 70 year old female with bowel obstruction secondary to adhesions is shown in Figure 3. Intravenous infusion of isotonic dextrose (D5W) or L-amino acids (3%) solutions were given in amounts shown in terms of calories delivered per day. A significant amount of protein was spared with the amino acid infusion as evidenced by a sharp improvement in nitrogen balance (ΔNu). The addition of carbohydrate to the amino acid infusate did not improve the nitrogen balance. When the administration of amino acid infusates was discontinued, the nitrogen balance became negative. The protein sparing which occurred with the infusion of amino acids is associated with a decreased level of insulin and blood glucose and increased levels of serum free fatty acids and ketone bodies.

A metabolic study in a fifty-two year old man with a duodenal fistula, subhepatic abscess and reactive hepatitis is shown in Figure 4. Serum glutamic oxalacetic transaminase levels averaged 1,800 mU/ml and bilirubin was 11 mg per cent. Although the clinical studies had to be interrupted early because of the patient's condition (day 9), the data clearly show that the nitrogen balance was better with the infusion of amino acids (days 14 to 17) than with glucose. When the infusate was changed from glucose (100 g per day) to amino acids (70 to 90 g per day) the nitrogen balance rapidly returned to normal. (See days 7 to 8 and 13 to 14). Furthermore, the addition

of glucose to the amino acid infusion (day 18) had an unfavorable effect although a negative tendency in the nitrogen balance was beginning on day 17 before glucose was added to the amino acid infusate.

Figure 13 gives the substrate profiles with the administration of different types of infusates as well as the daily nitrogen balances. When 100 g of glucose was delivered per day, the daily nitrogen deficits were 8.5 g. This figure is about half the nitrogen deficit seen in the initiation of a period of total starvation. Thus, the infusion of isotonic glucose solution had a definite protein-sparing effect, which is a well known fact. Nevertheless, the cumulative N loss over a four day period amounted to 28.9 g $\pm$ 4 g which is equivalent to the loss of about 2 pounds of muscle tissue. The substrate profile does not show the characteristic pattern of starvation, that is, elevated levels of free fatty acids and mild ketosis, even though the caloric balance is severely negative.

The addition of amino acids to the glucose infusion improved the nitrogen balance, and the nitrogen loss over a four day period amounted to 15 g $\pm$ 6 g. The substrate profile was not significantly altered from that observed with glucose alone.

In sharp contrast, the substrate profile during the administration of amino acids alone show the elevation of free fatty acids and ketone bodies which are typical of starvation. The nitrogen balance (ΔNu) during peripheral infusion of amino acids alone is practically

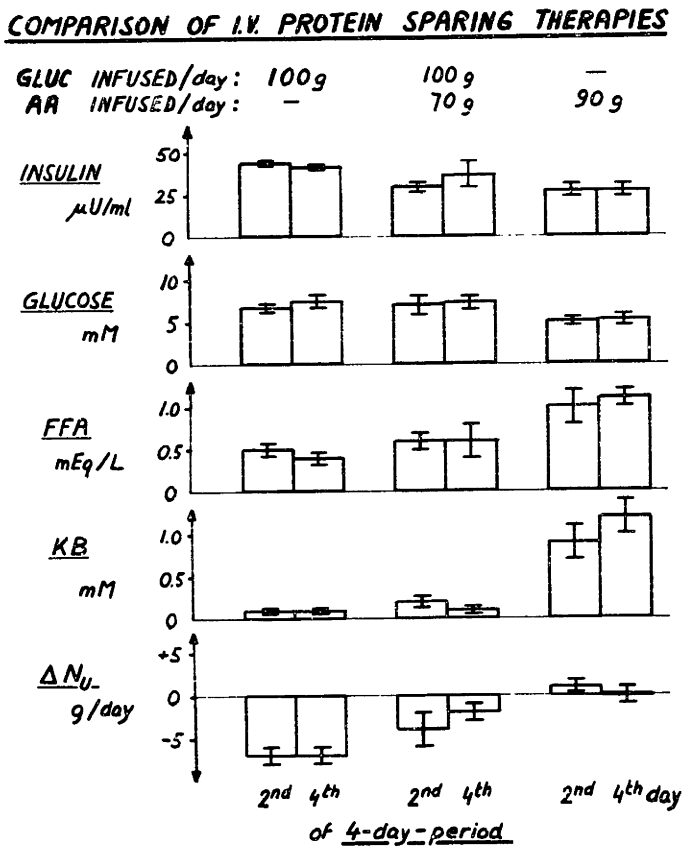


FIGURE 13: Comparison of I.V. Protein Sparing Therapies

Average data ± standard error for 10 patients.

Improvement in nitrogen balance (Δ Nu) in the group receiving amino acids alone was asscciated with increase in fat mobilization (higher free fatty acids, ketone bodies) and decrease in the glucose and insulin levels.

zero, demonstrating that amino acid infusion achieves protein-sparing to the extent that nitrogen loss was reduced to fecal and skin losses which are insignificant when compared with urinary nitrogen excretion.

The substrate profile data indicates that the success achieved with the infusion of an amino acid is due to adaptation of the metabolism which rapidly mobilizes its fat reserves as it does during total starvation. The limited effectiveness of peripheral protein-sparing therapy which includes glucose seems to be related to the interference of exogenous glucose with the physiologic adaptation to a state of severe negative caloric balance.

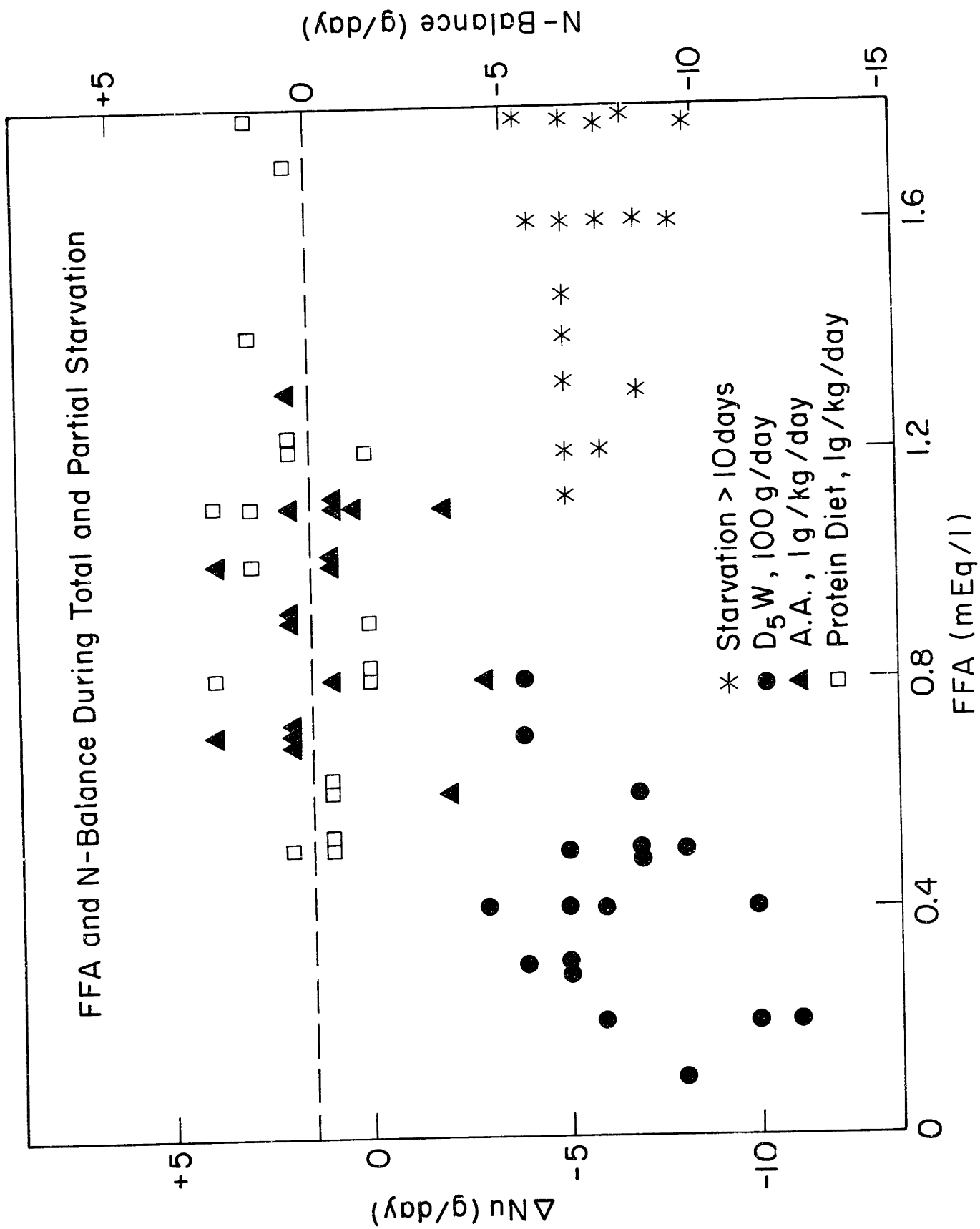
Free Fatty Acid, Ketone Bodies and Nitrogen Balance

In Figure 14 daily nitrogen balance (ΔNu) and serum free fatty acid levels are shown as determined in the four metabolic situations studied. The greatest nitrogen losses are seen to occur when free fatty acids are the lowest, wheich is typically the case in the surgical patients receiving D5W. The same patients when treated with amino acids infuions showed a significant increase FFA levels, from 0.4 ± 0.2 to 1.0 ± 0.2 mEq/L, ($p<0.01$) In the obese patients undergoing weight reduction FFA were 1.0 ± 0.4 mEq/L when receiving a limited amount of protein, and 1.4 ± 0.4 mEq/L when starvation was complete. After two to three weeks of adaptation the nitrogen balance in the patients undergoing total starvation shows a deficit of some 6 g per day, whereas on the weight reduction program with the

FIGURE 14: FFA and N-Balance During Total and Partial Starvation

Relationship between serum free fatty acids and nitrogen balance during various periods of negative caloric balance.

Improved nitrogen balance was associated with higher free fatty acids in these periods of semi-starvation.


FFA and N-Balance During Total and Partial Starvation
ΔNu (g/day)
N-Balance (g/day)
FFA (mEq/l)
+5
0
-5
-10
-15
0
0.4
0.8
1.2
1.6
Starvation > 10days
D5W, 100 g/day
A.A., 1 g/kg/day
Protein Diet, 1g/kg/day

limited intake of meat, net loss of nitrogen was significantly reduced ($p<0.01$).

Associated with elevation of FFA was the appearance of ketone bodies in the circulation. Figure 15 shows the correlation between ketosis and nitrogen balance. The surgical patients receiving intravenous glucose essentially had no ketonemia (serum β-hydroxybutyrate and acetoacetate $=0.1 \pm 0.05$mM). The prevention of ketosis by limited carbohydrate administration has been one of the expressed purposes of therapy with intravenous D5W. The same patients, when given isotonic amino acid infusions, developed ketosis within 36 hours, which was associated with considerable improvement in the nitrogen balance. Ketosis did increase with time during intravenous feeding with amino acids reaching values of some 2 mM.

Thus even after prolonged intravenous treatment with amino acids, ketone body levels did not approach those seen in the non-surgical group of obese receiving a comparable amount of protein in their weight reduction regimen. The surgical patients maintained comparatively higher insulin levels and the difference in the degree of ketosis can presumably be attributed to the antiketogenic action of this hormone.

Insulin, Glucose and Nitrogen Balance

The release of insulin by the pancreas increases with the concentration of glucose in blood. Figure 16 shows the relationship of glucose to insulin in the four physiological situations studied. The

limited intake of meat, net loss of nitrogen was significantly reduced ($p<0.01$).

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Insulin, Glucose and Nitrogen Balance

The release of insulin by the pancreas increases with the concentration of glucose in blood. Figure 16 shows the relationship of glucose to insulin in the four physiological situations studied. The

FIGURE 15: Ketone Bodies and N-Balance During Total and Partial Starvation

The appearance of ketone bodies in the blood was associated with improved nitrogen balance. Efficient protein sparing in surgical patients was associated with lower serum ketone body levels than in the weight reduction patients.

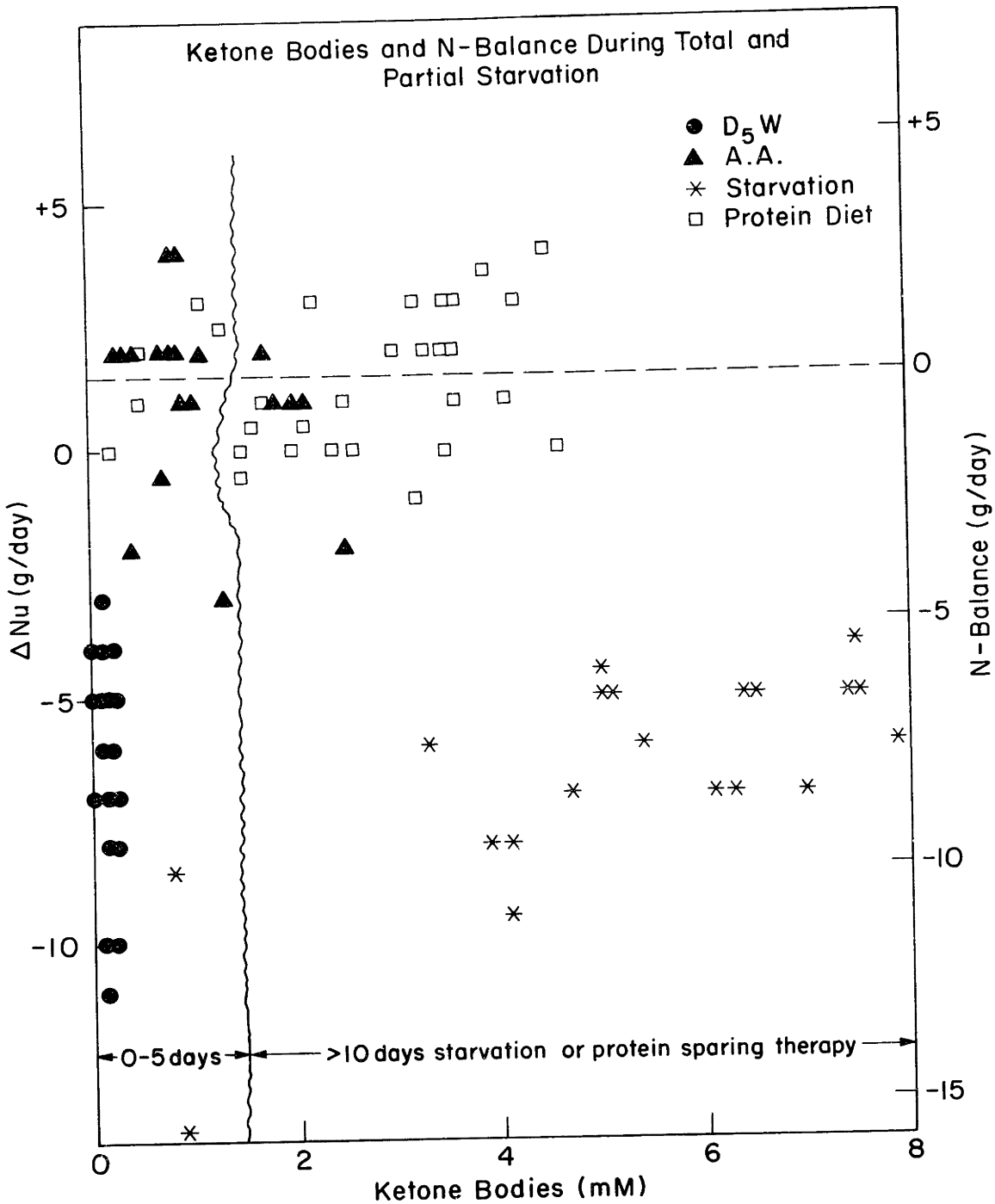
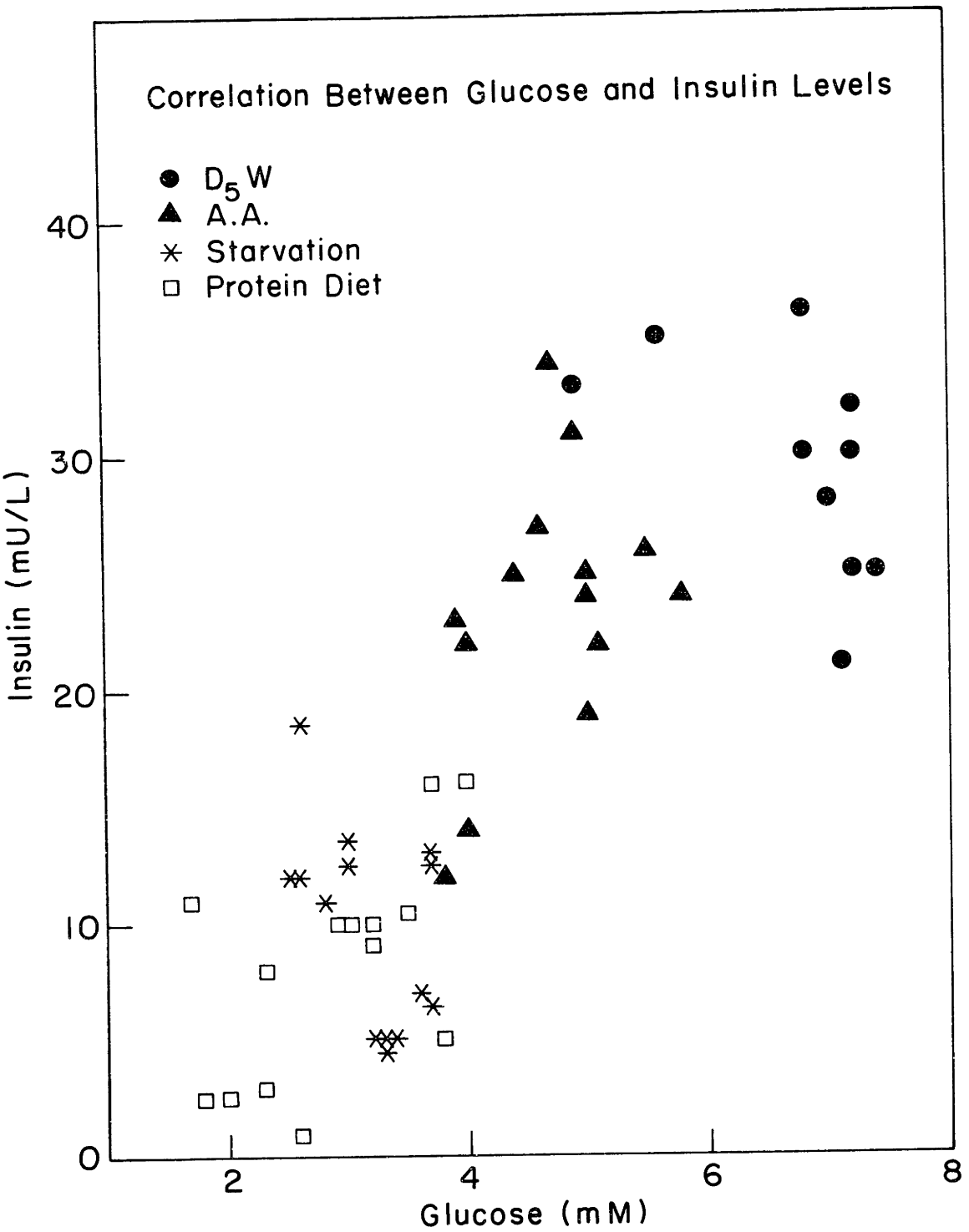

Ketone Bodies and N-Balance During Total and Partial Starvation
D5W
A.A.
Starvation
Protein Diet
+5
0
-5
-10
ΔNu (g/day)
N-Balance (g/day)
+5
0
-5
-10
-15
0-5 days
>10 days starvation or protein sparing therapy
0
2
4
6
8
Ketone Bodies (mM)

FIGURE 16: Correlation of Blood Glucose Levels with Serum Insulin Levels

The highest glucose and insulin levels were observed in the surgical patients receiving 100 g of glucose for 24 hours.

Insulin resistance in the surgical patients is suggested by elevated insulin levels during amino acid infusion as compared to protein ingestion in the weight reduction regimen.


Correlation Between Glucose and Insulin Levels
D5W
A.A.
Starvation
Protein Diet
Insulin (mU/L)
0
10
20
30
40
2
4
6
8
Glucose (mM)

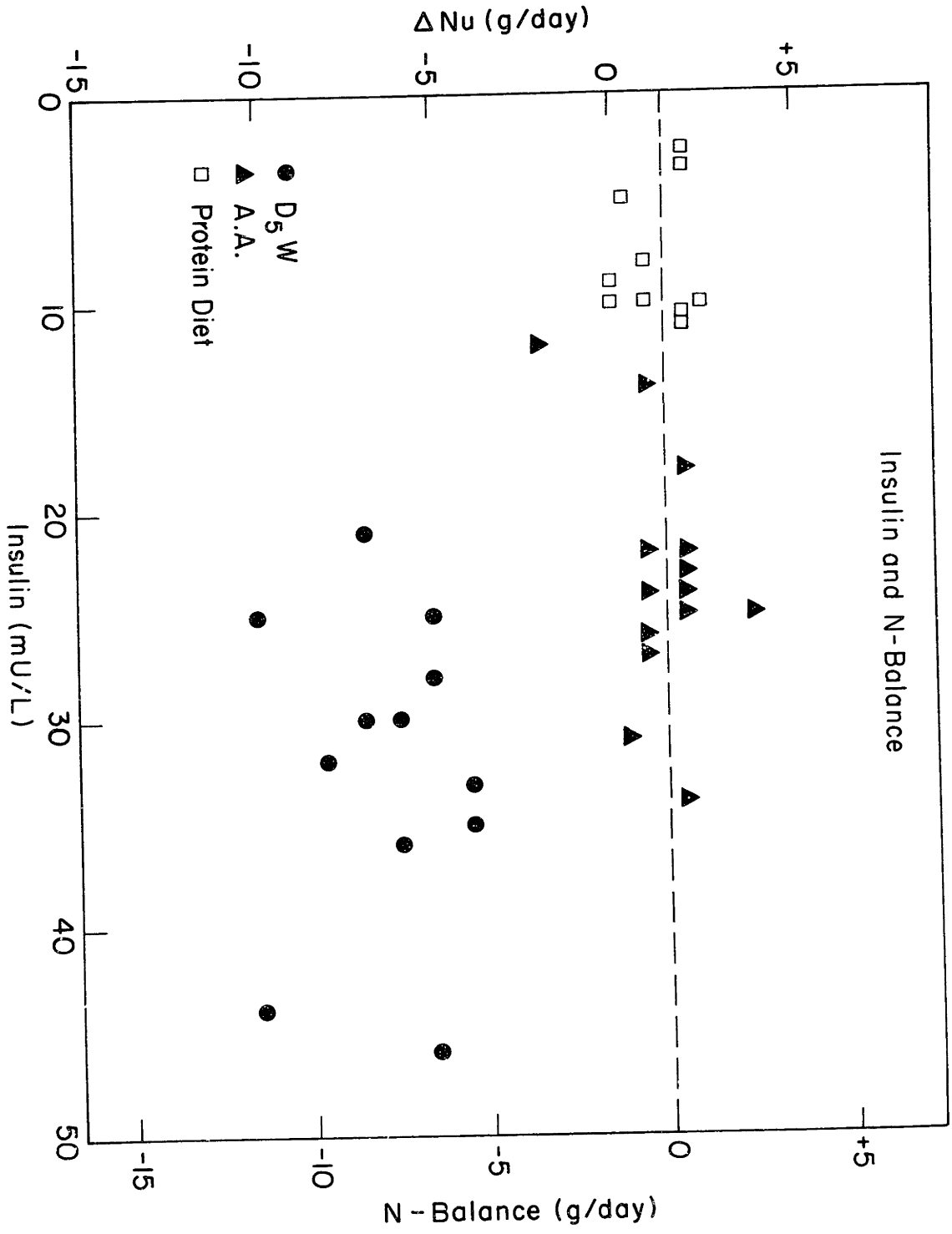
highest value for glucose and insulin were found in the patients receiving 5% dextrose in water. The substitution of glucose by amino acids in the infusate led to significant decreases in the level of glucose from 123 $\pm$ 15 to 85 $\pm$ 11 mg%: ($p < 0.01$), and insulin from 31 $\pm$ 6 to 23 $\pm$ 5 U/ml: ($p < 0.05$). In the weight reduction patients, supplying 40 to 80 g of protein per day caused only minimal and non significant changes in blood glucose (57 $\pm$ 8 instead of 52 $\pm$ 13 mg%) and insulin (9 $\pm$ 4 instead of 9 $\pm$ 4 uU/ml) levels.

Stimulation of insulin secretion by amino acids at this level of protein intake, and when blood glucose concentrations are low, is thus relatively weak. It is of interest to note that on comparable intakes of amino acids or protein, blood glucose and insulin levels were significantly higher in the surgical patients than in the individuals undergoing weight reduction ($p < 0.01$ and $p < 0.01$ respectively).

Since insulin is considered to be the key anabolic hormone, the relationship between insulin levels and nitrogen balances is illustrated in Figure 17. As previously described substitution of glucose by amino acid infusion has the effect of decreasing insulin levels but this is associated with a striking improvement of the nitrogen balance ($p < 0.01$)! The success of amino acid infusion as protein sparing in surgical patients during periods of semi-starvation intravenous feedings, and of limited protein intake in patients undergoing weight reduction, hinges on the ability of the absorbed amino acids to be utilized to replenish the amino acid pools which sustain protein synthesis

FIGURE 17: Correlation of Nitrogen Balance with Serum Insulin Levels

Elevated insulin levels were associated with the greatest nitrogen losses. Adequate levels of insulin during amino acid infusion and protein ingestion are evidenced by maintenance of nitrogen balance.


ΔNu (g/day)
-15
-10
-5
0
+5
Insulin and N-Balance
D5W
A.A.
Protein Diet
Insulin (mU/L)
0
10
20
30
40
50
N-Balance (g/day)
-15
-10
-5
0
+5

rather than being expended primarily for the production of energy.

Figure 18 summarizes the urinary nitrogen loss in a study conducted on a 32 year old female suffering from a perforated duodenal ulcer caused by a subphrenic abscess. Urinary nitrogen loss averaged 6.7 g/day while receiving 5% dextrose solution intravenously. The infusion of isotonic dextrose was replaced by the infusion of isotonic solutions of essential and nonessential amino acids, there was no significant increase in her urinary nitrogen excretion. After 17 days the patient returned to convention protein sparing therapy using 5% dextrose in water. Despite a show of reduction in nitrogen intake only a slight reduction in urinary nitrogen excretion took place. Thus the total of amino acid degradation during amino acid infusion with glucose is not much greater than during glucose infusion, however since the amino acid mixture supplies approximately 9 g of amino acid nitrogen per day the nitrogen balance can become essentially equal to zero. This protein sparing effect was associated with lowering of blood glucose from 7.2mM (130 ml%) to 3.8 mM (70ml%) and a corresponding insulin level decrease from 30 to 15 mU/ml. Free fatty acids and ketone bodies raised to 1 mM (=1mEq/liter) and 2.0 mM respectively. Fistula drainage decreased and the fistula was closed by the 25th day of treatment.

We have made similar observations in allowing an intake of 1 g of protein per kilogram body weight per day in starvation patients Figure 19. The urinary nitrogen excretion was nearly equal until the

FIGURE 18: Effect of Peripheral Intravenous Feeding

Comparison of protein sparing effect of isotonic glucose (D5W) and AA (3%).

No significant increase of urinary nitrogen occured.

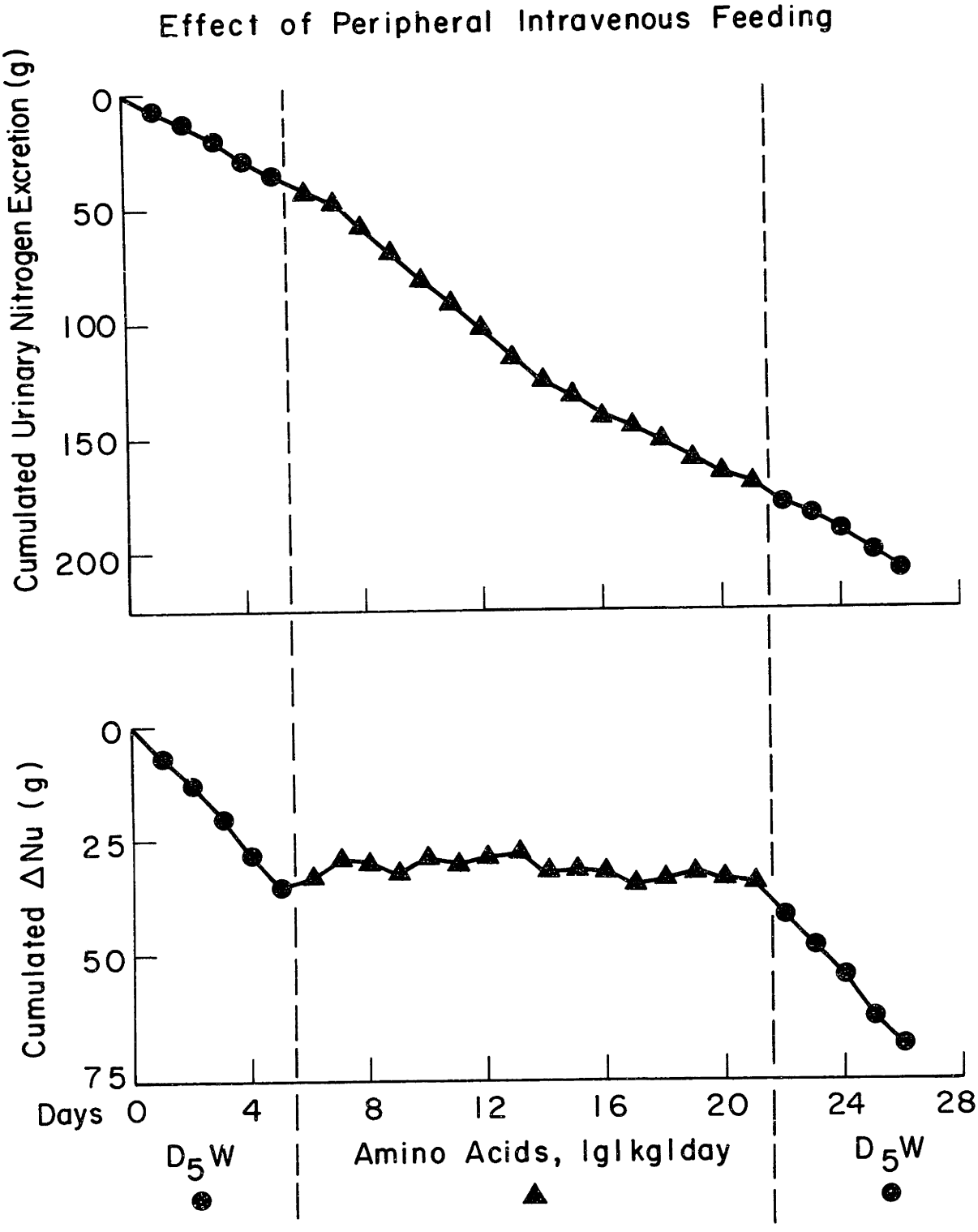
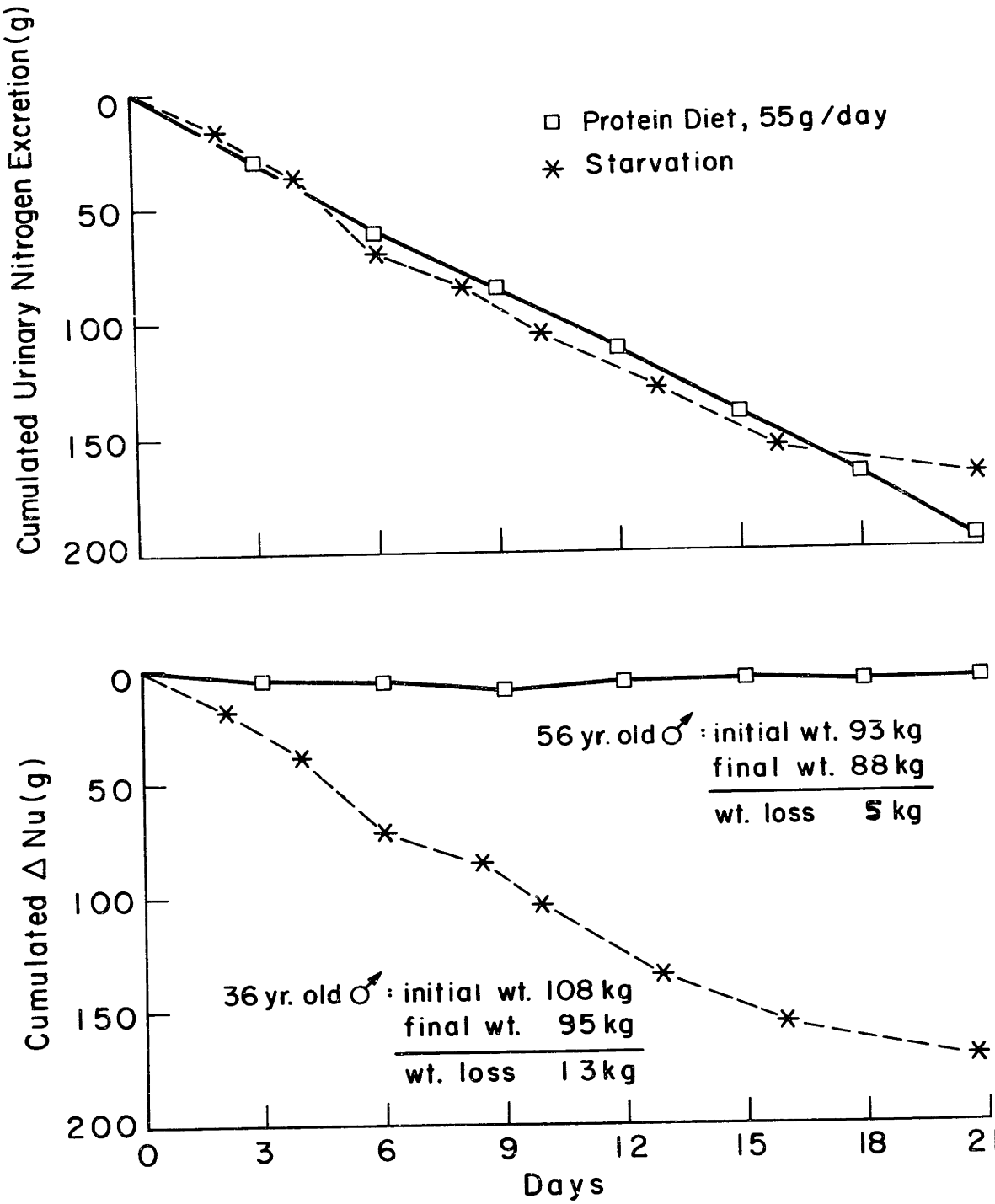

Effect of Peripheral Intravenous Feeding
Cumulated Urinary Nitrogen Excretion (g)
0
50
100
150
200
Cumulated ΔNu (g)
0
25
50
75
Days 0
4
8
12
16
20
24
28
D5W
Amino Acids, lg/kg/day
D5W

FIGURE 19: Accumulated Nitrogen Loss in Two Patients

Patients were on either starvation or a protein intake of 0.6 g/kg body weight/day.

Urinary nitrogen excretion was not increased by the protein intake until late starvation when adaptation resulted and decreased the gluconeogenesis from engogenous protein. The allowance of this limited protein intake essentially eliminated nitrogen loss observed in total starvation. Since an appreciable decrease in lean body mass occured with this regimen, weight loss was more than total starved control.


Cumulated Urinary Nitrogen Excretion (g)
0
50
100
150
200
□ Protein Diet, 55g/day
* Starvation
Cumulated Δ Nu (g)
0
50
100
150
200
56 yr. old ♂ : initial wt. 93 kg
final wt. 88 kg
wt. loss 5 kg
36 yr. old ♂ : initial wt. 108 kg
final wt. 95 kg
wt. loss 13 kg
0
3
6
9
12
15
18
21
Days

late phase of starvation when nitrogen excretion remained constant and approximately equal to the protein nitrogen ingested, whereas in total starvation further reduction in amino acid catabolism took place. On balance, the limited intake of protein prevents the protein losses which would be the consequence of complete starvation, while allowing for similar rates of reduction of the adipose tissue mass. Because no appreciable decrease in body cell mass occurs, the total weight loss is smaller than during total starvation. However, upon returning to an isocaloric intake, the lean body mass need not be rebuilt and the considerable regain of weight seen after total starvation is avoided. This may provide a physiological and psychological advantage for the patient in helping him to stabilize his weight after the period of weight reduction.

IV. DISCUSSION*

Metabolic Response to Caloric Deprivation

It is evident that in attempts to develop a rational approach to minimize loss of body cell mass in conditions of severely negative caloric balance, mobilization of endogenous fat stores must be of primary concern. The concept behind protein-sparing therapies should be specifically to take the fullest possible advantage of fat stores present in relative abundance in most patients. This important fact has previously received little consideration in comparison with the attention given hormonal factors capable of influencing the metabolism of carbohydrate and amino acids. These include the anabolic effect of insulin on protein catabolism (Cahill, 1971) and the catabolic effect of glucagon (Unger, 1971). The consequences of the antilipolytic action of insulin on nitrogen balance under conditions associated with caloric deprivation have not received much consideration so far.

Failure to mobilize endogenous fat stores leads to rapid consumption of body protein for energy production. The resulting rapid protein wasting and severely negative nitrogen balance is characteristic of many disease states. This is in striking contrast to physiological starvation.

*Discussion largely from Flatt and Blackburn, 1973; and Blackburn and Flatt, 1973; Blackburn et al., 1973a, 1973b, 1973c.

The metabolic changes that take place during total starvation have been extensively reviewed. The ability to derive substrates from endogenous fat is facilitated by the development of starvation ketosis. The latter becomes an important factor in the preservation of body protein during starvation.

Weight Reduction Regimen

These principles of metabolic adaptation to starvation are finding application in cases of obesity - the most prevalent nutritional problem in this country. Use of controlled starvation in weight reduction program has revealed various favorable and unfavorable aspects.

Rapid weight loss is certainly attractive to anyone who wants to lose weight. However the rate of weight loss decreases as the period of starvation extends because initially the weight loss is due largely to changes in fluid balance and intestinal content. In fact these initial effects are the basis for most of the criticism received against short-term starvation as a means of weight reduction.

The loss of hunger experienced during total starvation is an attractive component of this weight reduction program. It is largely related to the development of starvation ketosis, this metabolic significance has been revealed in the studies of Owen et al., (1967). However in total starvation there is significant loss of lean body mass particularly during the initial phase before the adaptation to starvation is complete. (200 g of nitrogen during three to four weeks corresponding to 1.25 kilograms of protein or about 6 kilograms of muscle tissue) This is related to the need for gluconeogenesis by

the liver and kidney. It is toward this unfavorable aspect of starvation that this investigation was directed.

In addition an important asset for effective weight reduction is simplicity as those who have dieted or those who have treated obesity well know. However, this can be a drawback, due to the fact that the patient has no occasion to learn important aspects that are necessary for sustained weight control once the patient resumes eating. This, coupled with considerable regain of lost fluid and rebuilding of lean body mass, which has been depleted, contributes significant disadvantages for total starvation as an adjunct to a weight reduction program.

The use of controlled starvation in weight reduction programs and in clinical studies of various metabolic programs have revealed very few major problems in connection with total starvation with the exception of muscle wasting and hyperuricemia. Controlled starvation is emphasized since it includes close medical supervision, and the provision of appropriate amounts of water, vitamins and minerals.

If instead of total starvation some food is ingested, a state of partial starvation prevails which differs from complete starvation according to the amount and type of nutrient ingested. Figure 20 shows the metabolic effects of carbohydrate on protein intake. Carbohydrate raises blood glucose levels directly while amino acids exert an indirect and therefore attenuated effect on blood glucose levels. Direct stimulation of insulin secretion by amino acids is relatively weak because of the low glucose levels (Mueller et al., 1971). Thus, compared to carbohydrate, protein leads to a more

moderate increase in insulin levels and to a lesser curtailment of fat mobilization. In other words, the ingestion of some protein does not affect ketosis, whereas, carbohydrate in the amounts of 50 to 100 g per day appreciably reduces ketosis. (c.f. Figures 5 and 6; Sapir et al., 1972) This explains the success for weight reduction of diets which provide protein but not carbohydrate, since these diets allow the development of starvation ketosis with its hunger suppressing effect (Stillman and Baker, 1967; Atkins, 1972; Bolinger, 1966). We have found that daily ingestion of 0.8 to 1 g of protein per kg of body weight produces a metabolic state similar to that of total starvation. The amount of urinary nitrogen excretion are comparable, indicating that the rate of gluconeogenesis are similar, with and without in limited protein intake (Figure 19). The ingestion of protein, however will compensate for the degradation of amino acid and the nitrogen balance will be close to zero; in protein depleted patients it may even become positive.

The loss of fat is comparable to that which can take place in total starvation. The hunger feelings are much reduced presumably due to development of starvation ketosis. It is an easy regimen to follow even for out-patients. It appears to be safe at least to the extent of our present studies which have now extended over a period of some two years. The only significant symptom is transient fatigue which has been commonly associated with ketonemia. Careful medical monitoring and the supplementing of the diet with appropriate

vitamins and minerals particularly potassium and calcium are essential for its safety and optimal effect.

Since there is essentially no loss of lean body mass the regime can be pursued for long periods of time. The decrease in body weight then is due almost exclusively to temporary loss of water and to a decrease in adipose tissue mass, the latter in the amounts of 200 to 250 g per day. This is of considerable significance as it may enable grossly overweight patients to return toward a normal body weight profile. This protein sparing regimen, when coupled with a good weight reduction program that emphasizes the role of activity, nutritional education, proper dietary and behavioral habits, is promising as a program for long term weight reduction.

Furthermore, the finding that nitrogen can be retained demonstrates that free fatty acids and ketone bodies even when they have to be delivered almost exclusively from endogenous fat stores are most effective in reducing amino acid degradation.

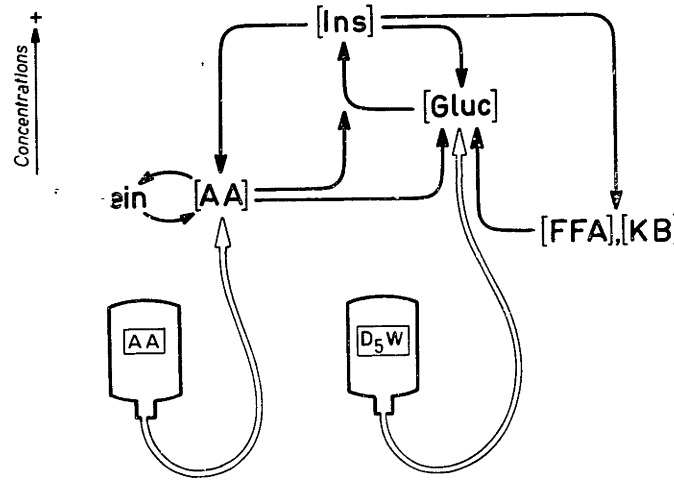


Figure 20: The metabolic Fuel Regulatory System and Protein Sparing Therapy: showing the effect of carbohydrate intake (e.g. 5% dextrose in water D5W) or protein (3% amino acid mixture AA) on the concentration of metabolic fuels and of insulin.

Under conditions of caloric deprivation rapid mobilization of endogenous fat stores are crucial if amino acids and endogenous protein are to be preserved efficiently. The figure shows the advantage administering amino acids for protein sparing, as this will lead to a lesser stimulation of insulin secretion and hence to a lesser reduction in fat mobilization than by giving glucose while at the same time replenishing the amino acid pools affording protein synthesis.

Protein-Wasting Associated with Disease

During periods of sepsis and disease, or after major trauma, the patient's nutrient intake often is far less than enough to offset the calorie expenditure, so that stress and partial starvation are superimposed in the disease state. In the absence of disease, the body adapts to calorie deprivation by the rapid mobilization of fat and development of ketosis, thus conserving body protein. During disease, however, fat mobilization does not proceed as rapidly (Blackburn and Flatt, 1973), and this may be an important factor in the excessive rate of protein catabolism that is typical of disease states.

When antilipolytic agents such as nicotinic acid or 3,5-dimethyl-isoxasole are administered to starving rats, a four fold increase in urea excretion is observed (Trout et al., 1967). Thus interference with the ability to mobilize endogenous fat stores under conditions of caloric deprivation can provoke the complex regulatory responses which allow increased protein catabolism and increased gluconeogenesis (Hasselblatt et al., 1971) in order to maintain the supply of substrates for energy production. It is even more interesting that a similar response occurs when the ability to mobilize endogenous fat stores is impaired by hypophysectomy (Engel and Kostyo, 1964), which leads to increased protein catabolism during fasting (McKee and Russell, 1968) even though several hormonal interactions are basically modified.

Figure 21 demonstrates the effect of sepsis superimposed on

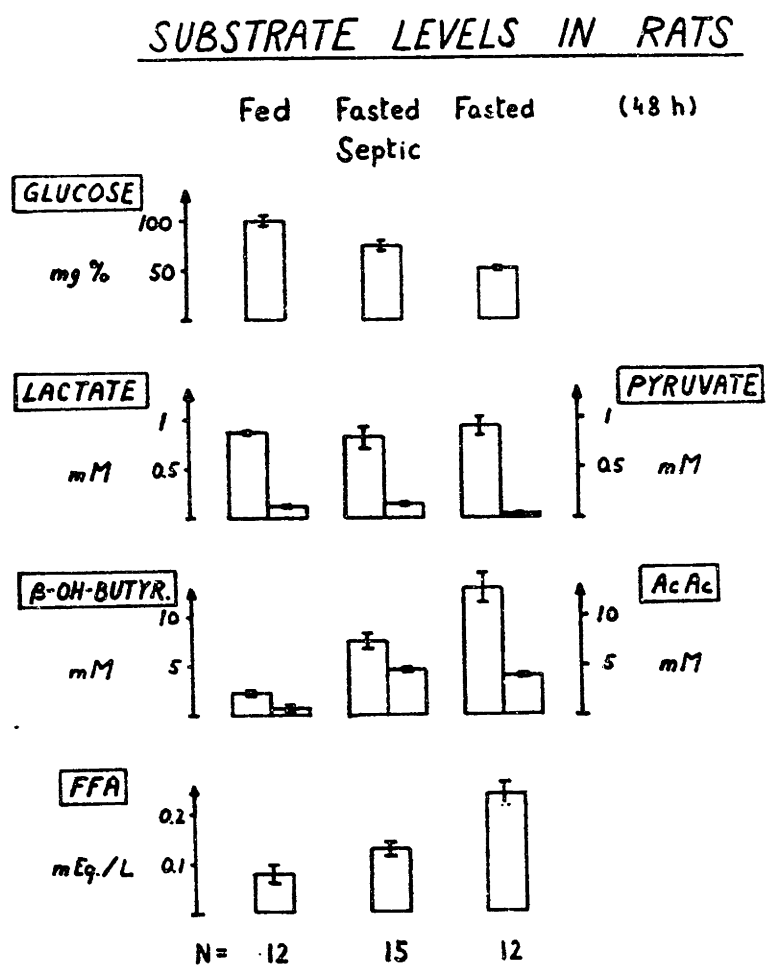


FIGURE 21: Substrate levels in fed, starved and starved-septic rats. The columns and the vertical bars show the average levels and the standard errors of various blood substrates determined enzymatically in deproteinized blood, or in the case of FFA, acidimetrically in plasma of 12 to 15 rats in each experimental group.

starvation in 150 g rats. The rats were made septic by cecal ligation and subjected to a two-day fast. Their free fatty acid and ketone body levels were only about half those in controlled animals subject to an equal fast but without sepsis. Moreover, the lactate-pyruvate ratio in the septic starved rats did not show the typical increase observed during fasting when fat became the major substrate for energy production (Krebs, 1967), and when conversion of pyruvate to acetyl-CoA is reduced (Jagow et al., 1968), to preserve it for gluconeogenesis. Sepsis thus appeared to reduce fat mobilization, while urinary nitrogen excretion increased to a level 50 percent higher than that in the starved control rats (Blackburn and Flatt, 1973a).

Despite the reduced utilization of endogenous fat, which implies increased utilization of glucose, glucose levels in the septic-starved rats was 50 percent higher than in the starved controls. This indicates that the rates of formation of gluconeogenesis precursors and the rate of gluconeogenesis can meet the increased demand for glucose under disease conditions.

The mechanisms by which disease may alter metabolic interactions so as to induce protein-wasting are implied in the scheme shown in Figure 22. During disease, insulin resistance occurs (Howard, 1955), and in order to regulate glucose levels distribution, increased amounts of insulin are needed. Under conditions of protein wasting, plasma levels of many amino acids are elevated (Blackburn and Flatt, 1973; Felig et al., 1970). This could be due either to a decrease

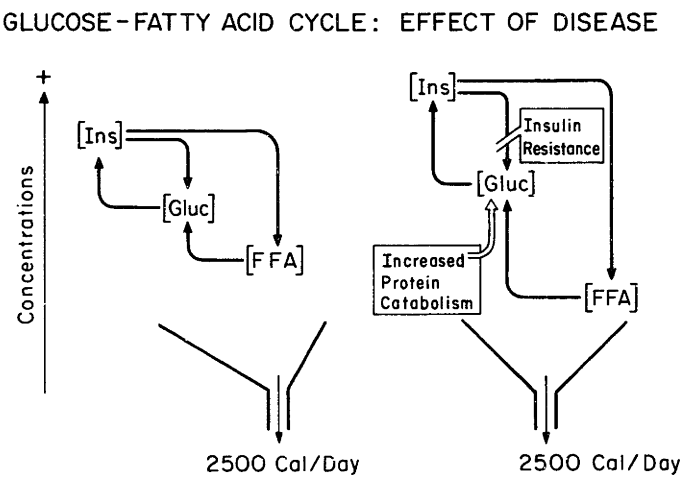


FIGURE 22: Arrows represent the effective glucose and increase the insulin level and of decreasing glucose levels. In addition the antilipolytic action of insulin is represented by an arrow pushing free fatty acids down. Finally free fatty acdis, by their sparing effect on the consumption of glucose tend to elevate the glucose concentration. In disease, glucose levels are elevated due to increases in protein catabolism and gluconeogenesis from amino acids. Because insulin resistance is associated withdisease, higher insulin concentrations are required to control blood glucose. Since insulin has an antilipolytic action, free fatty acid concentrations are lower than they are in comparable states of starvation without disease. The shape of the funnel serve to illustrate that in starvation free fatty acids are the major metabolic fuel whereas in disease a greater contribution of energy production is made by glucose.

in the peripheral tissues' responsiveness to insulin stimulation or to other causes such as the changes in the rate of protein synthesis and breakdown. Since FFA levels are low, there seems to be no comparable decrease in adipose-tissues sensitivity to the antilipolytic action of insulin (c.f. Figure 14 and 15; Blackburn and Flatt, 1973).

The increased requirement for insulin to control blood-glucose levels interferes with fat mobilization. Because endogenous fat makes such a major contribution to energy production, even a small reduction in fat mobilization creates a gap in the substrate supply which must be covered by increased oxidation of glucose or amino acids or both, and ultimately by the use of endogenous protein when nutrient intake is restricted. The relatively high rate of glucose oxidation in disease as compared to physiological starvation is well known (Long et al., 1971).

The seeming paradox of increased glucose oxidation in the face of insulin resistance can be resolved if one considers that glucose oxidation in muscle is regulated not only at the glucose uptake step, but also at several steps in the pathways through which glucose is catabolized (Randle et al., 1964; Scrutton and Utter, 1968). The reaction is markedly influenced by the availability of other fuels such as FFA and KB.

With low circulating FFA and KB levels, compounded by restricted uptake of circulating glucose, the supply of metabolic fuels to tissues such as muscle is reduced, except for amino acids whose

intracellular pool is continuously fed by protein breakdown. Only certain amino acids particularly the branched-chain or ketogenic type--can be degraded rapidly in muscle (Manchester, 1965). If the catabolism of some of the essential amino acids is increased, others will tend to accumulate and to escape into the circulation. Thus, disproportionately high levels of alanine are found in the plasma under conditions of protein wasting (Blackburn and Flatt, 1973a; Felig et al., 1970). Alanine is a major precursor for gluconeogenesis in the liver (Felig et al., 1970b), where this process may be enhanced by glucagon (Marliss et al., 1971; Unger, 1971). Since alanine stimulates the secretion of glucagon, high levels of circulating glucagon and high rates of gluconeogenesis are observed in the disease states (Cahill, 1971; Unger, 1971).

The condition of nonketotic hyperosmolar diabetes (Aneff and Carroll, 1971), is typically provoked by disease in patients with limited ability to secrete insulin. Insulin sufficient to prevent rapid fat mobilization and ketosis can be produced, but the levels may be inadequate, particularly in the face of insulin resistance, to allow enough glucose uptake by muscle to compensate for the limited availability of FFA. Insulin resistance induced by disease and excessive oxidation of certain amino acids in muscle could initiate a sequence of events leading to high rates of gluconeogenesis and the accumulation of glucose characteristic of the hyperosmolar syndrome.

High rates of release of glucogenic amino acids in protein-wasting conditions, particularly when combined with an intake of

some exogenous glucose, induce or maintain insulin levels high enough to prevent fat mobilization from reaching the rates which would prevail at a comparable degree of starvation without superimposed disease.

In addition to its function of producing glucose, especially for the brain, gluconeogenesis also serves to remove from the circulation glucogenic amino acids whose peripheral oxidation is slow. In converting these amino acids to glucose, gluconeogenesis makes them available for peripheral energy production even in muscle.

The rationale developed here to account for the rapid wasting of protein in disease, after trauma, or during sepsis, may be compared with other mechanisms which have been proposed, in particular: a) an increase in the need for glucose (Cahill, 1951; Long et al., 1951), and b) inhibition of protein synthesis or stimulation of protein catabolism by lack or excess of certain hormones or other as yet undefined substances (Cahill and Aoki, 1970). The prevalence of high glucose and insulin levels in disease, and the failure of isotonic glucose infusion to reduce protein catabolism to rates comparable to those found in starvation (Cahill, 1951; Long et al., 1951), would seem to argue against the first mechanism. On the other hand, the consequences of increased protein breakdown in relation to protein synthesis are similar to those noted with insulin resistance; increased release of amino acids from muscle, increased gluconeogenesis, elevated glucose levels, and elevated insulin levels, leading to a reduction in fat mobilization; and failure to improve nitrogen balance by administration of carbohydrate in limited amounts.

While a change in the relative rates of protein synthesis and breakdown would not account for the impairment of glucose tolerance (Howard, 1955), this resistance could arise for unrelated reasons.

Regardless of the exact sequence of events, the result is that the body functions with lower FFA and KB levels than are available in comparable starvation without disease. This means that more of the energy requirements will be met by carbohydrate and protein metabolism, a shift which will adversely affect nitrogen balance, particularly when the calorie intake is inadequate (c.f. Figure 12 and 13).

Protein-Sparing Therapy

The conventional metabolic care of severely ill patients include the administration of at least 100 g of carbohydrate per day (Gamble, 1946; Randall, 1969), by the peripheral intravenous infusion of 5% dextrose in water. The aim is to replace the glucose catabolized by the patient, and to spare his tissue proteins by reducing the need for gluconeogenesis as seen in Figure 23 (Cahill, 1971; Cahill and Aoki, 1970; Long et al., 1971).

The trouble is that glucose administration provokes an insulin response which impedes the mobilization of endogenous fat as a primary energy source. Some of the infused glucose is needed to make up for reduced oxidation of FFA and KB (c.f. Figure 25), thus sparing endogenous fat rather than protein. The extent to which caloric contribution from fat stores will be reduced depends on the degree of insulin resistance, which in turn is related to the severity of the injury, sepsis or disease, as well as the nutritional status. Thus the protein-sparing efficiency of small amounts of carbohydrate varies a great deal. At best in the moderately injured patient, nitrogen losses can be reduced only to some 7 to 8 g per day (c.f. Figure 24 and 25; Gamble, 1946).

The peripheral infusion of amino acids in addition to isotonic glucose seems to be of little value in improving nitrogen balance (Werner, 1947; Figure 24). The inflow of both glucose and amino acids elicits an especially sharp insulin response (Cahill, 1971; Floyd et al., 1966; Figure 25), which may reduce fat mobilization

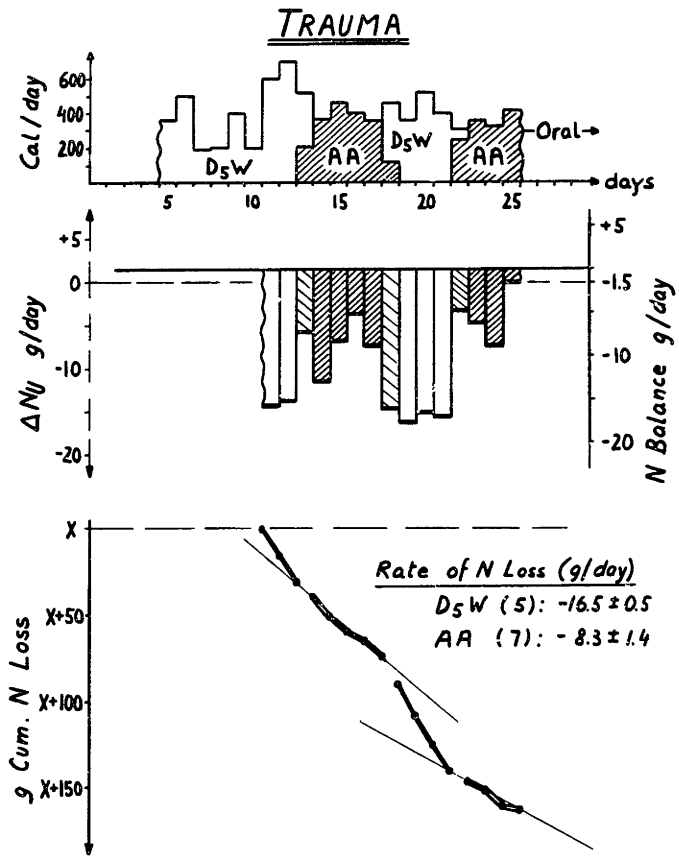


FIGURE 23: Effect of trauma and sepsis on man. 54 year old male with major blunt trauma and fractures from auto accident. Treatment with alternating peripheral intravenous fluid of 5% dextrose or 4% amino acids demonstrates varying protein sparing effect. Using urinary nitrogen data the calculated rate of nitrogen loss/day is significant ($p < 0.01$).

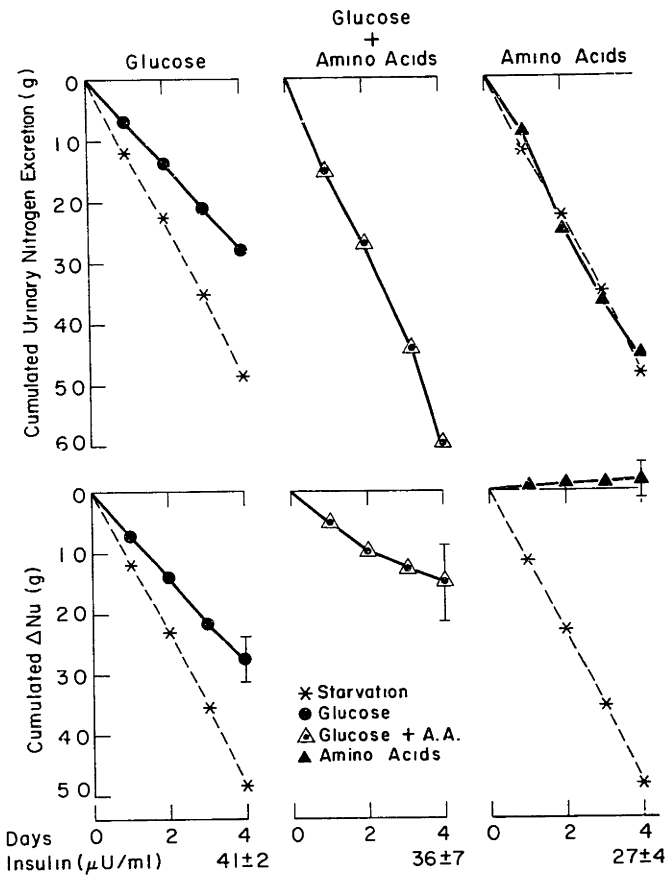


FIGURE 24: Effect of intravenous infusion of dextrose and/or amino acids. The accumulated urinary nitrogen excretion in 4 days in patients with moderate protein catabolism. The infusion of amino acids did not increase nitrogen excretion compared to starvation. Nitrogen balance was essentially zero. (Average ± S.D.)

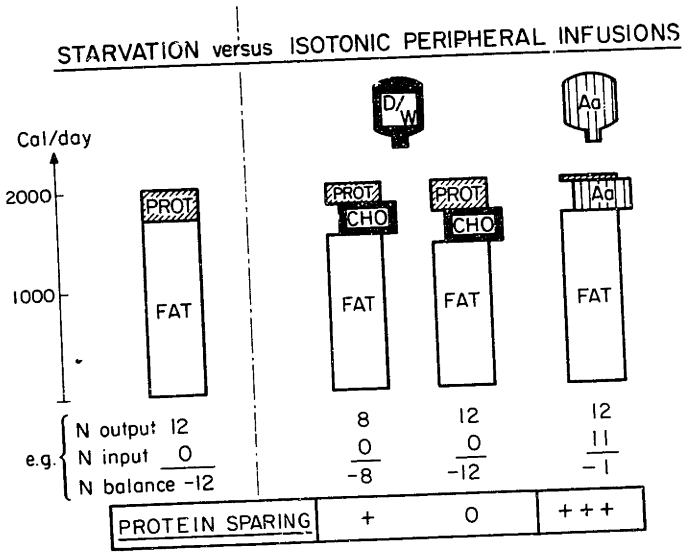


FIGURE 25: Due to the effect of glucose infusion of insulin secretion and hence on fat mobilization, the contributions made by endogenous fat to the caloric requirement would be reduced. Depending upon the degree of this reduction, the net amount of endogenous protein catabolism needed in addition to endogenous fat and exogenous glucose to cover the caloric requirements will be variable when compared to total starvation. With the infusion of amino acids alone, no appreciable antilipolytic effect is provoke, and the overall amino acid catabolism needed to make a contribution to energy metabolism is not greater than that in total starvation. Infused amino acids can thus replenish effectively the amino acid pool.

to such an extent that the infused nutrients need to be used mainly for energy production.

We have approached this problem by administering amino acids infusates containing no glucose, thus allowing the development of starvation ketosis. With energy production supported largely by FFA and KB oxidation, as in total starvation, the infused amino acids can be used to replenish the pools of amino acids available for protein synthesis.

Figure 20 shows the fuel-regulatory scheme as a basis for comparing the metabolic consequences of glucose or amino acid infusions. These two types of protein-sparing therapy differ in two main aspects: 1) their effects on glucose and insulin levels and hence on the intensity of the anabolic signal conveyed by insulin to muscle and adipose tissue, and 2) their direct relevance to the purpose of maintaining adequate amino acid pools for protein synthesis.

Under conditions of caloric deprivation, rapid mobilization of endogenous fat stores is crucial if amino acids and endogenous proteins are to be preserved efficiently. Figure 21 shows the advantage of administering amino acids for protein-sparing: This will lead to a lesser stimulation than when glucose is given, while at the same time the amino acid pools supporting protein synthesis will be replenished. (c.f. Figures 14, 16, 17).

The metabolic studies performed on patients receiving peripheral intravenous infusions indicate that protein-sparing therapy with amino acid infusates is far more effective and that it may be subject to

less variability in reducing protein losses than the conventional treatment with isotonic glucose solutions. The studies represent a reduction in nitrogen loss of some 8 to 10 g per day by using amino acids instead of glucose (Figure 23, 24, 25).

As soon as amino acid infusions are initiated, an appreciable inflow of amino acid nitrogen becomes available to offset nitrogen losses. This alone results in immediate improvement in nitrogen balance, even before the effects of starvation ketosis becomes operative. The 70 to 90 g of amino acids which can be administered intravenously by peripheral isotonic or near isotonic infusions provides 11 to 14 g of amino acid nitrogen. This is sufficient to achieve a satisfactory nitrogen balance even when amino acid degradation is not as efficiently reduced as when starvation ketosis is fully established. (Figure 26)

Hyperalimentation

In recent years a great deal of attention has been directed toward the techniques provided by complete parenteral nutrition by way of central venous catheters (Dudrick et al., 1970; Rea et al., 1970). This method allows the infusion of hyperosmolar solutions of glucose and amino acids -- hence the term "hyperalimentation". Because of its technical complexities this technique is recommended only for severely depleted patients and for infants who can not be fed by the digestive tract.

It is recognized that the daily intake of calories by the parenteral route must considerably exceed the normal daily requirements, if a positive nitrogen balance is to be achieved. While this

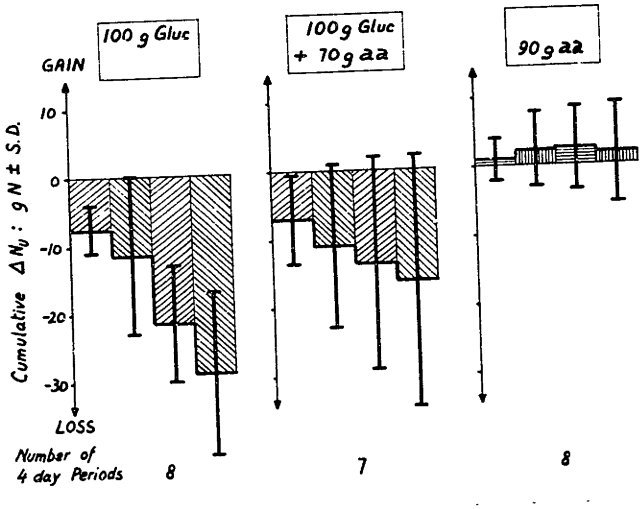


FIGURE 26: the accumulated nitrogen loss over 4 days in patients receiving isotonic infusions of, (1) 5% dextrose in water, and (2) dextrose in 3% amino acids, and (3) amino acids alone, (averages ± standard deviation)

is due largely to the strong catabolic trend associated with severe disease or injury, it should be noted that such a trend may also reflect inadequate nutrition. It is possible, moreover, to attribute the high caloric requirement in hyperalimentation to some disruption in the normal physiological interaction between metabolic fuels and insulin. The continuous infusion of a large amount of glucose to amino acids require that a high level of insulin be constantly maintained, by means of exogenous insulin if necessary. Thus the release of FFA from adipose tissue is largely prevented. High concentrations of insulin and glucose create conditions favorable for lipogenesis in adipose tissue (Winegrad and Renold, 1958; Masoro, 1962; Wiley and Leveille, 1970). It has been estimated that with high-carbohydrate diets, up to one-third of the ingested carbohydrate is converted to fat (Wertheimer, 1965), the stored calories being made available in the form of FFA during the post-absorptive periods of the day. Since appreciable release of FFA cannot occur when large amounts of glucose are infused continuously about a third of the glucose calories will be deposited in the form of adipose-tissue fat. In short, the sustained high insulin levels prevent the normal interplay of the metabolic fuels and exert a catabolic effect on protein.

On the other hand it has been found that nitrogen excretion in severely burned patients can be reduced dramatically by the infusion of large doses of insulin along with concentrated glucose solutions (Hinton et al., 1971). Here the decrease in amino acid

catabolism might be attributed either to an anabolic effect of in sulin, with glucose serving to prevent insulin-induced hypoglycemia, or to the effect of glucose itself, with insulin serving to insure the distribution of glucose. The second alternative is consistent with the general argument that protein wasting is caused by a relative lack of substrates other than amino acids to support energy production in muscle, a lack which can be overcome by massively enhancing the availability and uptake of glucose.

In this connection the problem of fat infusion is the object of renewed interest--ideally, fat could provide the chief source of energy, allowing a reduction in the amount of glucose administered, hence the degree of hypertonicity of the infusates. Emulsified fat preparations also would meet the need for polyunsaturated fatty acids which may arise during long-term parenteral alimentation (Maynard et al., 1972).

A consideration of the interplay of metabolic fuels and insulin suggests some further advantages to be derived from fat administration. Such emulsions usually are not mixed with concentrated glucose and amino acids, since these substances adversely affect the stability of the fat emulsion. Therefore the separate administration of the emulsion might allow time for a post-absorptive state to develop in the patient, with consequent mobilization of FFA from adipose tissue. A potentially significant benefit is related to the fact that fat in the diet, even in rather low amounts, considerably reduces the lipogenic effect of high-carbohydrate diets (Masoro, 1962; Hausberger and Milstein, 1954). With the regimen including fat,

therefore, conversion of glucose to fat could be restricted appreciably.

Hormones and Nitrogen Balance

Any hormones or drugs that can influence fat mobilization may affect nitrogen balance, favorably or unfavorably, especially during periods of restricted nutrient intake as previously reviewed. To some extent even the intermittent episodes of negative calorie balance which occur during each 24-hour period represent a challenge for the presentation of the lean body mass.

Among the hormones which favor nitrogen retention, growth hormone is of particular interest for its ability to stimulate fat mobilization (Greenbaum, 1953). This hormone has long been recognized as an antagonist of insulin (Young, 1953), an action which is significant in relation to the argument that an imbalance in the regulatory system, emphasizing the anabolic action of insulin, increases amino acid catabolism during disease.

The administration of steroid hormones, such as cortisone or dexamethasone, induces insulin resistance as judged by reduced glucose tolerance (Fajans and Conn, 1954) and the need to maintain higher insulin levels for longer periods in order to distribute a glucose load (Perley and Kitniss, 1966). Maintenance of elevated insulin levels for periods disproportionate with the nutrient intake could more than offset the mild lipolytic action of the steroid hormone themselves (Fain et al., 1963). Thus administered glucocorticoids produce varying effects on FFA mobilization and utilization which often vary protein synthesis and degradation in various parts

of the body. These actions could explain why adipose tissue is specifically increased in Cushing's disease (Forshin, 1968), or after long term treatment with exogenous steroids (Klink and Estich, 1964). When the ratio of muscle mass to adipose tissue mass has adapted, nitrogen balance may be maintained in these patients (Munro, 1964).

With the recent findings that prostaglandin levels are altered in certain diseases (Ramwell and Shaw, 1971), another group of substances emerge which, through their interference with fat mobilization (Steinberg et al., 1964) could be involved in the development of protein-wasting states.

Future Protein Sparing Therapy

The prospects for further improvement of protein sparing therapy based on the administration of peripheral isotonic amino acid solutions containing no glucose are promising. Different amino acids have different metabolic effects particularly in promoting the release of insulin and of glucagon, in supporting gluconeogenesis as well as ketogenesis which are related (Wieland, 1968; Flatt, 1972). It may therefore be possible to adjust the amino acid composition in the infusates so as to achieve the most effective metabolic leverage for protein sparing. Furthermore essential amino acids may be desirable in amounts which may appreciably increase the known requirements determined in the isocaloric state rather than under conditions of semi-starvation plus disease which are applicable in the treatment of patients. Knowledge on which to base the selection of particular amino acid

compositions is essentially lacking. Mixtures of synthetic L-amino acids such as those used in these studies are generally formulated to simulate the composition of protein of high biological values, such as egg and milk proteins (Table II and III). Nevertheless, clinical trials show that considerable and immediate improvement in nitrogen balance can be achieved with solutions of this type.

The nitrogen balance data shown clearly that the amino acid infusates tested were far more effective for protein sparing than the commonly used solutions of dextrose in water.

SUMMARY AND CONCLUSIONS

1. The delivery of 0.7 to 1.0 grams/kilogram/day of protein (orally or intravenously) as the sole nutrient was associated with marked improvement in the nitrogen balance in patients with moderately severe trauma and injury and in moderate to very obese patients. When compared to present intravenous therapy or total starvation.

2. This protein sparing effect was associated with blood levels of insulin, glucose, free fatty acid and ketone bodies similar to those seen in total starvation.

3. A schematic analysis of the metabolic fuel regulatory system shows that insulin is the key factor in the control of integration of the supply and consumption of substrates for energy production, health and disease.

4. The importance of unimpeded fat mobilization and the favorable effects of starvation ketosis during negative caloric balance is emphasized.

5. The close relationship between nitrogen balance and fat mobilization suggests that the favorable or unfavorable effects asserted by various metabolic fuels, hormones or drugs on protein metabolism may be related largely to their direct or indirect effect on fat mobilization.

6. The success of this new type of protein sparing therapy is attributed to the fact that the intake of protein stimulates insulin release less than carbohydrate. The antilipolytic action of the insulin is thereby reduced allowing for increased utilization of endogenous fat stores.

7. This concept is important in nutritional therapy designed to preserve lean body mass during periods of caloric deprivation and disease.

SUGGESTIONS FOR FUTURE RESEARCH

1) To extend the characterization of the complete "metabolic fuel substrate profile" including carbohydrate, fat and amino acid metabolites and the related hormones in acute ailments and obesity.

2) To evaluate the role of inadequate fat mobilization and ketogenesis on nitrogen loss during periods of disease or associated with insulin resistance or altered insulin secretion.

3) To determine the protein sparing effect of isotonic solutions of crystalline L-amino acids and protein diets administered with and without small amounts of glucose during periods of negative caloric balance.

4) To measure the rate of gluconeogenesis and ketogenesis during periods of protein catabolism.

5) To develop an optimal substrate composition for parenteral alimentation whether or not the regimen provides enough calories to cover the caloric requirements.

6) To determine the splanchnic and muscle exchange of metabolic fuels particularly with regard to amino acids during various physiological or pathological states.

7) To investigate the relationship between respiratory quotient (RQ) oxygen consumption (V̇.02), "substrate profile" and nitrogen balance.

8) To develop an effective obesity treatment program that would include this protein sparing concept.

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BIOGRAPHICAL NOTE

George L. Blackburn was born February 12, 1936, in McPherson, Kansas. He attended elementary and secondary schools in Joplin, Missouri, receiving his high school diploma in 1954. He was the recipient of an NROTC scholarship and attended Kansas University from 1954 to 1958. He then served as a Line Officer in the U. S. Navy with the Pacific Fleet and was the recipient of several Letters of Commendation. After his military service he returned to the University of Kansas Medical School and was graduated as a Doctor of Medicine, cum laude in 1965.

Upon graduation, Dr. Blackburn did his internship and surgical residency on the Harvard Surgical Service at the Boston City Hospital. After completing his general surgical residency he became an N.I.H. post-doctorate fellow in the Department of Nutrition & Food Science at Massachusetts Institute of Technology.

Dr. Blackburn is a Diplomat of the American Board of Surgery and member of several honorary and professional societies including Alpha Omega Alpha, New York Academy of Science and the American College of Surgeons.

He is the author and co-author of several original scientific articles and book chapters in the area of metabolism, particularly body composition, fluid and electrolytes and energy metabolism as it effects the surgical patient.

APPENDIX A

ENZYME ASSAYS FOR METABOLIC FUEL SUBSTRATE PROFILE

From: H.U. Bergmeyer (1971)

Sample Preparation (Blood Deproteinization)

Reagents

1. $HClO_4$ 30% (w/v)
2. KOH 20% (w/v)
3. Phenol Red Indicator

Procedure

1. Keep sample ice cold throughout procedure.
2. Collect blood in centrifuge tube containing an equal amount of cold perchloric acid ($HClO_4$) and mix well. (An alternate method is to heparinize the blood at time of collection, refrigerate, and then acidify at earliest possible time.)
3. Centrifuge for 10 minutes at 2000-3000 rpm.
4. Transfer supernatant to a graduated centrifuge tube and record volume (acidified serum, V_1).
5. Add 1 drop of phenol red indicator and vortex.
6. Add KOH dropwise until pale orange color is obtained (check with pH paper. pH should be 6.7-7.3) If end point is missed readjust with $HClO_4$.
7. Centrifuge as above and record total volume. (Neutralized serum, V_2)
8. Store sample frozen if it is not to be immediately assayed.

Blood Glucose Assay

Reagents

1. Tris Buffer (0.1M, pH 8.0, 10mM $MgCl_2$)

   Dissolve in 80-90 ml gdwi
   1.21 gm Trizma Base
   0.2 gm $MgCl_2$ $6H_2O$

   Adjust pH to 8.0 with HCl.
   Dilute to 100 ml with gdw.
   Store refrigerated.

2. ATP Solution

   Dissolve in 10 ml gdw:
   62 mg ATP

   Add 3 drops in NaOH.
   Store 0 C.

3. NADP Solution

   Dissolve in 2 ml 0.1% $NaHCO_3$:
   15.4 mg NADP

   Store 0 C.

4. Glucose-6-Phosphate Dehydrogenase

   1.43 mg protein/ml

5. Hexokinase

   0.75 mg protein/ml

Procedure

1. Pipette the following into a cuvette:
   2.0 ml Tris buffer
   0.1 ml ATP
   50 NADP
   0.1 ml deproteinized blood serum
   5 Glucose-6-Phosphate Dehydrogenase

2. Mix thoroughly.

3. Read the OD at 340 m , zeroing the instrument against glass distilled water.

4. Read the OD a second time. If the readings are stable, add: 5 Hexokinase.

5. Mix.

6. After approximately 10 minutes, read the OD several times until readings are stable.

7. For each series of experimental samples, include (in addition to a cuvette containing g. d. w.) a reagent blank, prepared by substituting 0.1 ml g. d. w. for the blood sample.

8. Rinse the cuvettes with dilute HCl, then g. d. w.

Calculations

moles/ml of glucose in the whole blood =

$$\frac{(OD_{340}(\text{sample}) - OD_{340}(\text{blank}) \times 2.26 \times Vol_2 \times 2}{6.22 \times 0.1 \times Vol_1}$$

where:

2.26 = total volume of solution in the cuvette
6.22 = absorbance in OD units of 1 mole/ml DADPH
0.1 = volume of deproteinized, neutralized blood added to the cuvette
Vol_1 = volume of acidic serum obtained
Vol_2 = volume of neutralized serum
2 = ratio of blood + $HClO_4$ to blood collected

Blood Lactate And β-Hydroxybutyrate Assays

Reagents

1. Hydrazine Buffer (pH 8.5)

   Add to 18.5 ml g.d.w.:
   1 ml Hydrazine Hydrate (85% solution O.K.)
   0.5 ml Conc. HCl (10N)

   Check pH
   Make fresh daily

2. Tris Buffer (0.1 M, pH 8.5)

   Dissolve in approximately 80 ml g.d.w.:
   0.01 moles Trizma Base (1.21 gm)

   Adjust pH to 8.5 with HCl
   Dilute to 100 ml with g.d.w.
   Store refrigerated.

3. NAD Solution

   20 mg NAD/2 ml g.d.w.
   Store frozen.

4. Lactate Dehydrogenase

   5 mg/ml

5. β-Hydroxybutyrate Dehydrogenase

   5 mg/ml

Procedures

β-Hydroxybutyrate Assay

1. Pipette the following into a cuvette:

   1.0 ml Hydrazine buffer
   1.0 ml Tris buffer
   0.1 ml NAD
   0.2 ml deproteinized blood serum

2. Mix thoroughly.

3. Read the OD at 340 m , zeroing the instrument against glass distilled water.

4. Read the OD a second time. If the readings are stable, add:

   20 -Hydroxybutyrate Dehydrogenase

5. Mix.

6. Allow the reaction go to completion (negligible OD change per minute).

Lactate Assay

7. Add 10 Lactate Dehydrogenase to the cuvette.

8. Mix.

9. After approximately 40 minutes, read the OD several times until readings are stable.

10. For each series of experimental samples, include (in addition to a cuvette containing g.d.w.) a reagent blank, prepared by substituting 0.2 ml g.d.w. for the blood sample.

11. Rinse the cuvettes with dilute HCl, then g.d.w.

Calculations

moles/ml of substrate in the whole blood =

$$\frac{(OD_{340}(\text{sample}) - OD_{340}(\text{blank}) \times CV \times Vol_2 \times 2}{6.22 \times 0.2 \times Vol_1}$$

where:

CV = total volume of solution in the cuvette:
   β -Hydroxybutyrate -- 2.32
   Lactate -- 2.33
6.22 = absorbance in OD units of 1 umole/ml NADH
0.2 = volume of deproteinized, neutrialized blood added to the cuvette
Vol_1 = volume of acidic serum obtained

Blood Pyruvate, Acetoacetate, and Glycerol Assays

Reagents

1. Potassium Phosphate Buffer (0.1 M, pH 7.0)

   Mix fresh daily:

   39 ml KH_2PO_4 (0.1M)
   61 ml K_2HPO_4 (0.1M)

   Check pH
   Potassium phosphate stock solutions may be prepared and stored at 0.5 C.

2. NADH solution

   2 mg NADH/1 ml g.d.w.

3. Lactate-Dehydrogenase

   5 mg/ml

4. Pyruvate Standard Solution

   18.7 mg Sodium Pyruvate/1 g.d.w.
   Make by serial dilution:
   A. 187 mg/100 ml g.d.w.
   B. 1 ml of A to 1-0 ml with g.d.w.
   Add 1 ml of B to each standard cuvette
   Make fresh daily

   (Theoretical OD change of 0.500 OD units -- sodium pyruvate concentration of 0.17 moles/ml)

5. Pyruvate Kinase (PK)

   10 mg/ml

6. Glycerol Kinase (GK)

   1 mg/ml

7. $MgCl_2$ (0.05 M)

8. Hydroxybutyrate-Dehydrogenase

   5 mg/ml

9. ATP-PEP solution

   $ATPNa_2H_2$ $4H_2O$ 10 mM (62mg/10 ml)

   Dissolve 62 mg in g.d.w. and adjust pH to 7.

   Then add:

   PEP, Tricyclohexylammonium salt -- 43 mg

   Bring volume to 10 ml with g.d.w.

10. Glycerol Standard Solution (0.1 mM)

   A. Dilute 0.73 ml Glycerol (glycerin) to 100 ml with g.d.w.

   B. Dilute 1 ml of A to 100 ml

   C. Dilute 1 ml of B to 10 ml

   Add 1 ml of C to each standard cuvette

   Measure volumes carefully

   A stock solution may be prepared.

Procedures

Pyruvate Assay

1. Pipette the following into a cuvette:

   1.0 ml Phosphate buffer
   1.0 ml deproteinized blood serum
   0.1 ml NADH

2. Mix thoroughly

3. Read the OD at 340 mu, zeroing the instrument against glass distilled water.

4. Read the OD a second time. If the readings are stable, add:
   10 Lactate-Dehydrogenase

5. Mix.

6. Read the OD until readings are again stable.

Acetoacetate Assay

7. To each cuvette, add: 10 of -Hydroxybutyrate Dehydrogenase.

8. Mix

9. Read the OD until readings become stable.

Glycerol Assay

10. Add:

    0.1 ml ATP-PEP solution
    5 PK
    0.1 ml $MgCl_2$
    and if OD readings are low:
    0.1 ml NADH

    (Be certain to make a note of this last addition)

11. Mix

12. Read OD until readings are stable.

13. Add 5 GK

14. Mix

15. Read OD until readings are stable.

16. Rinse the cuvettes with dilute HCl, then g.d.w.

17. For each series of experimental samples, include (in addition to a cuvette containing g.d.w.) a reagent blank, prepared by substituting 1.0 ml g.d.w. for the blood sample.

18. In addition, for each day's work, include two pyruvate standard and two glycerol standard runs. Prepare these cuvettes by substituting 1.0 ml of appropriate standard solution for the blood sample.

Calculations

UMOLES/ml of substrate in the whole blood =

$$\frac{(OD_{340}(\text{sample}) - OD_{340}(\text{blank}) \times CV \times Vol_2 \times 2)}{6.22 \times 1.0 \times Vol_1}$$

where:

6.22 = absorbance in OD units of 1 umole/ml NADH

1.0 = volume of deproteinized, neutralized blood added to the cuvette

Vol_1 = volume of acidic serum obtained

Vol_2 = volume of neutralized serum

2 = ratio of blood + $HClO_4$ to blood collected

OD_{340}(sample) = the appropriate absorbance change in the sample cuvette for the substrate being measured:

- Pyruvate -- the change resulting from addition of -OHbutyrate-DH
- Acetoacetate -- the change following addition of Ohbutyrate-DH
- Glycerol -- the change occuring after the addition of GK

OD_{340}(blank) = the change in absorbance of the reagent blank corresponding to the sample absorbance change.

CV = the total volume of solution in the cuvette:

- Pyruvate -- 2.11 ml
- Acetoacetate -- 2.12 ml
- Glycerol -- 2.33 ml (2.43 ml if additional NADH has been added)

The standard concentrations are calculated using this same equation with the omission of the factors DV, Vol_1, and Vol_2,

APPENDIX B

DETERMINATION OF URINARY NITROGEN BY MICRO-KJELDAHL DIGEST & AUTOMATED PHENOL-HYPOCHLORITE PROCEDURE

From: Fleck, A. (1967)

Reagents

1. Digestin: Concentrated H_2SO_4 (36N) + 7 gm Selenium Dioxide per liter of solution
2. Catalyst Mixture: Anhydrous K_2SO_4 and $CuSO_4$ in 9:1 ratio by weight
3. 0.5% Sodium hypochloride (aqueous)
4. 0.125 gm sodium nitroprusside (sodium ferrinitrosopentacyanide) diluted to 1 liter
5. Sodium Phenate reagent: 38 gm Na_3PO_4 $12H_2O$ + 29 gm NaOH + 60 gm Phenol disolved and diluted to 2 liters
6. Ammonium Sulfate Standards of 2 mg%, 4 mg%, 8 mg%, 10 mg% and 15 mg%

Procedure

1. Obtain specimens of 24 hr urines diluted to 3 liters (if 24 hr urines are over 3 liters the samples are processed pure)
2. Pipet 0.5 of urine specimen into Micro-Kjeldahl flask containing approximately 1 mg. of catalyst mixture
3. Two samples are prepared for each specimen
4. Add 1 ml of Digestin to each flask
5. Heat vigorously for 3 hours.
6. Allow flasks to cool and add water to just below 15 ml mark
7. Allow to cool again and dilute to exactly 15 ml. Vortex each flask thoroughly
8. Place approximately 2 ml of each sample on a single channel Technicon Autoanalyzer and the automated phenol-hypochlorite method for colorimetric determination of ammonia in digest.
9. Use one set of standards for every 36 samples.
10. After programing standard curve into Wang computer, enter Optical Densities to obtain answers (in gm N per day).

APPENDIX C

CREATININE & UREA DETERMINATION

Creatinine from: modification of Folin and Wu (text) "Practical Physiological Chemistry" by Hawk, Osen and Summersin, The Blakiston Co., 12th Ed., page 506.

Urea from: modification of Marsh, W.H. Fingerhut, B. and Miller, H., Clinical Chemistry 11:624, 1965

Reagents

Creatinine

1. Sodium Hydroxide 0.5N
2. Satured Picric Acid

Urea

1. Color Reagent
   a. 67ml 2.5 gm% Diacetyl Monozime
   b. 67ml 0.5 gm% Thiosemicarbazide
   c. dilute to liter
2. Working Acid
   a. 2 ml of Ferric Chloride/Phosphoric Acid
      1) Ferric Chloride 15 gm
      2) Phosphoric Acid (85%)
      3) H_2O (q.) 450 ml.
   b. 2000 ml Sulfuric Acid

Procedure

1. Obtain samples of 24 hr urine diluted to 3 liters (if 24 hr urine over 3 liter samples are processed pure)
2. Place 2 ml of sample in Technicon samples autoanalyzer, using one set of standards for every 38.
3. Autoanalyzer will then run automatically colorimetric determinations of urea N and creatinine, giving results in Optical Density.
4. After programming in standards from Wang computer obtain results in grams urea N and grams creatinine per day.

APPENDIX D

NON-ESTERIFIED FATTY ACIDS

From: Dole and Meinertz (1960)

Reagents

1. Palmitic Acid Standard: (M.W. = 256.4 g/mole)

   19.2 mg palmitic acid diluted to 100 ml. with heptane.
   (0.75 milliequiv. /liter.)

2. Dole's Extraction Mixture

   800 ml isopropyl alcohol
   200 ml heptane
   20 ml 1 Normal H_2SO_4 (conc. H_2SO_4 = 36N= 18M)

3. Thymol Blue Indicator

   Stock Solution:
   0.100gm. is dissolved in 21.5ml. 0.10N NaOH using a mortar and pestle. Transfer to a 100 ml volumetric flask and dilute to 100ml. with Isopropanol. (Dark green color)

   Working Solution:
   Place 5ml. of stock solution in 100ml. volumetric flask and dilute with Isopropanol. Color should be orange-yellow at first and therefore must be adjusted to bright yellow with a few drops of 0.02N NaOH.

4. 0.02 Normal NaOH

5. 0.05% H_2SO_4 (0.6ml. conc. H_2SO_4 to 2 liters with distilled H_2O.

Procedure

1. Pipet 1 ml. of sample with blowout or T.D. volumetric pipet. Then pipet 1 ml. of standardized palmitic acid for each standard and 1 ml. of CO_2-free water for each blank.

2. Add 5 ml. of Dole's Extraction mixture to each tube. Mix on the Vortex Genie. Let stand for 10-30 minutes.

3. To Blank and unknowns add 3 ml. of Heptane and 2 ml. of distilled water. Mix. To standard add 2 ml. of Heptane and 3 ml. of distilled water. Mix.

4. Pipet top (heptane-NEFA) layer into medium size test tubes with Pasteur pipets.

5. Add 2 ml. of 0.05% H_2SO_4. Mix. Then centrifuge for 2 min. at 2000 RPM.

6. Transfer 3 ml. aliquots of upper phase to the small test tubes. Then add 1 ml. of indicator.

7. Titrate with N_2 bubbling through the solution, to a pale yellow end point. (For titrant, NaOH, add 0.15 ml. stock solution into erleyer flask 3/4 filled with CO_2-free water.

APPENDIX E

DOUBLE IMMUNOASSAY FOR INSULIN

By: Soeldner (1965)

Materials*

1. Buffer: 1% BSA Borate buffer (Shelf life - 1 week at 4 C)

   A. For 1 liter borate buffer, pH 8.5

      1 l H_2O
      8.25 gm boric acid
      2.70 gm NaOH
      3 ml 12N HCl
      Shelf life: indefinite at 4 C

   B. Bovine Standard Albumin should be added to make 1% solution on day of assay.

*(Unless otherwise noted, all further dilutions should be made in 1% BSA Borate buffer)

2. Insulin standards of 0, 5, 10, 20, 50, 100 & 150 uU/ml should be prepared.

   Approximately 5 mg of Schwartz-Mann recrystalized porcine insulin (Cat. no. 7518, 100 mg for $8.00) at 25.9 U/ml should be weighed and diluted to a stock of 150 uU/ml (If any insulin solution is cloudy, 1 or 2 drops of 1N HCl should be added to clear the solution). Stock insulin standards have a shelf life of roughly 1 week at 4 or several months at -20 .

3. Insulin in plasma samples is unstable with changing temperature. Accordingly, repeated freezing and thawing of plasma samples should be avoided.

4. I^{125} - Insulin
   I^{125} - Insulin stock or roughly 50 uU/ml (.1 ml/sample @ 5 uU/sample) should be prepared. Labeled insulin should be divided into daily aliquots and frozen upon arrival. (e.g., 10 ml of 775 uU/ml I^{125} - Insulin from the Joslin Clinic was divided into 20 .5 ml aliquots each sufficient for .5 ml x 775 uU/ml 5 uU/sample = 77 samples)
   For each assay, a frozen aliquot should be thawed and diluted to 50 uU/ml.

5. Anti-Insulin Serum of 1:7500 dilution should be prepared for each assay. 1 ml of Pentex anti-porcine insulin serum (Cat. no. GPH 400, $40.00) should be diluted with redistilled water to 10 ml upon arrival, after which 100 .1 ml aliquots should be divided and frozen for daily use. For each assay, the .1 ml aliquot of 1/10 dilution should be further diluted to 1:7500.

6. Normal Guinea Pig Serum of 1:100 dilution should be prepared for each assay. For each assay .1 ml of Pentex Normal guinea pig serum (Cat. no. P10315, 15 ml for $10.00) should be diluted to 10 ml.

7. Anti-Guinea Pig Serum of 1/4 dilution should be prepared for each assay.
Pentex Anti-guinea pig gamma globulin (Cat. no. RM508, 5 ml lyophilized for $20.00) should be reconstituted and sufficient serum should be diluted to 1/4 for daily use.

Procedure

1. Reaction 1: Insulin + Anti-Insulin Anti-Insulin Complex.
In an ice bath the following reagents should be combined
   a. .6 ml Buffer
   b. .2 ml Insulin standard or sample
   c. .1 ml 50 uU/ml I^{125} - Insulin (i.e. 5 uU/sample)
   d. .1 ml 1:7500 Anti-Insulin Serum

   The reaction mixture should be turned at room temperature for 2 hours and then overnight in the cold room.

2. Reaction 2: Anti-Insulin Complex + Anti-Guinea Pig Serum Precipitable Complex
After a short (1 min.) centrifugation to remove any liquid from the test tube caps, the following reagents should be added to the reaction mixture:
   a. .1 ml 1:100 Normal Guinea Pig serum
   b. .1 ml 1/4 Anti-Guinea Pig Serum

   The mixture should be turned for 1 hour at room temperature followed by at least 48 hours (preferrably 72 hours) in the cold room.

3. Precipitation and Separation: After another short centrifugation to remove liquid from the test tube caps, the test tubes should be opened and then centrifuged (Sorvall, 4000 rpm) for 30 minutes. A visible precipitate should form. The supernatant should be carefully decanted into another test tube and both parts counted in a gamma scintillation spectro meter, allowing a 50 CPM background for I^{125}.

REPRESENTATIVE SIGNIFICANCE TEST ON TEN PATIENTS

(By Least Significant Difference[1])

	$\bar{X}_{D5W} - \bar{X}_{D5W + AA}$	$\bar{X}_{D5W} - \bar{X}_{AA}$	$\bar{X}_{D5W + AA} - \bar{X}_{AA}$
Insulin (uU/ml)	9.39	15.35**	5.96
Glucose (mM/ml)	.005	2.045**	2.04**
Free Fatty Acids (mEq/ml)	-.161	-.640**	-.478**
Ketone Bodies (mM/ml)	-.045	-.885**	-.930**
Nitrogen Balance (g/day)	-4.09*	-7.675**	-3.885*
Cumulative Nitrogen Loss (gm)	-13.4*	-31.0**	-17.6**

[1]C.W. Dunnet. J. American Stat. Assoc., 50:1955.

*p .05

**p .01

Calculations

For each sample:

Bound = CPM precipitate

Free = CPM supernatant

Total = Bound + Free

Bound/Total ratio = CPM pre/ (CPM sup + CPM pre)

A standard curve should be prepared by plotting B/T ratio on the ordinate and log (Ins) on the abscissa. For each unknown, insulin values can be read off the standard curve.

APPENDIX F

SUMMARY OF METABOLIC DATA ON TEN PATIENTS

(Mean ± S.D.)[1]

	D5W	D5W + AA	AA
Insulin (mU/ml)	42.04 (±21.61)	32.66 (±17.38)	26.7 (± 12.51)
Glucose (mM/ml)	7.10 (± 2.50)	7.09 (± 1.93)	5.05(± 5.05)
Free Fatty Acids (mEq/ml)	.420(± .19)	.58 (± .39)	1.06(± 4.63)
Ketone Bodies (mM/ml)	.105(± .10)	.15 (± .09)	1.04(± .65)
Nitrogen Balance (g/day)	-7.15 (± 3.19)	-3.06 (± 4.74)	.53(± 2.60)
Cumulative Nitrogen Loss (gm)	-28.4 (±11.19)	-15.0 (±18.65)	2.6 (± 7.12)

[1]Paired t-tests of 48 hour and 96 hour samples within treatment groups were all insignificant except for the following cases:

on D5W: rise in glucose at 96 hours over 48 hours,

on D5W & AA: decline in ketones at 96 hours over 48 hours,

on AA: rise in ketones at 96 hours over 48 hours.

APPENDIX G

CALCULATION OF INTRAVENOUS NITROGEN AND DEXTROSE INPUT

Daily records of input and output are kept for patients receiving intravenous feedings. From these records total daily input can be calculated.

NAME	gm glc	cal glc	gm N	gm Prot	cal Prot	Total Cal
Protein Sparing Solutions						
Amigen, 5%			6.4	40.	170	170
Freamine, 3%			4.8	30.	120	120
$Freamine_E$, 3%			3.6	30.	120	120
Dextrose 5%	45	166.5				166.5

Calories per gram		Nitrogen content
glucose	3.75	
		6.65 g FA amino acids contains 1gN
amino acids	3.9	8.85 g FA_E amino acids contains 1gN

APPENDIX H

INTRAVENOUS PROTEIN SPARING THERAPIES - GROUP II

	HCT %	WBC x103	Temp F^{o}	BUN mg%	Creat mg%	SGOT I-U	Bili mg%	LDH I.U.	ALK-P Body Unit
K.A.	31-50	8-17.9	97-100	17-52	1.3-1.4	70-90	.5-6.5	275-350	60-75
J.B.	36-42	12	99-103	8-21	1.5	70-120	.8-1.6	135-175	60-85
R.B.	7-20	7.5-23		20-27	1.0-1.1	26-85	.7-2.3	315-415	115-375
W.C.	39-40		98.6-100	7-16	1.2	148	5.3	215	120
A.D.	36-41	13.6	100-102	2-13	0.5-0.7	16-50	0.8-2.7	173-215	70-283
T.G.	38-40	8-9	98-101	40-60	2.2-2.6	68-168	0.6-0.8	295	240
W.M.	26-38	14	99-103	4-13	0.8-1.5	65-74	0.6	127-225	60-76
J.P.	30-41	13.0	98.6-101	7-11	0.4-4.6	23-94	0.6-1.1	126-170	46-102
A.V.	31-41		98.6-101	21-30	0.7-2.0	55-65	0.6-0.9	125-260	75-100

INTRAVENOUS PROTEIN SPARING THERAPIES - GROUP I

	% HCT	x103 WBC	°F Temp	mg% BUN	mg% Creat.	I-U SGOT	mg% Bili	I.U. LDH	Body Unit ALK-P
JS	30-38	10	98 -101	18	.9	150-190	0.7-1.7	135	60-82
LM	31-34	8-10	98.6-101	4-19	1.0	34-38	0.3-0.7	185-200	75-90
FC	31-39	5-11	98.6-101	25-46	.9-1.4	86-93	3.1-5.6	250	105
CW	34-37		100 -103	28-47	1.8-2.4	650-3600	1.7-8.3	400-500	172-216
IC	27-34								
JY	25-35	9-13.5	100 -103.5	15-40	0.8-1.0	62-75	2.4-6.3	360	50-90
BH	34-35		98.6-100	3-12	0.9	60-150	0.6		250
RW	22-28	8-18	99 -103	24	2.1-1.3	32	1.0	20	190-210
LS	39-44	4-5	98 -100	5-23	0.8-1.2	130-180	0.6-0.6	130-150	226-450
SW	28-32	3-6	97 -99	6-15	0.8-1.0	60-80	0.6-0.8	130-140	75-90

Starvation + P.S. After 21 Days

Patient	Age/Sex	Wt (kg)	Hgt	Surface Area (M²)	Associated Condition	Medication	Diet g prot/kg/day	Wt Loss (kg)/M²	Fat Loss (kg)/M²	LBM Loss (kg)/M²
J.C.	35 y.o. M	108	71.5"	2.1	—	—	0	6.2	3.4	2.8
J.T.	M	112	70"	2.2	—	—	0	6.1	3.1	3.0
M.R.	38 y.o. F	93	66"	1.9	—	—	0	5.3	1.9	3.4
C.C.	18 y.o. F	116	62"	2.0	Prader-Willi Syndrome	Anivar	0.5	4.0	3.4	0.6
J.F.	20 y.o. M	115	71"	2.2	—	—	0.5	5.0	3.7	1.3
G.D.	51 y.o. F	98	67"	2.0	—		0.6	2.0	1.6	0.4
N.J.	55 y.o. M	78	67.5"	2.0	[illegible] abnormality	—	0.6	2.6	2.0	0.6
L.R.	69 y.o. F	90	64"	1.9	depleted hypothyroidism hypercholesterolemia	thyroid	0.6	3.7	4.0	-0.3
B.M.	32 y.o. F	84	65"	1.8	depression	—	0.65	2.9	1.4	1.5
C.J.	31 y.o. M	165	70"	2.6	—	—	0.8	3.8	4.4	-0.6
M.S.	22 y.o. F	74	64"	1.7	hypercholesterolemia type II	—	0.8	2.6	2.1	0.5
N.W.	60 y.o. M	148	69"	2.5	—		0.8	2.3	1.7	0.6
K.D.	20 y.o. F	127	65.5"	2.2	—	—	0.8	1.9	2.2	-0.3
M.F.	47 y.o. M	112	70.5"	2.2	—	—	1.0	4.1	3.4	-0.7
E.S.	47 y.o. F	152	70"	2.5	diabetes [illegible]	insulin	1.0	5.2	7.6	-2.4

Starvation

	Mean	± SD	SE	n = 3
Wt Loss/M²	5.9	0.39	0.23	
Fat Loss/M²	2.8	0.65	0.46	
LBM Loss/M²	3.1	0.65	0.46	

P.S. 0.5 – 0.65 g/kg/day

	Mean	SD	SE	n = 6
Wt Loss/M²	3.4	1.00	0.45	
Fat Loss/M²	2.7	1.05	0.47	
LBM Loss/M²	0.7	0.60	0.27	

P.S. 0.8 – 1.0 g/kg/day

	Mean	SD	SE	n = 6
Wt Loss/M²	3.0	1.21	0.40	
Fat Loss/M²	2.8	0.98	0.49	
LBM Loss/M²	1.2	0.47	0.23	

Range after 3 days on diet

Patient	Days on Diet	Wt Loss (lbs)	BP Systolic / Diastolic	Glucose (mg%)	Na / K	Ca / Mg	CO_2 / Cl	Phos	Hct / Hbg	WBC	Alb. / T.P.	SGOT / LDH	Uric Acid	BUN	Ser. Creat.	Cl	T.G.	Ins	KB	FFA
J.C.	38	20	112-110 / 80-70	80-40	145-136 / 4.7-3.6	8.9-8.3 / —	22-18 / 102-90	—	46-34 / 15.0-11.5	6200-4200	4.4-3.2 / 8.1-6.5	— / —	23.0-8.0	8-4	1.4-1.2	268-180	100-73	7-4	7.9-6.1	3.8-2.6
J.T.	27	14	145-120 / 100-90	85-70	142-139 / 4.1-3.2	10.2-10.0 / —	24-22 / 103-97	3.8-2.2	/ 15.8-14.4	6400-4200	4.7-3.5 / 8.1-6.5	43-27 / 89-74	14.3-6.6	11-7	1.3-1.2	213-183	113-109	19-11	6.4-3.3	1.7-1.0
M.R.	26	11	130-90 / 85-60	103-80	144-185 / 4.6-4.6	9.4-8.9 / —	22-15 / 105-103	4.0-3.5	41-35 / 13.2-12.0	9600-6800	4.0-3.9 / 7.0-6.6	— / —	14.0-12.8	9-6	1.0	258-203	164-120	18-12	6.4-3.9	1.7-1.6
C.C.	182	31	135-95 / 84-60	90-54	148-135 / 4.6-3.7	10.2-8.7 / 1.7-1.5	27-20 / 104-94	4.3	48-38 /	6400-4800	4.4-2.8 / 8.2-6.7	32-16 / —	14.0-8.8	16-7	1.6-0.9	214-137	112-71	14-5	5.8-2.5	1.4-1.0
J.F.	21	10	100 / 70-60	95-68	141 / 5.0-4.2	— / —	28-24 / 100-97	—	47 / 15.2	—	5.0-4.7 / 6.8-6.4	21 / —	12.0-11.8	14-12	1.6-1.5	192-149	158-134	16-7	5.0-2.0	1.5-0.4
G.D.	21	4	124-90 / 80-70	73-65	146-139 / 5.4-4.4	10.0-9.6 / —	28-25 / 105-98	4.9-4.6	41-35 / 13.8-11.6	8260-3660	4.8-3.9 / 7.9-7.0	27-17 / 106-102	9.1-7.5	18-11	1.8-0.8	220-181	225-187	11-2	2.0-1.3	1.7-0.8
N.J.	21	5	150-110 / 80-70	88-56	140-136 / 4.2-3.7	10.4-10.0 / —	29-26 / 98-94	4.7-4.3	46-45 / 15.0-14.4	7700-4500	4.5-4.2 / 7.9-6.5	22-17 / —	8.8-7.6	12-10	1.0-0.8	214-195	134-107	5-3	2.4-0.5	1.8-0.5
L.R.	29	8	150-120 / 78-60	75-61	144-140 / 4.6-3.3	10.0-8.4 /	26-20 / 100-97	3.1	35-31 / 11.4-10.3	7900-5400	4.4-3.7 / 7.6-6.4	25-22 / —	9.7-5.8	21-17	1.5-0.7	488-274	287-210	18-7	4.8-2.2	1.4-0.8
B.M.	87	11	118-108 / 70	102-95	145-140 / 4.5-2.9	9.6 / —	27-26 / 100	2.3	44-41 / 14.8-13.4	6500-5800	4.7-4.5 / 7.7-7.1	26-18 /	6.9-5.2	9-7	0.9-0.8	278-187	108-72	80-3	1.6-0	1.6-1.0
C.J.	38	15	170-115 / 110-60	103-87	141-129 / 4.4-4.2	— / 1.6-1.5	26-24 / 105-101	—	42-41	5000-3800	4.7-2.9 / 7.6-6.8	50-38 / —	13.1-12.5	12-7.0	1.3-1.2	288-278	175-129			
M.S.	21	4	90-80 / 60-40	64-56	143-141 / 4.7-3.7	— / —	29-26 / 104-96	—	39	5600	5.2-4.6 / 8.1-6.6	43-31 /	15.9-12.7	17-14	1.4-1.2	390-282	151-106	10-9	4.6-1.1	1.2-0.6
N.W.	60	23	150-110 / 96-70	146-86	146-137 / 5.2-2.0	— / 1.97	31-24 / 102-99	—	48-42	9900-4500	4.6-3.7 / 7.8-6.2	47-17 / —	12.9-6.6	19-13	1.2-1.0	226-126	213-132	18-4	2.5-0.8	1.5-0.8
K.D.	70	18	150-120 / 88-55	81-74	145-140 / 4.4-3.6	— / 1.6-1.5	25-21 / 105-100	—	41-35	5600-3750	4.4-3.6 / 7.7-6.5	43-15 / —	11.1-5.6	23-10	1.0-0.8	157-113	96-57	20-2		
M.F	30	12	150-120 / 95-60	97-76	149-146 / 5.4-4.5	— / —	27-22 / 105-98	—	48-43	9200-6700	5.5-4.9 / 8.2-6.9	42-17 / —	12.0-9.8	17-13	1.3-1.2	327-227	195-130		1.7-0.5	
E.S.	72	27	170-130 / 100-70	201-86	144-139 / 4.7-3.5	9.8 / 1.62-1.58	29-22 / 104-94	4.4-2.7	45-29	10,200-6100	4.2-3.3 / 7.6-6.5	47-7 / —	13.1-9.2	20-11	1.1-0.8	238-141	258-150	99-5	5.0-1.6	

Ranges after 3 days on diet

Patient	Days on Diet	Wt. Loss (kg)	B.P. Systolic / Diastolic	Glucose (mg%)	Na / K	Ca / Mg	CO_2 / Cl	Phos	Hct / Hbg	WBC
J.C.	38	20	112-110 / 80-70	80-40	145-136 / 4.2-3.6	8.9-8.3 / —	22-18 / 103-90	—	46-34 / 15.0-11.5	6200-4[illegible]
J.T.	27	14	145-120 / 100-70	85-70	142-139 / 4.1-3.2	10.2-10.6 / —	24-22 / 103-97	3.8-3.2	/ 15.8-14.[illegible]	6[illegible]00-48[illegible]
M.R.	26	11	130-90 / 85-60	103-80	144-135 / 4.6-4.0	9.4-8.9 / —	22-15 / 105-103	4.0-3.5	41-35 / 13.2-12.[illegible]	9[illegible]00-68[illegible]
C.C.	182	31	135-95 / 80-60	90-64	148-135 / 4.6-3.7	10.2-9.7 / 1.7-1.5	27-20 / 104-94	4.3	43-35	6400-4[illegible]
J.F.	21	10	100 / 70-60	95-68	141 / 5.0-4.2	— / —	28-24 / 100-97	—	47 / 15.2	—
G.D.	21	4	124-90 / 80-70	73-65	146-139 / 5.4-4.4	10.0-9.6 / —	28-25 / 103-98	4.9-4.6	41-35 / 13.8-11.[illegible]	8[illegible]50-36[illegible]
N.J.	21	5	150-110 / 80-70	88-56	140-136 / 4.9-3.7	10.4-10.0 / —	29-26 / 98-94	4.7-4.3	46-45 / 15.0-14.[illegible]	7900-46[illegible]
L.R.	27	8	150-120 / 70-60	75-61	144-140 / 4.6-3.3	10.0-8.9	26-20 / 100-97	3.1	35-31 / 11.4-10.[illegible]	7900-59[illegible]
B.M.	87	11	118-108 / 70	108-95	145-140 / 4.5-3.9	9.6 / —	27-26 / 100	2.3	44-41 / 14.8-15.4	6[illegible]00-58[illegible]
C.J.	38	15	170-116 / 110-60	103-87	141-139 / 4.4-4.2	— / 1.6-1.5	26-24 / 105-101	—	42-41	5[illegible]00-3[illegible]
M.S.	21	4	90-80 / 60-40	64-56	143-141 / 4.7-3.7	— / —	29-25 / 104-96	—	39	65[illegible]0
N.W.	60	23	150-110 / 96-70	146-86	146-137 / 5.2-2.0	— / 1.97	31-24 / 102-99	—	48-42	9[illegible]-45[illegible]
K.D.	70	18	150-120 / 80-55	81-74	143-140 / 4.4-3.6	— / 1.6-1.5	25-21 / 105-100	—	41-36	56[illegible]-39
M.F.	30	12	150-120 / 95-60	99-76	147-140 / 5.4-4.5	— / —	27-22 / 105-98	—	48-43	9[illegible]-4[illegible]
E.S.	73	27	170-130 / 100-70	201-86	144-139 / 4.7-3.5	9.8 / 1.59-1.58	29-22 / 104-94	4.4-2.9	45-39	10,200-6[illegible]

days on diet

K	Ca / Mg	CO_2 / Cl	Phos	Hct / Hbc	WBC	Alb. / T.P.	SGOT / LDH	Uric Acid	BUN	Ser. Creat.	Chol	T.
3.6 / 2-3.6	8.9-8.8 / —	22-18 / 103-90	—	46-34 / 15.0-11.5	6200-4800	4.4-3.2 / 8.1-6.5	— / —	23.0-8.0	8-4	1.4-1.2	268-180	1
3.9 / 1-3.2	10.2-10.0 / —	24-22 / 103-97	3.8-3.2	/ 15.8-14.6	6400-4800	4.7-3.5 / 8.1-6.5	43-27 / 87-74	14.8-6.6	11-7	1.3-1.2	213-183	11
3.5 / 6-4.0	9.4-8.9 / —	22-15 / 105-103	4.0-3.5	41-35 / 13.2-12.0	9600-6800	4.0-3.7 / 7.0-6.6	— / —	14.0-12.8	9-6	1.0	258-203	16
3.5 / 3.7	10.2-9.7 / 1.7-1.5	27-20 / 104-94	4.3	43-35	6400-4800	4.4-2.8 / 8.9-6.7	32-16 / —	14.0-5.8	15-7	1.0-0.9	214-137	11
-4.2	— / —	28-24 / 100-97	—	47 / 15.2	—	5.0-4.7 / 6.8-6.4	21 / —	12.0-11.8	14-12	1.6-1.5	192-147	15
3.9 / 4.4	10.0-9.6 / —	28-25 / 103-98	-4.6	41-35 / 13.8-11.6	8250-3	4.3-3.9 / 7.9-7.0	27-17 / 106-102	9.1-7.5	18-11	1.3-0.8	220-181	22
3.6 / 3.7	10.4-10.0 / —	29-26 / 98-94	4.7-4.3	46-45 / 15.0-14.4	7700-4800	4.5-4.2 / 7.9-6.5	22-17 / —	8.8-7.6	12-10	1.0-0.8	214-195	18
4.0 / 3.3	10.0-8.9	26-20 / 100-97	3.1	35-31 / 11.4-10.3	7900-5800	4.4-3.7 / 7.6-6.6	25-22 / —	9.7-5.8	21-17	1.5-0.7	488-274	2
4.0 / 3.5	9.6 / —	27-26 / 100	2.3	44-41 / 14.8-13.4	6500-5800	4.7-4.5 / 7.7-7.1	26-18	6.9-5.2	9-7	0.9-0.8	278-189	10
3.9 / 4.2	— / 1.6-1.5	26-24 / 105-101	—	42-41	5000-3000	4.7-3.9 / 7.6-6.8	50-38 / —	13.1-12.5	12-10	1.3-1.2	288-228	17
4.1 / 2.7	— / —	29-25 / 104-96	—	39	5500	5.2-4.6 / 8.4-6.6	43-31 / —	15.9-12.7	17-14	1.4-1.2	390-282	15
3.7 / 2.0	— / 1.97	31-24 / 102-99	—	48-42	7900-4500	4.6-3.7 / 7.8-6.2	47-17 / —	12.9-6.6	19-13	1.3-1.0	226-126	2
3.6 / 3.6	— / 1.6-1.5	25-21 / 105-100	—	41-35	5600-3750	4.4-3.6 / 7.7-6.5	43-15 / —	11.1-5.6	23-10	1.0-0.8	157-113	9
4.5 / 4.5	— / —	27-22 / 105-98	—	48-43	9200-6900	5.5-4.9 / 8.2-6.9	42-19 / —	12.0-9.8	17-13	1.3-1.2	327-227	19
3.9 / 3.6	9.8 / 1.59-1.58	29-22 / 104-94	4.4-2.9	45-39	10,200-6100	4.2-3.3 / 7.6-5.9	47-7 / —	13.1-9.2	20-11	1.1-0.8	238-141	25

BC	Alb. T.P.	SGOT LDH	Uric Acid	BUN	Ser. Creat.	Chol	T.G.	Ins	KB	FFA
00-4800	4.4-3.2 8.1-6.5	— —	23.0-8.0	8-4	1.4-1.2	268-180	100-73	7-4	7.9-6.1	3.8-2.6
00-4800	4.7-3.5 8.1-6.5	43-27 87-74	14.3-6.6	11-7	1.3-1.2	213-183	113-109	19-11	6.4-3.3	1.7-1.0
0-4800	4.0-3.7 7.0-6.6	— —	14.0-12.8	9-6	1.0	258-203	164-120	18-12	5.4-3.9	1.7-1.6
-4300	4.4-2.8 8.9-6.7	32-16 —	14.0-5.8	16-7	1.0-0.9	214-137	112-71	14-5	5.8-2.5	1.4-1.0
	5.6-4.7 6.8-6.4	21 —	12.0-11.8	14-12	1.6-1.5	192-147	156-124	16-7	5.0-2.0	1.5-0.4
0-3600	4.3-3.9 7.9-7.0	27-17 106-102	9.1-7.5	18-11	1.3-0.8	220-181	226-167	11-2	2.0-1.3	1.7-0.8
0-4500	4.5-4.2 7.9-6.5	22-17 —	8.8-7.6	12-10	1.0-0.8	214-195	184-107	5-3	2.4-0.5	1.8-0.5
-5500	4.4-3.7 7.6-6.6	25-22 —	9.7-5.8	21-17	1.5-0.7	488-274	287-210	18-9	4.5-2.2	1.4-0.8
0-5500	4.7-4.5 7.7-7.1	26-18	6.9-5.2	9-7	0.9-0.8	278-189	108-72	30-5	1.6-0	1.6-1.0
-3000	4.7-3.9 7.6-6.8	50-38 —	13.1-12.5	12-10	1.3-1.2	288-228	175-129			1 . 0
00	5.2-4.6 8.4-6.6	43-31 —	15.9-12.7	17-14	1.4-1.2	390-282	151-106	10-9	4.6-1.1	1.2-0.6
-4500	4.6-3.7 7.8-6.2	47-17 —	12.9-6.6	19-13	1.3-1.0	226-126	213-132	18-4	2.5-0.8	1.5-0.8
-3900	4.4-3.6 7.7-6.5	43-15 —	11.1-5.6	23-10	1.0-0.8	157-113	95-57	20-2		
-6900	5.5-4.9 8.2-6.9	42-19 —	12.0-9.8	17-13	1.3-1.2	327-227	195-130		1.7-0.5	
-6100	4.2-3.3 7.6-5.9	47-7 —	13.1-9.2	20-11	1.1-0.8	238-141	258-150	97-5	5.0-1.5	

Starvation + P.S. After 21 Days

Patient	Age/Sex	Wt (kg)	Hgt	Surface Area (M^2)	Associated Condition	Medication	Diet g prot/kg/day	Wt Loss/ (kg) / M^2	Fat Loss/ (kg) / M^2	LBM (kg
J.C.	35 y.o. M	108	71.5"	2.1	—	—	0	6.2	3.4	2.
J.T.	M	112	70"	2.2	—	—	0	6.1	3.1	3.
M.R.	38 y.o. F	93	66"	1.9	—	—	0	5.3	1.9	3.
C.C.	18 y.o. F	116	62"	2.0	Prader-Willi Syndrome	Anivar	0.5	4.0	3.4	0
J.F.	20 y.o. M	115	71"	2.2	—	—	0.5	5.0	3.7	1.
G.D.	51 y.o. F	98	69"	2.0	—	—	0.6	2.0	1.6	0
N.J.	55 y.o. M	98	67.5	2.0	Sella turcica abnormality	—	0.6	2.6	2.0	0.
L.R.	69 y.o. F	90	64"	1.9	depleted hypothyroidism hypercholesterolemia	thyroid	0.6	3.7	4.0	-0
B.M.	32 y.o. F	84	65"	1.8	depression	—	0.65	2.9	1.4	1.
C.J.	31 y.o. M	165	70"	2.6	—	—	0.8	3.8	4.4	-0.
M.S.	22 y.o. F	71	64"	1.7	hypercholesterolemia type II	—	0.8	2.6	2.1	0.
N.W.	60 y.o. M	148	69"	2.5	—	—	0.8	2.3	1.7	0.
K.D.	20 y.o. F	127	65.5"	2.2	—	—	0.8	1.9	2.2	-0.
M.F.	47 y.o. M	112	70.5"	2.2	—	—	1.0	4.1	3.4	-0
E.S.	47 y.o. F	152	70"	2.5	diabetes hypertension	insulin	1.0	5.2	7.6	-2.

Starvation

Wt Loss/M² (kg)	Fat Loss (kg)/M²	LBM loss (kg)/M²
6.2	3.4	2.8
6.1	3.1	3.0
5.3	1.9	3.4
4.0	3.4	0.6
5.0	3.7	1.3
2.0	1.6	0.4
2.6	2.0	0.6
3.7	4.0	-0.3
2.9	1.4	1.5
3.8	4.4	-0.6
2.6	2.1	0.5
2.3	1.7	0.6
1.9	2.2	-0.3
4.1	3.4	-0.7
5.2	7.6	-2.4

	Mean	± SD	± SE	n = 3
Wt loss/M²	5.9	0.39	0.28	
Fat loss/M²	2.8	0.65	0.46	
LBM Loss/M²	3.1	0.65	0.46	

	Mean	SD	SE	n=6
	P.S.	0.5 - 0.65 g/kg/day		
Wt loss/M²	3.4	1.00	0.45	
Fat loss/M²	2.7	1.05	0.47	
LBM loss/M²	0.7	0.60	0.27	

	Mean	SD	SE	n=6
	P.S.	0.8 - 1.0 g/kg/day		
Wt loss/M²	3.0	0.81	0.40	
Fat loss/M²	2.8	0.98	0.49	
LBM loss/M²	0.2	0.47	0.23	