Clinical Trials for the Treatment of Secondary Wasting and Cachexia

Nutritional and Metabolic Endpoints[1,2]

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ABSTRACT None of the metabolic indicators which have been used to date provides a single or necessarily ideal endpoint for interventional management in wasting disorders. Some of these indicators may provide better endpoints for the acute rather than the chronic wasting conditions. In addition, it is imperative that more than one endpoint be selected to be assured that there is concordance in the findings. However, prior to the selection of any endpoint measure, the investigators involved must be fully cognizant of the potential pitfalls and errors that can occur in every one of the selected methodologies. In anticipating these potential problems, developing strategies for the interpretation of the data is critical at the outset of any interventional management strategy. The manufacturers, the regulators and the investigators involved in the interventional management of chronic and acute wasting disorders must agree on the endpoints to be used and these endpoints must provide the most appropriate and valid information. Selection of nutritional and metabolic endpoints must be in part dependent on the disease process involved, the potential magnitude of the interventional effect and must be utilized in the context of a carefully designed experimental protocol with a well focused question(s). J. Nutr. 129: 273S–278S, 1999.

KEY WORDS: • *wasting* • *cachexia* • *metabolic indicators*

Protein wasting and malnutrition are a frequent consequence of a number of disease processes which health care providers manage on a daily basis. Appropriate management of these patients is imperative if we are going to improve the morbidity and mortality as well as their quality of life. During periods of catabolic illness, endogenous nutrient substrates are mobilized resulting in the loss of body fat and depletion of glycogen pools. In contrast to triglycerides and glycogen, no significant pool of free amino acids exist within body tissues to function as a potential reservoir for essential amino acids and nitrogen to sustain an individual through a period of severe and/or protracted illness. All proteins in mammalian species exist as either structural or functional proteins. Profound loss of body protein is incompatible with life. Acute illnesses, such as severe injuries resulting from a motor vehicle accident, large surface areas burns or superimposed sepsis and/or surgical trauma on the backdrop of an underlying illness, result in a rapid depletion of body protein. If acute losses exceed 30% of total body protein or chronic losses, 50% , it is highly unlikely that the individual will survive.

The ultimate goal in identifying nutritional metabolic endpoints is to be able to provide therapies or interventional strategies which will decrease the mortality and morbidity of a given disease process, as well as, to improve the patient's quality of life, decrease the length of institutional stays and thus, reduce costs. In the current environment of health care reform, any of these parameters which move in a positive direction may ultimately be an appropriate endpoint. As a result, all of our medical therapies and management in such catabolic conditions, from a nutritional and outcome standpoint, should be directed first, at preserving or restoring body protein, and secondly, at restoring body energy stores in the forms of fat and carbohydrate. This will hopefully provide the individual with the best opportunity to survive, presuming that their underlying condition can be treated.

The focus of this manuscript is to identify potential metabolic endpoints, which might be utilized in the design of drug or therapeutic interventional trials of individuals with wasting conditions to provide meaningful and significant indications of success or failure of any interventions. This, potentially, involves a large body of literature which reports the metabolic

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TABLE 1

Nutritional and metabolic endpoints considered

Linear Growth in Children
Immunocompetency
Circulating Proteins
Nitrogen Balance Studies
Amino Acid Kinetics
Energy Expenditure
Study Design

indicators of malnutrition as they relate to individuals suffering from classical nutritional deficiencies of protein alone (kwashiorkor) or protein and calories (marasmus). Over the last twenty years, a significant body of literature has developed largely due to the efforts of a number of pioneer investigators in the area of hospital nutrition and nutritional support (Bistrian 1977, Blackburn and Thornton 1979). Prior to 1980, most patients with underlying wasting diseases were supported with infusions of fluids, electrolytes and carbohydrates (Bistrian 1977, Blackburn and Thornton 1979, Harvey et al. 1981). Only with the advent of total parenteral nutrition did issues of availability of amino acid, amino acid nitrogen and essential fatty acids become a practical reality of acute care management of patients with acute catabolic illnesses (Bernstein 1995).

With the development of numerous new therapies ranging from new categories of pharmaceutical agents to recombinant hormones to organ transplantation, the ultimate morbidity and mortality of a number of disease entities has dramatically changed. In the management of these conditions, it is, however, important to identify appropriate metabolic and nutritional endpoints upon which pharmaceutical companies, regulatory agencies and health-care providers can judge relative success or failures of new interventional strategies. Aside from body composition which has already been discussed, a number of metabolic endpoint candidates will be considered (Table 1). It should be pointed out that several comprehensive reviews of a number of these and other nutritional and metabolic endpoint measures have been recently published and they are recommended to the interested reader (Manning and Shenkin 1995, Shenkin et al. 1995).

No single metabolic or nutritional endpoint exists which will be universally applicable under the wide spectrum of wasting disorders which should be considered for interventional management. For example, the course of management of a small cell carcinoma of the lung, a highly aggressive neoplasm, can hardly be compared with the long-term chronic debilitating condition of growth hormone deficiency of the adult which is associated with less significant muscle wasting and osteopenia (De Boer et al. 1995). In addition, some neoplasms of the apparent same type are highly invasive and rapidly progressive, while others are far more indolent and of significantly less health risk. In addition, the superimposition of additional risk factors in the course of disease management will most likely significantly alter morbidity and mortality, e.g., surgical intervention, infection, septicemia, etc. The endpoint measures might be approached differently, if a specific intervention is intended to preserve body protein and nutrient stores as opposed to restoring them (i.e., preventive versus salvage and restoration). And finally, the endpoint(s) to be selected in part depend upon the anticipated changes based on the relative potency or effect of the interventional therapy. As a result, it is easy to see that no single metabolic or nutritional endpoint will be appropriate under all circumstances. Great care must be taken to define clearly meaningful and appropriate endpoint measures for the population being investigated in the development of any interventional strategy.

LINEAR GROWTH IN CHILDREN

This discussion will not deal with changes in body composition as they have already been presented. However, linear growth in children is a complex, dynamic, anabolic process that clearly involves energy and accretion of protein, calcium, and other nutrients. This is perhaps one of the more sensitive indicators of nutritional and metabolic health in children and one which is frequently disturbed by underlying chronic medical conditions ranging from bronchopulmonary dysplasia, cyanotic congenital heart disease, AIDS, renal failure and cancer to inflammatory bowel disease and rheumatoid arthritis. In addition, interventional managements themselves may alter linear growth in children. For example, children, who because of their underlying illness are required to take high doses of glucocorticosteroids have decreased growth velocities, delayed bone age, osteopenia and relative short stature. Interventional management has been explored in some of these conditions using recombinant growth hormone (rhGH), which has restored normal growth velocities and in some cases returned the children to their normal growth isobar (Stefanidis 1996) in individuals who have not yet fused their epiphyseal growth centers. Thus, linear growth can be a very appropriate and meaningful metabolic and nutritional endpoint. Unfortunately, this can not be used in adults because they have fused their epiphyses and are no longer sustaining linear growth.

IMMUNOCOMPETENCE

Immunocompetence is a frequent co-morbidity factor in individuals with wasting and/ or catabolic conditions. In situations in which protein and energy malnutrition have occurred, the absolute lymphocyte concentration is found to be decreased. This decrease is distributed to all categories of lymphocytes not just T-cells (Manning and Shenkin 1995, Shenkin et al. 1995).

Of particular note is the well-established decrease in delayed hypersensitivity known to occur in situations of protein energy malnutrition (Bistrian et al. 1975, Chandra 1972, Dominioni and Dionigi 1987). This is most easily assessed using intradermal injections of antigens to challenge an individual's immune recall. Intradermal injections of antigens of Candida albicans or streptococcal bacteria or sensitization to chemical compounds known to induce delayed hypersensitivity (e.g., dinitrofluorobenzene) have traditionally been used as methodologies for determining delayed hypersensitivity. The presence of anergy in a patient with wasting and malnutrition is a grave prognostic indicator. Such immunocompromise in the face of underlying protein and energy malnutrition places the host at significant risk for infections and septicemia. As discussed below, such infections will result in even further significant protein wasting. Under these circumstances, the individual is unable to mount an adequate immune defense and is at high risk of dying. Anergy in the face of protein wasting generally reflects the severity of the illness and not necessarily the nutritional status of the patient (Manning and Shenkin 1995; Shenkin et al. 1995).

PLASMA PROTEINS

A large number of studies have pursued the relative importance of circulating proteins in monitoring the nutritional

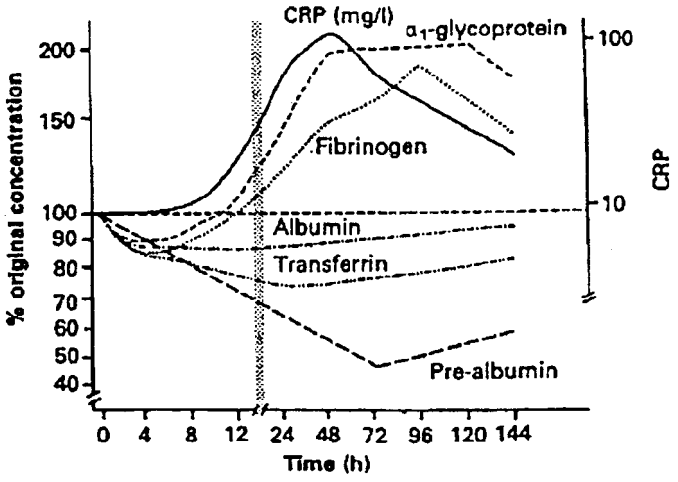

FIGURE 1 Response of plasma proteins after injury. Plasma proteins as nutritional indicators in the perioperative period. (From Fleck 1988, with permission.)

status of patients. A number of circulating plasma protein concentrations are dramatically altered with injury, trauma, infection and protein wasting conditions. The plasma concentrations of some proteins are decreased in response to such insults, while those of other proteins are increased (Fleck 1988) (Fig. 1). Negative protein indicators include prealbumin, insulin-like growth factor I (IGF-I), transferrin and albumin. The plasma protein (excluding IGF-I), whose concentration is most dramatically decreased is prealbumin (transthyretin) which has the shortest half life, followed by transferrin and then albumin. Of these negative indicating proteins, albumin and transferrin are stated to have the best accuracy and precision in predicting protein calorie malnutrition under conditions of both acute and chronic conditions followed by prealbumin and IGF-I. However, it must be pointed out that in terms of sensitivity and specificity for protein-energy malnutrition, these four circulating proteins are only "good to fair" (Manning and Shenkin 1995). Thus, a decrease in the concentration of these circulating proteins is most appropriately utilized as adjunctive correlations to other metabolic endpoints and clinical findings.

In contrast to the proteins mentioned above, acute-phase reactant proteins play an instrumental role in the normal immune defense mechanisms at both the local and systemic levels. In the presence of protein or protein and energy malnutrition, the synthesis of these circulating peptides places additional burdens on the host. It is now recognized that tissue damage, as a result of trauma or infection, results in the release of specific cytokines from tissues and invading white cells. The release of cytokines such as interleukin 1, interleukin 6, and tumor necrosis factor results in local vasodilatation, increased capillary permeability and local edema. If significant infection, trauma, etc., occur, these cytokines can result in a systemic reaction including fever, leukocytosis, increased erythrocyte sedimentation rate, increased ACTH and cortisol concentrations, activation of complement and clotting cascades (Manning and Shenkin 1995). With the appearance of systemic symptoms, most patients will become anorectic and refuse to eat even with encouragement. Collectively, these factors serve to increase dramatically the nitrogen and protein losses of any individual. In the clinical setting of protein energy malnutrition, this leads to significant stress and is associated with significantly increased morbidity and mortality.

The acute phase reactants, however, are poor indicators of protein and energy malnutrition and of wasting. They are more appropriate indicators of acute illness, trauma, or acute catabolic stress and are associated with the rapid mobilization of body protein and nutrients to respond to tissue injury. It should be pointed out that some of the additional factors induced by the cytokines are significantly catabolic in their own right. It is well established that circulating cortisol in high physiologic concentrations increase the rates of protein breakdown (Simmons et al. 1984). Fever increases the metabolic rate and leads to further mobilization of nutrient and amino acids, and accelerates nitrogen losses. Even in the face of malnutrition, the acute phase reactant protein will return to near normal concentrations once a superimposed infection is brought under control (Jahoor et al. 1997); thus, the response of acute phase reactant proteins provides no clear indication of nutritional status but is an indicator of the severity of an acute event.

The timing in the increase in the acute phase proteins is largely dictated by their individual synthetic rates and biologic half lives. C reactive protein, serum amyloid A, alpha-1 antichymotrypsin will increase over a 6- to 10-hour period. Those of alpha-1 antitrypsin, alpha-1 acid glycoprotein, haptoglobin and fibrinogen will increase over a period of five to seven days, whereas those of slower turning over proteins, complement factors C3 and C4, prothrombin, and ceruloplasmin increase over the course of one to two weeks. Depending on the duration of the underlying stress one may not see a complete rise in all of the above listed proteins. Some of these proteins are uniquely enriched in sulfur containing amino acids which exist in relatively low concentrations in muscle. As mentioned above, patients with trauma or infection, regardless of their protein status, are anorectic and have decreased intake of both calories and proteins. Thus, the only source for the synthesis of these acute phase proteins under such circumstances is the scavengering of these key and essential amino acids from other body proteins such as muscle. Since these sulphur containing amino acids are poorly represented in muscle tissue, significantly greater amounts of body protein must be degraded to recover sufficient amounts of these key amino acids to sustain the synthesis of these acute phase proteins (Reeds et al. 1994).

NITROGEN BALANCE

Nitrogen balance is the classical metabolic indicator of protein metabolism. This straightforward technique serves as the relative "gold standard" or reference method for many other nutritional indicators and endpoints. The concept of nitrogen balance is simple to comprehend, but not always easy to measure. It represents the difference between the total nitrogen intake (whether oral or parenteral) and the total nitrogen losses which include urinary, fecal, skin, hair and other nitrogen losses. Urinary urea nitrogen makes up approximately 70-80% of the total nitrogen losses and therefore, as a single indicator, is extraordinarily useful. However, some investigators have reported daily variances in urinary nitrogen excretion and in the relative composition of the nitrogen containing compounds in urine over the course of five to seven sequential days even under ideal study conditions (Shenkin et al. 1995). "Nitrogen balance" measures net nitrogen balance and provides no information about changes of whole body protein synthesis or protein breakdown. Thus, at an identical nitrogen intake, increased nitrogen losses could be the result of an increase in protein breakdown, a decrease in protein synthesis or some combination of the two. Although the concept of nitrogen balance is straightforward, precise measurements

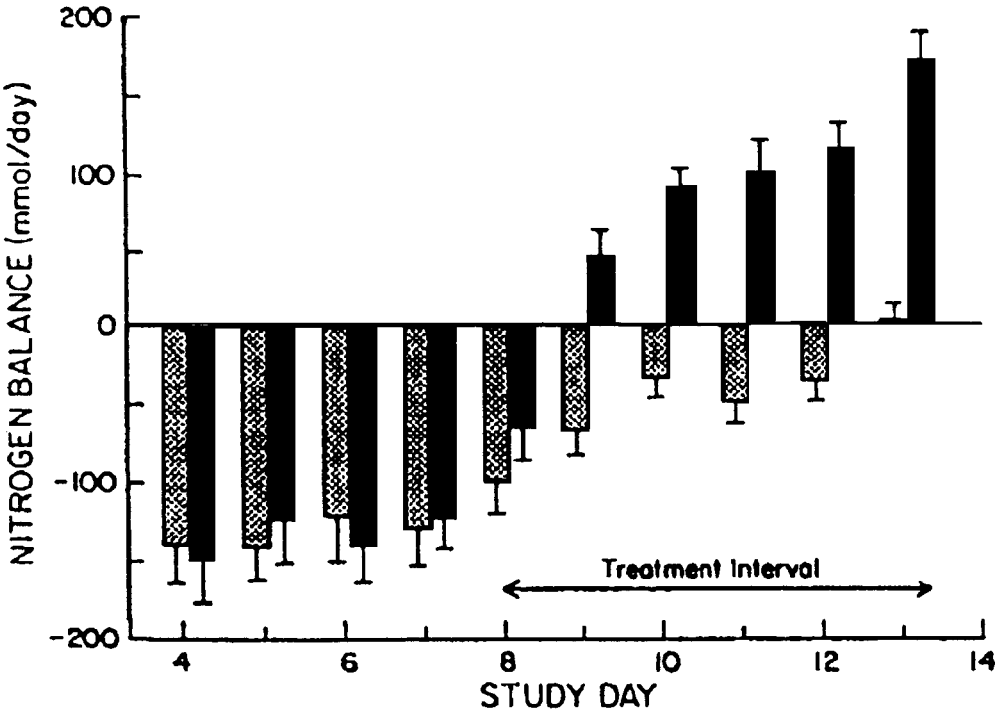

FIGURE 2 Changes in nitrogen balance in response to infusion of IGF-1 (shaded bars) or combined treatment with GH and IGF-1 (solid bars). Daily nitrogen balance was calculated by subtracting the sum of the 24-h urinary urea nitrogen and the estimate of stool and integument losses (245 mmol) from the daily nitrogen intake. The results are expressed as the mean ± SD for seven subjects for the last 4 d of each diet and treatment week. The difference in nitrogen balance between treatment groups were significant ($P < 0.01$) on days 9–13. (Kupfer et al. 1993, with permission.)

require accurate measurements of complete 24-hour urine and stool collections; this is not trivial outside of the setting of a clinical research unit. In addition, accurate measurements of nitrogen intake can usually be done in a hospital setting but are nearly impossible at home since dietary records and recall are notoriously unreliable.

Despite all of the potential difficulties, nitrogen balance continues to be a very useful and cost-effective endpoint indicator for interventional studies. Dr. Kupfer and coworkers (Kupfer et al. 1993) demonstrated the utility of nitrogen balance studies in recent studies examining the effects of both growth hormone and IGF1 in partially fasted normal subjects. Nitrogen balance was measured prior to and following growth hormone and/or IGF1 administration in this patient population. Figure 2 demonstrates a clear and dramatic change in nitrogen balance in response to rhGH and IGF-I or IGF-I alone.

AMINO ACID KINETIC STUDIES

Over the past two decades, the use of both stable and radioactive isotope tracers of amino acids in human investigations has gained both popularity and utility. Administration of a selected isotopically labeled essential amino acid and measuring its dilution by unlabeled amino acid in the plasma space provides an estimate of the rate of entry of the unlabeled amino acid and a measure of whole body proteolysis. Stable isotopes provide us with a variety of potential labeled compounds when deuterium (^{2}H), nitrogen (^{15}N), carbon (^{13}C) and/or oxygen (^{18}O) are incorporated into the molecule of interest. The advantage of these stable isotope tracers is that a number of them can be infused simultaneously and that they can be used in infants and pregnant women without risk. A number of investigators , including ourselves, have utilized radioactive tracers of amino acids, glucose and fatty acids using ^{14}C and/ or ^{3}H. These can be used alone or in combination with stable isotope tracers (Horber and Haymond 1990). However, they can not be used in children or pregnant women because of the unknown but potential risk of radiation exposure.

Utilizing these techniques collectively, it is possible to identify changes in amino acid and protein metabolism in response to an intervention within a period of hours. In early studies carried out in our laboratory, changes in the rates of protein breakdown were identified within four hours of the infusion of a high physiologic concentration of cortisol using a stable isotope tracer of leucine. Also, Dr. Fryburg and coworkers demonstrated an effect of rhGH and insulin in forearm human muscle after four hours using radioactive tracers of phenylalanine (Fryburg et al. 1992). Whether these changes will be sustained and predict improvement in the nutritional status of a patient remains to be fully proven.

Using a combination of stable and radioactive tracers of leucine, we demonstrated an acute effect of recombinant human growth hormone in offsetting the protein catabolic effects of high-dose prednisone over a period of 7 to 10 days in normal volunteers in both the fed and fasted condition (Horber and Haymond 1990). In the course of these studies, we demonstrated that the isotope dilution estimates of nitrogen loss were similar to those determined by classical nitrogen balance techniques (Figs. 3 and 4). An additional advantage of stable and/or radioactive amino acid tracers is the ability to estimate the fractional synthesis of specific or whole tissues proteins (De Feo et al. 1991).

Finally, a number of isotope models exist to predict changes in substrate metabolism. Each has its potential strengths and weaknesses, and the user must be fully knowledgeable in these issues to design an appropriate and meaningful interventional trial using these techniques. Utilizing a carefully designed protocol, these technics can be potentially very useful in assessing the impact of a particular interventional therapy.

INDIRECT CALORIMETRY

It would be incomplete not to address indirect calorimetry as an endpoint. Indirect calorimetry is a method in which

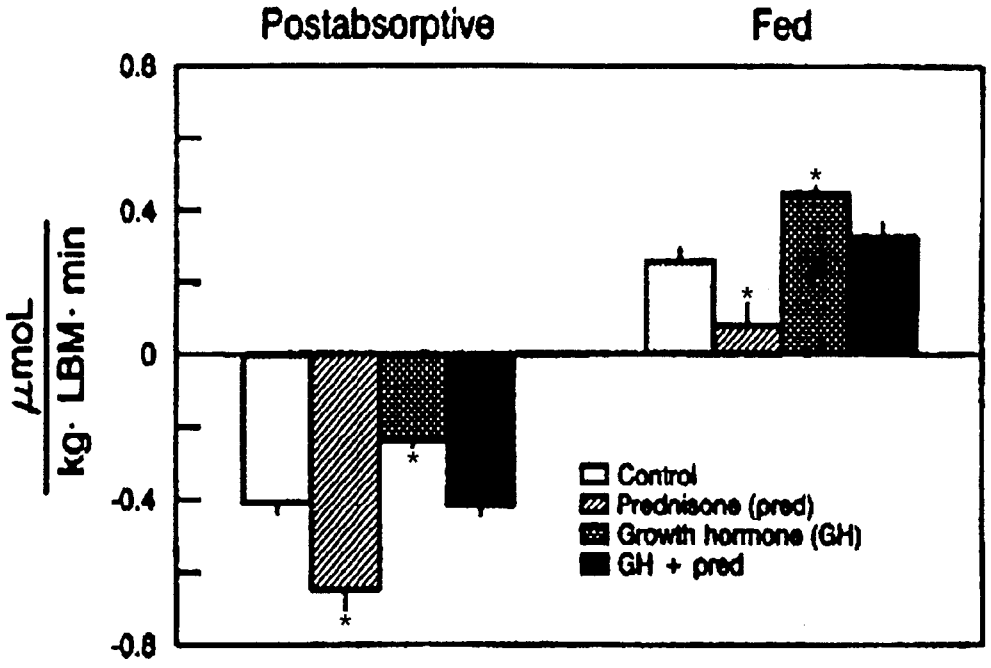

FIGURE 3 Leucine balance in the postabsorptive (left side) and fed state (right side) in the placebo treated subjects (control), subjects treated with prednisone (pred), recombinant DNA human growth hormone (GH), and combined treatment with rhGH and prednisone. *P < 0.001 when compared to placebo treated subjects by ANOVA. (Horber and Haymond 1990, with permission.)

oxygen consumption and CO_2 production are measured. Utilizing standard equations, the individual's metabolic rates can be estimated and, by knowing the excretion rate of nitrogen, estimates of relative rates of macronutrient oxidation can be made. Under conditions of fasting, fatty acids are the preferred fuel and there is relative sparing of protein and glucose. Estimation of the nutrient fuels oxidized under a variety of circumstances can be of immense importance in designing interventional strategies.

The total metabolic rate is increased primarily in circumstances associated with increases in acute phase proteins such as trauma, body burns, septicemia and fever. Thus, under these circumstances energy expenditure might be a methodology by which response to specific therapy (antibodies, antipyretics, etc.) might be measured over the short term. In situations of wasting in which protein and energy malnutrition occurs, indirect calorimetry measurement reflect decreased rates of total energy expenditure, which would teleologically be in keeping with sparing relative body stores. It should be pointed out, however, that under non-steady state conditions such as feeding and fasting, changes in the rates of gluconeogenesis, ketogenesis (or the accumulation or elimination of any metabolic intermediate in plasma such as lactate and pyruvate) can introduce significant errors in both the total energy expenditure, as well as, in the partitioning of macro nutrient fuel oxidation.

IMPORTANCE OF STUDY DESIGN

Several points of caution should be considered in selecting or utilizing any single nutritional or metabolic endpoint(s). It is imperative that the specific interventional management not interfere with the selected endpoint measures. An example of therapeutic interference is the use of rhGH in a variety of

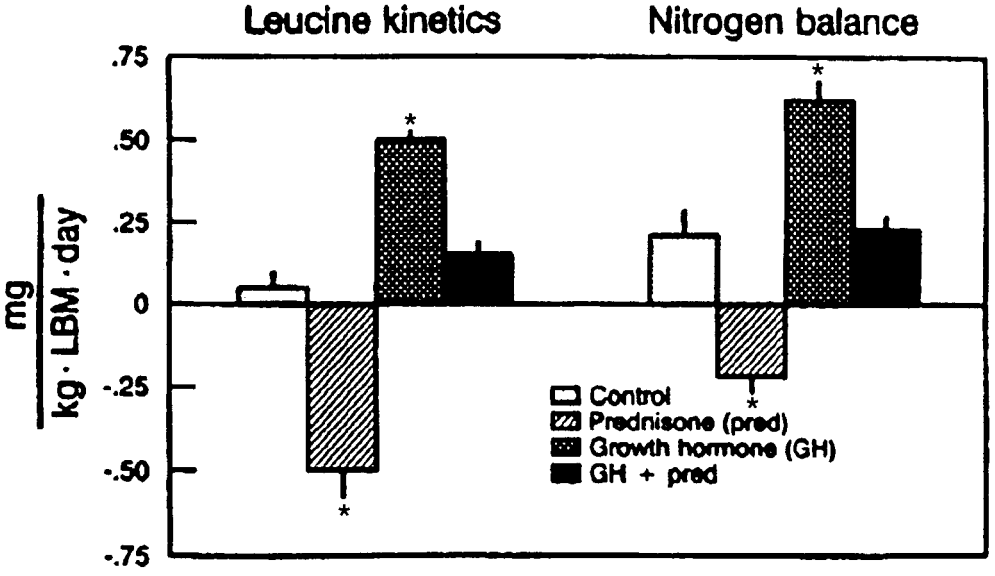

FIGURE 4 Protein balance calculated from leucine kinetic data (left side) and nitrogen balance (right side) in placebo treated subjects (control), patients treated with prednisone (pred), human growth hormone (GH), and combined treatment with human growth hormone and prednisone. Values are given as milligram of protein per kilogram LBM per day (see Methods for details). *P < 0.001 when compared to placebo treated subjects by ANOVA. (Horber and Haymond 1990, with permission.)

metabolic conditions. Large dose administration of growth hormone can lead to fluid retention which can interfere with a variety of estimates of lean body mass (De Boer et al. 1995). Thus, an increase in both intra- and extracellular fluid volume can lead to increased estimates of lean body mass and thus, compromise the ability to measure accurately the impact of rhGH.

Finally, it should be pointed out that any therapeutic intervention may not be continuous. For example, rhGH and/or anabolic steroid intervention will improve nitrogen balance and amino acid metabolism acutely regardless of the endpoint used. Fortunately, the patient has some ability to modify his/her response (e.g., down regulation, decreased gene expression, etc.) to pharmacologic doses of rhGH otherwise, with a sustained increase in nitrogen balance over a long period of time; and, if carried to its logical conclusion, the patient would become just a mass of protein or nitrogen. Thus, within some period of time following the introduction of an interventional management, metabolic indicators will most likely return towards normal if the intervention is successful. It must be recognized that normalization of these endpoints may be appropriate and desirable if they correlate with clinical improvements in the patients and other endpoint parameters.

Individual strategies and endpoint measures must be predicated on the basis of the anticipated progression of wasting, the disease process involved and morbidity that might be anticipated in the absence of intervention. In a situation of a rapidly progressing disorder any interventional changes that would alter metabolic rate, nitrogen balance (or amino acid metabolism) and immunocompetence may well have some impact on morbidity and/or mortality of such conditions. In contrast, in diseases involving more insidious onset of wasting such as chronic demylenating diseases, slowly progressing AIDS, loss of normal endogenous hormones (growth hormone, estrogen and testosterone) in isolation or as part of the normal aging process, require a different approach to the assessment of an interventional management. In these latter situations, one might consider using measures of body composition and indicators of function and quality of life.

SUMMARY

None of the metabolic indicators discussed above provides a single or necessarily ideal endpoint in planning interventional management in wasting disorders. Some of these indicators may provide better endpoints for the acute rather than the chronic wasting conditions. In addition, it is imperative that more than one endpoint be selected to be sure that there is concordance in the findings. However, prior to the selection of any endpoint measure, the investigators involved must be fully cognizant of the potential pitfalls and errors that can occur in every one of the above selected methodologies. In anticipating these potential problems, developing strategies for the interpretation of the data is critical at the outset of any interventional management strategy.

In conclusion, the manufacturers, the regulators and the investigators involved in the interventional management of chronic and acute wasting disorders must agree on the endpoints to be used and these endpoints must provide the most appropriate and valid information. Selection of nutritional and metabolic endpoints must be in part dependent on the disease process involved, the potential magnitude of the interventional effect and must be utilized in the context of a carefully designed experimental protocol with well focused questions.

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