Identification of Functional Immunological Indicators of Nutritional Status During Acute Nutritional Deprivation

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Optimal functioning of the immune system is necessary for the host to be capable of mounting a sufficient immune response, especially in times of sickness and injury. Acute bouts of starvation may compromise immune function, and subsequently lead to increased susceptibility to infection. Immunocompetence has been suggested as a functional indicator of nutritional status as the function of the immune system relies upon nutrient dependent metabolic pathways and the provision of adequate nutrient substrates to synthesize its components. The sensitivity of monocyte phagocytic activity, major histocompatibility complex (MHC) class II expression, and fibronectin concentration were studied in 23 healthy cats during a 7 day period without food followed by a 7 day refeeding period. Blood samples were obtained for plasma fibronectin analysis and immune cell function tests on days 0, 4, 7, 11, and 14. A turbidimetric immunoassay was used for determination of plasma fibronectin concentration. Monocyte phagocytosis and MHC class II expression were measured using flow cytometric techniques. Weight, lymphocyte number, percent lymphocytes, white blood cell number, and serum albumin concentration were monitored throughout the study. Phagocytic activity, MHC class II expression, weight, lymphocyte number, percent lymphocytes, and white blood cell (WBC) number, decreased significantly (p<0.05) during the starvation period. Fibronectin concentration increased significantly (p<0.05) by day 4 of starvation. During refeeding there was a significant increase (p<0.05) in MHC class II expression, fibronectin concentration, weight, lymphocyte number, percent lymphocytes, and white blood cell number. Phagocytic activity decreased significantly (p<0.05) by day 11 of refeeding. Pearsons correlation analysis revealed a positive
correlation (p<0.05, r=.2682) between weight change and phagocytosis. There was a positive correlation (p<0.05, r=.3588) between monocyte number and MHC class II expression, and between monocyte number and WBC number (p<0.05, r=.3506). Results indicate that maintenance of immune function is dependent upon the provision of continuous nutritional intake by the host. Plasma fibronectin, monocyte phagocytosis, MHC class II expression, and other immunological measures of health status were sensitive to acute alterations of nutritional intake and subsequent refeeding. Both phagocytic activity and MHC class II expression were found to be reliable indicators of nutritional status during acute nutritional deprivation. These data suggest that short periods of food deprivation may significantly decrease immune response.
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<td>Protein Energy Malnutrition</td>
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<tr>
<td>DTH</td>
<td>Delayed Type Hypersensitivity</td>
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<tr>
<td>CBCD</td>
<td>Complete Blood Count with Differential</td>
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<td>MC</td>
<td>Mononuclear Cells</td>
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<td>PMNC</td>
<td>Polymorphonuclear Cells</td>
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<td>HBSS</td>
<td>Hanks Balanced Salt Solution</td>
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<td>MHC</td>
<td>Major Histocompatibility Complex</td>
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<td>FITC</td>
<td>Fluorescein Isothiocyanate</td>
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<td>FBS</td>
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<td>PBS</td>
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Chapter 1

INTRODUCTION

Malnutrition among hospital patients remains a common problem in North America (Giner et al. 1996; McWhirter et al. 1994). Many patients enter hospitals in a malnourished state or are at risk of becoming malnourished (Gallagher-Allred et al. 1996). During hospitalization, patients often suffer from disease-related anorexia, and/or food may be withheld for several days prior to and following major surgical procedures. During those times in which patients suffer acute bouts of food deprivation, malnutrition may arise or worsen and attenuate depression of the immunological function of the patient (Chandra, 1993). The immune system is a network of cells and organs that is highly sensitive to environmental and physical changes. Optimal functioning of all of the cells of the immune system is necessary in order for the host to be capable of mounting a sufficient immune response, especially in times of sickness or injury. Identifying those patients who are malnourished in order to provide prompt nutritional intervention to improve nutritional status is critical. Because of its dependence on the provision of adequate nutrients, measurement of immune status has been suggested as a potential indicator of nutritional status (Chandra 1981, 1991). Specific tests of immune function, however, have not been identified for the assessment of nutritional status.

Malnourished hospitalized patients suffer more complications, more infections, stay in the hospital for longer durations, and have higher mortality rates than well nourished patients (Gallagher-Allred et al. 1996; Giner et al. 1996). Acute bouts of food deprivation in hospitalized patients may induce a state of malnutrition, compromise immune function, and subsequently lead to increased incidence of complications (Neuvonen et al. 1984). Nutritional intervention in these individuals is therefore critical for improving their nutritional status, immune function, and subsequent outcome. Identifying the most sensitive indicators of nutritional status is extremely important in the nutritional assessment process. Immune function tests have been proposed as good indicators of nutritional status because they are altered early in states of undernutrition, and they are sensitive to overall nutritional status rather than deficiency of individual specific nutrients (Puri et al. 1985). Currently, however, a set of functional immunological parameters indicative of nutritional status have not been identified.

The purpose of this research was to identify immune function parameters that may be used as sensitive indicators of compromised nutritional status. It is necessary to identify and establish such indicators since current nutritional assessment techniques lack sensitivity for
identifying changes at the cellular level that result from undernutrition. Although current literature provides evidence that there is a strong association between nutritional status and immune function, it fails to propose specific immune function tests that may be used for nutritional assessment purposes. Hence, it was the purpose of this research to measure the sensitivity of select immune function tests to acute nutritional alterations in order to establish the validity of their use as indicators of nutritional status. A cat model for acute starvation was used as their anatomy is closer to a human being in uniformity of size than many other available animals (Walker, 1982), and they lack the regulatory adaptive mechanisms to utilize alternative pathways and substrates.

**Research Objectives:**

1. To determine the response of monocyte phagocytic activity, fibronectin concentration, and monocyte major histocompatibility complex (MHC) class II expression to 7 days of starvation, and 7 days of refeeding in healthy cats.

2. To identify relationships among changes in body weight, and monocyte phagocytosis, MHC Class II expression, and fibronectin concentration during a 7 day period of acute starvation and a 7 day period of refeeding.
Chapter 2

LITERATURE REVIEW

Malnutrition Among Hospital Patients

Malnutrition remains a common problem among hospitalized patients, significantly affecting their outcome. A survey of eight studies involving 1,327 hospitalized adult patients found the incidence of malnutrition to be between 40% to 55% (Gallagher-Allred et al. 1996). Data from this survey showed that malnourished, surgical patients were two to three times more likely to suffer from increased risk of complications and higher rates of mortality than those surgical patients who were adequately nourished. Possible factors that account for the prevalence of malnutrition among hospitalized patients are: failure to monitor food intake; withholding meals because of diagnostic tests; failure to recognize increased nutritional needs due to injury or illness; and failure to appreciate the role of nutrition in the prevention and recovery from infection (Giner et al. 1996). Malnutrition often goes undiagnosed in hospitalized patients. Mowe et al. (1991) reported that in a sample of 121 elderly patients, of whom 66 were identified as malnourished by a review of medical records, only 24 were given this diagnosis upon admission.

Patients who are at risk for malnutrition are usually the sickest patients who require early identification of risk and early nutritional intervention to prevent life threatening complications. The consequences of malnutrition may include an impairment of the immune system and subsequent inability of the body to mount a sufficient immune response. Giner et al. (1996) found an increased incidence of complications in those hospitalized patients identified as being malnourished. Of 110 geriatric patients, Sullivan et al. (1990) found that the impact of malnutrition was independently related to the risk of developing life threatening complications and death. In a review of studies relating malnutrition to increased mortality, Dempsey et al. (1988) found that malnutrition in hospital patients was strongly associated with poor outcome. Haydock et al. (1986) found that compared to normally nourished patients, those patients with mild or severe protein energy malnutrition had impaired wound healing. Furthermore, Pinchocofsky et al. (1985) found that nutritional status deteriorated with increasing length of
hospital stay. These data provide evidence that nutritional status significantly affects the outcome of hospitalized patients.

The prevalence of malnutrition among hospitalized patients has a substantial economic impact. Reilly et al. (1988) studied the length of stay and cost of patients who were identified as having a likelihood of malnutrition. Those with a likelihood for malnutrition had longer mean length of stays in the hospital. When converted to relative cost, it was estimated that total hospital costs were between $1738 and $3557 higher per patient when compared to adequately nourished patients. A survey by the Nutrition Care Management Institute (Tucker et al. 1996) reviewed the results of 22 studies involving malnutrition and its association with several variables, including length of hospital stay. Patients with malnutrition had a prolonged length of stay of approximately 5 days as compared to adequately nourished patients. Robinson et al. (1987) reported that overall, malnourished patients had costs three times greater than the normally nourished patient. Tucker et al. (1996) reported results on the effect of early nutritional intervention and length of stay from the Malnutrition Cost Survey performed by the Nutrition Care Management Institute. Those patients who received early nutritional assessment and therefore early nutritional intervention, had shorter hospital stays. It is apparent that early nutritional intervention will likely prevent or depress malnutrition, reduce hospital costs, and lead to shorter hospital stays.

**Nutritional Assessment Methods**

“Good” nutritional status may be defined as a diet sufficient to meet or exceed the needs of the individual which keeps the composition and function of the otherwise healthy individual within the normal range (Jeejeebhoy et al. 1990). This equilibrium is disturbed by three processes: decreased intake, increased requirements, and altered utilization. When equilibrium is disturbed by these processes, metabolism is altered, function is impaired, and loss of body tissue ensues (Jeejeebhoy, 1994). Indicators of nutritional status have been classified historically as biochemical, clinical, anthropometric, and dietary. These classifications reveal little as to the usefulness of these indicators in prediction of nutritional status. For the purpose of evaluating nutritional status in research, an indicator must have the potential to respond to a nutritional intervention in a fashion that can be identified with good statistical power (Habicht et al. 1990). Nutritional status indicators are measures of dysfunctions caused by inadequate nutrient supply to the cells, or they are measures of nutrient content or stores in the body (Habicht et al. 1990). For the purposes of this research, a “good” indicator of nutritional status is therefore considered one which is sensitive, consistent, accurate, reliable, and predictive of acute dietary alterations.
Most of the diagnostic techniques used for assessing nutritional status lack sensitivity and accuracy. Current nutritional assessment practices include mostly static measures. These include routine laboratory tests that are part of serum chemistry analysis (e.g. total serum proteins, serum albumin, transferrin), anthropometric measures (e.g. height, weight, skinfold thickness), and clinical examination. While these measures may be appropriate for the evaluation of chronic nutritional status, they lack sensitivity in detecting acute alterations in nutritional status.

Static measures commonly used to assess nutritional status include visceral protein analyses. There are several disadvantages to using such measures in assessment of nutritional status. Circulating protein levels may be independently affected by hydration status, other diseases, infection, drugs, or hormonal status (Solomons, 1985). Furthermore, laboratory determinations of biochemical levels of proteins represent static indices of total-body nutrition status (Solomons, 1983).

Albumin is a visceral protein which requires an adequate supply of exogenous amino acids for its synthesis. Since albumin has a half life of 20 days, a deficit is considered a measure of long term rather than acute protein undernutrition. Forse et al. (1980) studied the relationship between albumin and body composition. Although body cell mass correlated significantly with serum albumin concentration, when patients were divided into three groups based on changes in body composition over time, albumin did not consistently reflect significant body composition changes. The authors concluded that albumin was a poor indicator of nutritional status because of its poor sensitivity and specificity to significant body composition changes. In a study by O’Keefe et al. (1988) albumin was not a useful indicator of nutritional status but rather, was more useful as an indicator of degree of sickness or risk of mortality. In a study by Reinhardt et al. (1980), hypoalbuminemia was linearly correlated with mortality rate in a population of 2,060 patients. Serum albumin alone has been shown to be an insensitive and inaccurate indicator of nitrogen balance or protein status (Starker et al. 1982; Church et al. 1987).

Transferrin, which has a shorter half life than albumin (8 days), has been used as a biochemical indicator of nutritional status. Roza et al. (1984) studied transferrin for its potential as a measure of nutritional status in 74 surgical patients receiving total parenteral nutrition. Transferrin was not a sensitive or accurate indicator of nutritional status. Church et al. (1987), found that transferrin was only 67% sensitive in detecting a positive nitrogen balance. During a 24 day reduction in energy and protein intake in 16 obese women, albumin and transferrin did not change while prealbumin decreased significantly by day 6 (Shetty et al. 1979). Transferrin is not sensitive to dietary alterations or predictive of improving nutritional status.

Prealbumin has been proposed as a sensitive indicator of visceral protein status because it has a very short half life (2 days) and has as a component the amino acid tryptophan which is
only found in the diet. Vanlandingham et al. (1982) compared nitrogen balance and prealbumin levels in patients receiving intravenous nutrition. They found that patients in positive nitrogen balance had higher prealbumin levels, while those in negative nitrogen balance had lower prealbumin levels. Church et al. (1987) found that prealbumin was a 93% positive predictive value for positive nitrogen balance in patients receiving intravenous nutrition. Bernstein et al. (1996) compared prealbumin to serum albumin and total protein in the nutritional evaluation of 545 surgical and medical patients. They found that albumin and total protein failed to reflect current status or change in nutritional status while prealbumin was a good indicator of current and changing nutritional status. Together, these data demonstrate that the proteins albumin, total protein, and transferrin, are not sensitive to changes in nutritional status, nitrogen balance, and/or short term energy deprivation seen commonly in hospitalized patients. Although prealbumin is a more sensitive indicator of current and changing nutritional status, it does not provide a comprehensive assessment of nutritional status.

Of the anthropometric measurements frequently used, weight loss is the most sensitive to changes in exogenous nutrient intake and is the most reproducible. The association between body weight and total body protein mass and energy content make body weight an indirect marker of protein mass and energy stores. In a prospective study of 398 surgical patients, Gianotti et al. (1995) found that after pre- and post-operative evaluation of weight loss, serum albumin and total lymphocyte count, weight loss was the only nutritional indicator that retained prediction of post-operative complications. Bellantone et al. (1990) found that in 43 patients who underwent a total gastrectomy, weight loss was associated with the development of post-operative nutrition associated complications. Seltzer et al. (1982) found after surveying 4,382 surgical patients with regards to weight loss, an absolute weight loss of greater than 10 pounds was positively correlated with incidence of mortality. When food intake is less than nutrient losses, a negative nutrient balance occurs manifested by a loss in body weight. Weight loss is sensitive to acute dietary alterations, and is associated with increased morbidity and mortality.

Skinfold thickness is commonly used as a physical measure of nutritional status. Because skinfold thickness has large reference ranges of normal values, and inaccuracy in measurement is common, it lacks sensitivity to diagnose altered nutritional status (Butters et al. 1996). Fuller et al. (1991) evaluated intra-observer variability in the measurement of body composition with six observers and 12 healthy adult subjects. They found that there were variations of 11-18% in the measurement of skinfold thickness, and only .01-.05% variability in measurement of weight. Fernandez et al. (1993) studied the use of anthropometric indices for assessing nutritional status on hospital admission in 45 male surgical patients. They found that after grouping the patients as either undernourished or adequately nourished according to body
mass index scoring, there were significant differences in the anthropometric indices of mid-arm circumference and triceps skinfold between the groups. Ten of these patients were followed for eight days postoperatively, and although they were found to have energy deficient intakes, there were no significant changes in mid-arm circumference or triceps skinfold. While these measures may be useful indicators of chronic nutritional status, they may not be appropriate for the determination of acute nutritional status as they lack accuracy and sensitivity.

Clinical exams also possess little diagnostic value as they only detect severe, chronic nutrient deficiencies (Allen, 1990). Obvious physical signs and clinical symptoms of nutrient deficiency appear only late, in the final stages of nutritional depletion. These clinical symptoms should generally not be allowed to evolve before they are recognized and treated in medically managed patients.

Functional indicators of nutritional status may be preferable to static indices as they show defective function at the cellular level despite an apparent adequate nutritional status (Solomons et al. 1983). This may provide the opportunity to identify those patients at risk for malnutrition prior to obvious signs and symptoms associated with current nutritional assessment practices. Immunocompetence has been suggested as a functional indicator of nutritional status.

Malnutrition is associated with a progressive decline in immune function (Manning et al. 1995). Immune function has been studied in models of nutritional deprivation in an effort to establish its sensitivity to acute changes in nutritional status. A variety of immune cells have been shown to be affected by short-term nutritional deprivation and may therefore be potentially useful as measures of nutritional status.

**Overview of the Immune System**

The immune system is a network of organs and cells that functions in the defense of the host against foreign antigens. The cells of the immune system are known as leukocytes. Leukocytes can be further subdivided into cells of innate immunity and cells of acquired immunity. Innate immunity is the nonspecific immunity that one is born with that functions in providing the body’s first line of defense against an invading antigen. Cells involved in innate immunity are monocytes, macrophages and polymorphonuclear neutrophils (Roitt et al. 1996). All of these cells possess the unique capability of phagocytosing or engulfing and processing foreign antigens. Another cell of innate immunity is the natural killer cell which nonspecifically kills tumor and virus infected cells. Acquired immunity is a specific immune response which has memory and is mediated by lymphocytes. This type of response functions in the identification of
a previously encountered foreign antigen and subsequent production of a rapid immune response.

Acquired immunity can be subdivided into cell mediated immunity and humoral immunity. T lymphocytes function in cell mediated immunity while B lymphocytes function in humoral immunity. Both T and B lymphocytes interact together to provide the immune response. T lymphocytes are produced in the thymus and may be divided into T-helper cells and cytotoxic T cells, each of which serve individual and distinct functions in the immune response. B cells are produced in the bone marrow, and when activated by an antigen, they become plasma cells capable of producing one of several classes of antibodies: immunoglobulin G (IgG), immunoglobulin A (IgA), immunoglobulin M (IgM), immunoglobulin E (IgE), and immunoglobulin D (IgD).

The immune system also possesses a complement system. The complement system exists as a series of protein molecules that upon activation function in a cascade of events which facilitate inflammation, opsonization, and lysis of pathogens. The most abundant complement protein is identified as the C3 protein which associates with the antigen-antibody complex (Roitt et al. 1996).

Antigen recognition by immune cells occurs when the antigen is presented on the surface of a molecule in association with the Major Histocompatibility Complex (MHC). There are two classes of MHC molecules: class I and class II. Class I MHC molecules are present on all immune cells while class II MHC molecules are present on certain specialized cells such as macrophages and B cells (Roitt et al. 1996). MHC class II molecules present foreign peptides to T-helper cells after monocytes/macrophages engulf and process the antigen so that the immune response may occur. Also of importance is a glycoprotein called fibronectin. Among its many proposed functions, fibronectin may play an important role in the enhancement of the phagocytic process of macrophages. A relationship between monocyte phagocytic capability, MHC class II expression, and fibronectin may therefore exist. The capability of a cell to phagocytize, process, and subsequently present a foreign peptide to T cells in association with MHC class II may be dependent on plasma fibronectin concentration.

**Monocyte Phagocytosis**

Mononuclear phagocytes are one of the most important groups of phagocytic cells. These cells develop from the bone marrow stem cells, and their main function is to bind, internalize, and destroy particles. Monocytes are developed in the bone marrow from colony forming units (Roitt et al. 1996). These monocytes then randomly travel to various sites in the
body (in the absence of localized inflammation) where they undergo transformation into immature macrophages as a result of tissue specific stimuli. Here, macrophages become activated by several possible factors, including inflammation, and perform the critical function of engulfing and destroying foreign or waste particles. In an immune response to infection, macrophages often are the “first line of defense” against the invading foreign microorganism. Macrophages can ingest the foreign organism, then process and present its antigen to lymphocytes to activate the immune response. Macrophages secrete many factors which stimulate other immune functions to mobilize immune cells and stimulatory products in order to continue the immune response against the infectious substance. Phagocytosis can be mediated by opsonins which are substances which can be deposited on the antigen to facilitate its binding to the macrophage. In a review of the feline immune system, Lin (1992) suggested that feline macrophages are similar to other mammalian macrophages; however, in cats the removal of blood-borne pathogens is effected primarily by pulmonary macrophages as opposed to spleen or liver macrophages in the human. Any loss of ability of phagocytic cells to perform their function would be detrimental to the host’s capability to fight infection. In particular, lack of appropriate exogenous nutrients may affect the function and effectiveness of these cells.

**MHC Class II**

The MHC is a region of genetic loci involved in recognition and rejection of foreign and non-self antigens. There are two major classes of the MHC: class I and class II molecules. All class I and class II MHC have similar genetic make-up and structure. The MHC is highly polymorphic in that no two individuals will have the same exact MHC (Roitt et al. 1996). Class I MHC molecules are expressed on all living cells and are involved in presentation of foreign peptides to cytotoxic T cells to promote an immune response. Class II molecules are only found on macrophages and B cells. Their function is to present foreign peptides to T helper cells.

Foreign antigens are therefore engulfed, processed, and peptides are expressed on the surface of macrophages in conjunction with the MHC class II molecule. Antigen presentation on these immune cells translates into an immune response and is therefore critical to the overall ability of a host to fight off infection. Willett and Callanan (1995), reported that the feline MHC class II molecules are similar to the human MHC class II molecules with the exception that they are only expressed on T cells in the feline.
Fibronectin

Fibronectin is a large glycoprotein found in the extracellular space of most tissues. (Mosher, 1984). Fibronectin can be divided into two types: insoluble and soluble. Insoluble fibronectin is present in extracellular structures while soluble fibronectin is found in plasma. Plasma fibronectin is thought to have adhesion properties which bind cells to collagen, collagen to fibrin, and of interest to this research is its ability to function as an opsonin in the binding of pathogens to immune cells. Particularly, fibronectin appears to bind to receptors on leukocytes and act as an opsonic glycoprotein (Saba et al. 1983). It appears that fibronectin promotes phagocytic binding and uptake of foreign molecules and waste from tissue injury. Boughton et al. (1984) studied the in vitro affect of varying amounts of fibronectin supplementation on monocyte phagocytosis function. Researchers found that there was a dose response effect in that as the concentration of fibronectin increased, the phagocytic activity of monocytes increased. These investigators also found that fibronectin accounted for 60% of the opsonic activity of plasma. Pommier et al. (1983) found that addition of fibronectin to monocytes produced a dose related increase in ingestion of foreign particles. Associations between fibronectin deficiency and various disease states such as acute respiratory distress syndrome, sepsis, multiple organ failure, and starvation have been suggested in previous studies (Saba et al. 1983).

The measurement of fibronectin in non-human species has only recently been pursued. Hirschberger et al. (1996) studied the use of a commercial test kit for nephelometric measurement of human fibronectin in dogs and cats. In agreement with previous reports, there was cross-reactivity between feline plasma fibronectin and anti-human fibronectin antiserum, validating the use of this kit for the determination of feline fibronectin concentrations.

Immunocompetence in Malnutrition

A variety of immune cells may be affected by undernutrition and therefore may provide an indication of nutritional status. Immune function is dependent upon metabolic pathways that require various nutrients as critical cofactors. The synthesis of immune cells and associated proteins is dependent on the provision of appropriate and adequate nutrients. The metabolic adaptations to brief periods of fasting include proteolysis of body proteins and may result in defective functioning and synthesis of components of the immune system. Many studies have been performed on people with protein-energy malnutrition (PEM) to determine which immune parameters or components are most often affected by nutritional status. Those that have been identified include T lymphocytes and their subsets, specific immunoglobulins, the complement
system, fibronectin, and the phagocytic activity of monocytes (Chandra, 1993). Delayed type hypersensitivity (DTH) is also reduced during malnutrition (Puri et al. 1985).

Chandra (1991) reported that the measurement of T lymphocytes and their subsets, as well as DTH were sensitive indicators of nutritional status. In six infants diagnosed with PEM, Chandra et al. (1982) reported a decrease in the number of lymphocytes, and a decrease in the number of both T helper and cytotoxic T cell subsets. Dowd et al. (1986) found a decrease in lymphocyte proliferation in malnourished hospital patients, and McMurray et al. (1981) found that in 32 infants who became mild to moderately malnourished in the first two years of life, lymphocyte proliferation decreased by 50% from baseline. Holstein bull calves fed a protein and energy malnourished feed for four weeks showed depressed lymphocyte proliferation by day 10 of the study (Griebel et al. 1987). Lymphocyte proliferation, lymphocyte number, and subsets of lymphocytes, are all adversely affected by PEM.

Abnormalities of DTH skin testing have been reported in malnourished hospitalized patients and in malnourished children. McMurray et al. (1981) also reported that in malnourished children who were previously well nourished there was a significant reduction in DTH. Forse et al. (1981) studied DTH in 257 patients who received total parenteral nutrition support. A significant relationship was found to exist between nutritional status and DTH skin testing. Fakhir et al. (1989) found significantly depressed lymphocyte counts and depressed DTH in malnourished children. These data show that depressed DTH response is associated with PEM.

The complement system has not been widely studied during PEM. Chandra (1975) found that the function of the complement system, particularly C3, was depressed in 35 malnourished children. Ozkan et al. (1993) found that in 29 patients with PEM, C3 levels were significantly lower than in the well nourished control group. These data provide evidence that the complement system is negatively affected by PEM.

In murine models of PEM, macrophage function has been shown to be significantly impaired (Hill et al. 1995; Redmond et al. 1991). Welsh et al. (1996) found that phagocytic cell function in 37 malnourished surgical patients was significantly depressed. In a study by Reynolds et al. (1992), protein-deprived mice had significantly impaired macrophage activation as compared to mice on an isocaloric, protein fortified diet. Macrophage function appears to be negatively influenced by PEM.

Antigen presentation has also been studied in response to malnutrition. As previously described, in order for an antigen to be presented by an immune cell, it must be in association with an MHC class II molecule. Therefore, antigen presentation may be an indicator of MHC Class II expression. Redmond et al. (1995) studied the effect of PEM on antigen presentation in
macrophages in mice. They found antigen presentation on these cells to be defective during PEM. In a study by Conzen et al. (1988), antigen presentation was found to be defective during chronic protein deprivation in mice. Welsh et al. (1996) studied phagocytosis and MHC class II expression in response to in vitro interferon-gamma simulation in monocytes taken from 37 malnourished patients and 25 well-nourished controls. There was a significant loss in phagocytic function and MHC Class II expression in the malnourished group as compared to the control group. In a second part of the study, 10 malnourished patients received five days of total parenteral nutrition which resulted in a significant increase in MHC class II expression. These data show that MHC class II expression was sensitive to chronic nutritional deprivation and nutritional repletion.

Plasma fibronectin has been considered for use in assessment of nutritional status based on studies that demonstrate low fibronectin levels in states of acute nutritional deprivation and PEM (Benjamin, 1989). Yoder et al. (1987) simultaneously measured fibronectin, prealbumin, and albumin in 20 hospitalized infants with PEM upon admission and then every week until patients achieved full nutritional recovery. Researchers found that fibronectin, prealbumin, and albumin were all depressed upon admission; however, only fibronectin and prealbumin were sensitive to one week of nutritional repletion. Both fibronectin and prealbumin continued to increase with nutritional repletion during the hospital stay. In a study by Sandberg et al. (1985), fibronectin levels in 58 malnourished children were significantly lower than healthy controls. Higher fibronectin levels in these children translated into improved survival rate. In a study of nutritionally depleted hospitalized patients initiated on nutrition support, fibronectin rose significantly in response to 1-4 days of nutrition support (McKone, 1985). Fibronectin appears to be responsive to nutritional depletion and repletion.

In states of PEM, it is apparent that cellular immune function is depressed. Particularly, T lymphocyte cellular proliferation and function is depressed, T lymphocyte subset expression decreases, the complement system is affected, macrophage and subsequent phagocytic function is depressed, and DTH is reduced. MHC class II expression and fibronectin concentrations have also been found to be depressed in PEM. Clearly, the continuous provision of nutrition to the host is required to maintain proper immunological status. The depression of immune function during PEM will decrease the ability of the malnourished host to mount a sufficient immune response when faced with invading pathogens.
Immune Function and Acute Starvation

Less widely studied have been the effects of acute bouts of starvation on cellular immune function. Conflicting results are prevalent in current literature describing the relationship between acute starvation and immune function. For example, in studies involving the fasting of rats or mice for 4 to 9 days, the number of lymphocytes was significantly lower than in the non-fasted control group (Ogawa et al. 1993; Moriguchi et al. 1989). However, in human subjects fasted from 3 to 14 days, no significant decrease in lymphocyte number has been observed (Neuvonen et al. 1984; Wing et al. 1983). Measurement of the total number of leukocytes has also provided conflicting results in cases of acute starvation. Neuvonen et al. (1984) found no change in the number of leukocytes after a 3-day fast in 10 human subjects. However, Wing et al. (1983) reported that although the mean number did not decrease significantly, a 14-day starvation diet resulted in a decrease in the total number of leukocytes in 12 of 15 human subjects. Becker et al. (1992) found that in pigs fasted for 48 hours, there was a significant decrease in the number of leukocytes. Currently, the use of lymphocyte number and total number of leukocytes as sensitive indicators of acute nutritional deprivation is unclear.

Lymphocyte proliferation has been shown to be depressed during acute bouts of starvation. Wing et al. (1983) reported a significant decrease in lymphocyte proliferation in 15 obese subjects who were on a starvation diet for 14 days. Neuvonen et al. (1984) found that lymphocyte proliferation was depressed significantly in human subjects fasted for three days. Similarly, Holm et al. (1976) reported depressed lymphocyte proliferation in 14 healthy subjects starved for 10 days. These results suggest that lymphocyte proliferation is depressed in response to acute nutritional deprivation.

DTH has been suggested as an indicator of nutritional status in individuals with PEM, but has not been widely studied under conditions of short term starvation. Wing et al. (1983) reported an enhanced response to antigens during DTH testing in humans fasted for 14 days while Holm et al. (1976) found no difference between human subjects fasted for ten days and non-fasted control subjects. Conversely, Nohr et al. (1985) reported that DTH was significantly reduced in rats fasted for three days as compared to non-fasted controls.

Macrophage function has been shown to be depressed during acute starvation. Sakai et al. (1990) showed that in rats starved for six days, macrophage function was enhanced by day 2 of starvation but was suppressed by four days of starvation. Similarly, Moriguchi et al. (1989) found that in rats fasted for nine days, macrophage function increased within two days of fasting but then decreased significantly for the remaining seven days of the study. Dillon et al. (1982) found that in rats starved for five days, phagocytic function was significantly depressed as
compared to normal controls. The function of macrophages may be dependent on the provision of adequate nutrition as it has been shown to decrease in response to short-term nutritional deprivation. The measurement of the phagocytic ability of macrophages may, therefore, be a sensitive indicator of nutritional status during nutritional deprivation.

Fibronectin has been studied in states of starvation or fasting to determine its sensitivity as a nutritional assessment parameter. Howard et al. (1984) studied 12 adult females placed on a starvation diet for five days and monitored the resulting fibronectin levels. Mean fibronectin levels increased slightly during the first two days of starvation followed by a 25% decrease by day 5. In 18 human subjects fasted for 21 days, plasma fibronectin levels were significantly depressed by day 7 of starvation and remained low until food consumption resumed (Scott et al. 1982). In rabbits fasted for 48 hours, plasma fibronectin levels decreased significantly (Cheslyn-Curtis et al. 1990). In 10 male subjects starved for 4-5 days, plasma fibronectin values significantly decreased by day 2 of starvation (Chadwick et al. 1986). Soper et al. (1984) studied fibronectin levels and reticuloendothelial function in rats who were starved for five days as compared to control chow fed rats. Researchers found that in the starved rats, fibronectin values were significantly lower than controls and opsonin dependent phagocytic function was depressed. The authors suggested a deficiency of circulating opsonins (namely fibronectin) may mediate the dysfunction in phagocytosis. Dillon et al. (1982) found that both phagocytic activity and plasma fibronectin concentration in rats starved for five days was significantly depressed compared to normal, fed controls. When plasma was incubated with supplemented fibronectin, phagocytic activity was restored. Fibronectin concentration correlates with other measures of nutritional status, and is depleted in response to nutritional deprivation. Fibronectin concentration appears to be a sensitive indicator of nutritional status.

Although MHC class II expression has been studied in models of PEM, the effect of acute starvation on MHC class II expression has not been studied. As previously mentioned, both phagocytosis and fibronectin were influenced in models of acute nutritional deprivation. Because MHC class II expression is dependent on the ability of macrophages to phagocytose foreign antigens, it follows that if phagocytic ability was depressed, MHC class II expression was also likely depressed. MHC class II expression may therefore be a sensitive indicator for nutritional status assessment.

During states of acute starvation, it seems that cellular function of the immune system is affected; however, the nature of these responses is unclear. Conflicting results in current literature point to the need for further research in this area. Potential effects of acute starvation on the immune system include depressed lymphocyte number and proliferation, depressed leukocyte number, depressed DTH response, reduced macrophage activity, depressed MHC class II
expression, and decreased plasma fibronectin. The depression of any component of the immune system may increase the susceptibility of the host to infection. Acute nutritional deprivation appears to result in adverse effects on immune function and may provide a basis for studying the sensitivity of specific immune function tests to nutritional status.

The phagocytic ability of macrophages, plasma fibronectin concentration, and MHC class II expression have been shown to be sensitive to nutritional status. Current research indicates that both phagocytic activity and plasma fibronectin concentration are sensitive to acute nutritional deprivation. The relationship between phagocytic activity and fibronectin concentration, and previous evidence reporting their sensitivity to acute dietary alterations, lead to the use of these variables as immunological indicators of nutritional status in this study. The measurement of MHC class II expression on monocytes was chosen as an immunological indicator of nutritional status due to its relationship to phagocytosis, evidence that its expression is decreased in PEM, and because current literature lacks studies of MHC class II expression during acute nutritional deprivation. The determination of the sensitivity of these immune function tests will serve to identify if they are potentially useful indicators of acute changes in nutritional status.
ABSTRACT

The sensitivity of monocyte phagocytic activity, major histocompatibility complex (MHC) class II expression, and fibronectin concentration were studied in 23 healthy cats during a 7-day fast followed by a 7-day refeeding period. Immune cell function tests, and body weight were determined on days 0, 4, 7, 11, and 14. Phagocytic activity, weight, lymphocyte number, white blood cell number, MHC class II expression, and percent lymphocytes decreased (p<0.05) during the starvation period. Fibronectin concentration increased (p<0.05) by day 4 of starvation then decreased by day 7. During refeeding there was a increase (p<0.05) in MHC class II, fibronectin, weight, lymphocyte number, percent lymphocytes, and white blood cell (WBC) number when compared to day 7. Macrophage phagocytic activity decreased (p<0.05) on day 11, followed by an increase on day 14. There was a positive correlation (p<0.05, r=.3588) between monocyte number and MHC class II expression, and monocyte number and WBC number (p<0.05, r=.3506). There was a positive correlation (p<0.05, r=.2682) between weight change and phagocytic activity. In conclusion, MHC class II expression, and phagocytosis were found to be reliable indicators of nutritional status during acute nutritional deprivation; however, fibronectin was not found to be a reliable indicator of nutritional status as it lacked consistency in its response to nutritional depletion and repletion.
INTRODUCTION

Malnutrition remains a common problem among hospital patients in North America (1,2). During hospital stays, patients often suffer from disease related anorexia, and/or are held off food for several days prior to and following major surgical procedures. Acute bouts of food deprivation in hospitalized patients may induce a state of malnutrition, compromise immune function, and subsequently lead to increased incidence of complications (3). Malnourished hospitalized patients suffer more complications, more infections, stay in the hospital for longer durations, and have higher mortality rates than well-nourished patients (1,4). Optimal functioning of the immune system is necessary for the host to be capable of mounting a sufficient immune response, especially in times of sickness or injury. Identifying those patients who are malnourished in order to provide prompt nutritional intervention to improve nutritional status is critical. The status of the immune system has been suggested as a potential indicator of nutritional status; however, specific tests of immune function which are sensitive to nutritional status, specifically acute starvation, have not been clearly identified.

Functional indicators provide the opportunity to identify those patients at risk for malnutrition prior to obvious signs and symptoms associated with current nutritional assessment practices. Functional indicators are thought to be sensitive to changes in nutritional status by identifying defective function at the cellular level despite an overall apparent adequate nutritional status (5). The function of the immune system relies upon nutrient dependent metabolic pathways and the provision of adequate nutrient substrates to synthesize its components.

The capability of monocytic immune cells to phagocytize foreign antigens and present them in association with the major histocompatibility complex (MHC) molecules are potential functional indicators of nutritional status. Macrophage function is depressed in response to acute starvation (6,7). MHC class II expression has not been widely studied in times of acute starvation; however, studies in protein-deprived mice and malnourished surgical patients have shown this to be sensitive to nutritional status (8,9). A recent addition to the list of immune protein measurements sensitive to nutritional status is plasma fibronectin concentration. Plasma fibronectin is a glycoprotein with a short half life of 4-24 hours. The primary function of fibronectin appears to be related to its adhesion capabilities, including phagocytic binding and uptake of foreign antigens. Plasma fibronectin appears to facilitate the uptake of such particles.
by functioning as an opsonic glycoprotein. Plasma fibronectin has been considered for use in assessment of nutritional status based on studies that demonstrate low fibronectin levels during periods of acute nutritional deprivation (10,11,12). In vitro fibronectin supplementation has been shown to promote ingestion of foreign antigens by monocytes in a dose response fashion (13,14). There may therefore be a relationship between MHC class II expression, phagocytosis, and fibronectin.

The objective of this research was to determine the sensitivity of specific immune function tests to acute starvation. More specifically: are monocyte phagocytic capability, MHC class II expression, and plasma fibronectin concentration sensitive to acute nutritional alterations?

MATERIALS AND METHODS

Animals and Facilities

Twenty-three (4-7 years age) healthy, adult, domestic shorthaired, neutered cats were used in this study. Cats were housed in individual steel cages (24” X 24”) and kept in a climate and light controlled environment. Cats were current on vaccinations, deworming, and were fed a nutritionally complete dry dieta to maintain an optimal body weight and condition score prior to the study.

Experimental Protocol

Study protocols were approved by the Virginia Polytechnic Institute and State University Animal Care Committee. The cats were randomly divided into three groups of 8, 8, and 7 cats each. One group at a time was held off-food for seven consecutive days, and then refed their normal, portion-controlled diets to meet calculated daily energy requirements. Fresh water was available at all times. During treatment cats were housed in a separate room under the same conditions as the remaining colony cats. Blood samples were collected on days 0, 4, 7, 11 and 14. Fluid loss from blood collection was replaced subcutaneously with 30 ml/lb lactated ringers solution. Hydration status was monitored daily by checking tackiness of mucus membranes and skin elasticity. Body weight was recorded on all blood collection days. Complete blood count with differential (CBCD) as well as albumin, alkaline phosphatase, blood urea nitrogen, and total protein were monitored on all blood collection days to evaluate the health of the cats.

a Science Diet®, Feline Maintenance Light. Hills Pet Nutrition, Inc., Topeka, KS.
**Blood Collection and Processing**

On each sample collection day (days 0, 4, 7, 11, 14), blood was collected via jugular venipuncture. Whole blood samples were collected from each cat into one 7 ml and one 3 ml EDTA Vacutainer® tube for analysis of immune function tests and CBCD, respectively. Blood was also collected into a 3 ml Vacutainer® tube containing sodium heparin for serum chemistry analysis. Serum chemistry and CBCD were analyzed by the Clinical Pathology Laboratory at the Virginia-Maryland Regional College of Veterinary Medicine. All immune function tests except fibronectin were performed on fresh, whole blood.

For fibronectin analysis, one ml of whole blood was centrifuged for 20 minutes at 1150 x g to separate plasma. Two hundred fifty microliters of plasma was extracted and placed in a microcentrifuge tube. Aprotinin (Boehringer Mannheim Corp. Indianapolis, IN), a protease inhibitor, was added to block enzymatic breakdown of the fibronectin protein. The samples were frozen at -4°C until analysis.

Mononuclear (lymphocytes and monocytes) cell (MC) populations in whole blood samples were separated and purified using a double density Ficoll separation technique (15). All procedures were performed under sterile conditions, in a laminar flow hood. Briefly, 3 ml of 1.119 Histopaque (Sigma St. Louis, MO) was added via a 20 gauge needle to a 15 ml conical tube, followed by layering 3 ml of Histopaque 1.077 (Sigma St. Louis, MO) over the 1.119 layer. Six milliliters of whole blood was then carefully layered via an automatic pipette over the 1.077. After the lid was placed securely on the conical tube, it was centrifuged at 725 x g for 30 minutes at room temperature with no brake. Separation of MC cells occurred during centrifugation resulting in two identifiable interfaces in the conical tube. The MC cell interface was aspirated via a Pasteur pipette and delivered to a separate 50 ml conical tube.

Mononuclear cells were washed twice with Hanks balanced salt solution (HBSS) (Gibco/Brl, Grand Island, NY) then centrifuged for ten minutes at 200 x g. Cells were then washed in complete media (87 ml RPMI 1640, 10 ml fetal bovine serum, 1 ml L-glutamine, 1ml Sodium pyruvate, 1 ml Pen-strep) and centrifuged for 20 minutes at 450 x g to remove any platelets. After the complete media wash, cells were brought up in 2 ml HBSS for cell counting.
Cells were counted with a hemocytometer, and adjusted to appropriate concentrations for immunoassays.

**Measurement of Monocyte MHC Class II Expression**

Mononuclear cells were adjusted to a concentration of $1.0 \times 10^6$ cells/ml in HBSS. Half of the MC cells were incubated with primary MHC class II monoclonal antibody [anti MHC class II for feline - TH14B, diluted 1:10 in HBSS, VMRD, Pullman, WA] (MHC +) while the control cells were incubated with no antibody (MHC-). Cells were centrifuged at 200 x g, supernatant was poured off, and cells were resuspended in HBSS. All cells were then incubated with Fluorescein Isothiocyanate (FITC) labeled secondary antibody (Jackson ImmunoResearch, West Grove, PA), and resuspended in HBSS. Cells were then centrifuged at 200 x g, and resuspended in paraformaldehyde. The cells were then read on a flow cytometer (Flow Coulter Epics Excel, FL.) set to emit at 525 nm. Determination of the expression of MHC class II molecules was measured as the mean channel fluorescence and the percent cells resulting from the subtraction of the MHC- sample from the MHC+ sample.

**Monocyte Phagocytosis Assay**

The phagocytic capability of monocytes was determined by measuring fluorescent polystyrene bead uptake (16). Mononuclear cells were adjusted to a concentration of $1 \times 10^6$ cells/ml in Krebs Ringer Bicarbonate - gelatin solution. Cells were divided into two tubes labeled for 4°C and 37°C and 100 μl fetal bovine serum and 20 μl Fluoresbrite® beads (Polysciences, Warrington, PA) were added to each tube. Tubes were then incubated at their respective temperatures for 60 minutes. Phosphate buffered saline - gelatin - EDTA solution was then added to each tube to terminate the reaction, cells were centrifuged at 200 x g, and then resuspended in HBSS. Determination of phagocytic activity was assessed as the mean channel fluorescence and the percent of cells resulting from the subtraction of the 4°C control sample from the 37°C sample after being read on the flow cytometer set to emit at 525 nm.
Fibronectin Assay

Analysis of plasma fibronectin concentration was performed using an in vitro turbidimetric immunoassay for quantitative determination of human fibronectin (Boehringer Mannheim Indianapolis, IN). Standard solutions (0, 37, 133, 236, 346, 642, 948 μg/ml) were thawed and both standards and samples were warmed to room temperature. Standards were measured on a spectrophotometer (Milton Roy Spectronic 1001 Plus) and a standard curve was constructed. Absorbance was read at 60 seconds and again at 10 minutes at 365 nm. Absorbance change was then determined by subtracting absorbance measurement at 60 seconds from that at 10 minutes. Sample absorbance measurements were performed in exactly the same manner as standards. Absorbance change was then used to extrapolate the plasma fibronectin concentration from the standard curve.

Statistical Analysis

For each parameter measured, a dependent t-test was used to determine significant differences between baseline values and each subsequent measure, and between day 7 and each subsequent measure. Pearson’s correlation analysis was performed to assess linear relationships between weight and immune function tests, and monocyte number and immune function tests. Pearson’s correlation analysis was also used to assess linear relationships among the immune function tests measured. Weight was used as a measure of nutritional health with which the immune function tests were compared in order to determine if they were similar in sensitivity to acute nutritional deprivation.

RESULTS

All 23 cats completed the study without complication. The differences in mean monocyte number, percentage of phagocytic activity, mean percentage of cells expressing MHC class II, body weight, and fibronectin concentration are shown in Table 1. Results from the dependent t-test analysis of differences between days are presented in Figure 1 (MHC class II), Figure 2 (fibronectin concentration), and Figure 3 (Phagocytosis) as mean differences from baseline.
TABLE 1. Summary of Mean and Standard Deviation Values for Monocyte Number, Percent Phagocytosis, MHC class II Expression, Weight, and Fibronectin Concentration.

<table>
<thead>
<tr>
<th></th>
<th>Baseline</th>
<th>Day 4</th>
<th>Day 7</th>
<th>Day 11</th>
<th>Day 14</th>
</tr>
</thead>
<tbody>
<tr>
<td>Monocyte No. (x 10^3 /µl)</td>
<td>.334±.2626</td>
<td>.255±.2043</td>
<td>.267±.2702</td>
<td>.534±.7010#</td>
<td>.421±.4833</td>
</tr>
<tr>
<td>Phagocytosis (%)</td>
<td>11.73±7.81</td>
<td>7.62±3.90*</td>
<td>7.56±3.03*</td>
<td>5.96±3.16*#</td>
<td>7.05±3.41*#</td>
</tr>
<tr>
<td>MHC class II expression (%)</td>
<td>37.11±15.80</td>
<td>33.02±13.31</td>
<td>27.94±12.15*</td>
<td>47.71±17.85#</td>
<td>32.89±14.00#</td>
</tr>
<tr>
<td>Weight (lb.)</td>
<td>10.13±1.71</td>
<td>9.18±1.54*</td>
<td>8.77±1.50*</td>
<td>9.07±1.50*#</td>
<td>9.10±1.48*#</td>
</tr>
<tr>
<td>Fibronectin (µg/ml)</td>
<td>96.75±33.36</td>
<td>123.67±32.47*</td>
<td>98.38±20.75</td>
<td>112.53±24.60#</td>
<td>123.67±42.21*#</td>
</tr>
</tbody>
</table>

*significantly different from baseline (p<0.05)  
#significantly different from day 7 (p<0.05)

The differences in mean albumin, mean lymphocyte number, mean percent lymphocytes, and mean WBC number are shown in Table 2.

TABLE 2. Summary of Mean and Standard Deviation Values for Albumin, Lymphocyte Number, Percent Lymphocytes, and White Blood Cell Number.

<table>
<thead>
<tr>
<th></th>
<th>Baseline</th>
<th>Day 4</th>
<th>Day 7</th>
<th>Day 11</th>
<th>Day 14</th>
</tr>
</thead>
<tbody>
<tr>
<td>Albumin (g/dl)</td>
<td>3.05±.150</td>
<td>3.09±.166</td>
<td>3.08±.161</td>
<td>2.99±.177*#</td>
<td>2.98±.204*#</td>
</tr>
<tr>
<td>Lymph. No. (x 10^3 /µl)</td>
<td>2.91±1.22</td>
<td>2.34±.849*</td>
<td>2.41±1.20</td>
<td>3.14±1.73#</td>
<td>2.88±1.20#</td>
</tr>
<tr>
<td>% Lymphocytes</td>
<td>30.59±14.09</td>
<td>27.45±9.71</td>
<td>24.57±8.59*</td>
<td>27.96±9.79#</td>
<td>28.70±10.52#</td>
</tr>
<tr>
<td>WBC No. (x10^3 /µl)</td>
<td>10.97±5.27</td>
<td>9.20±4.25*</td>
<td>10.34±4.49</td>
<td>11.28±4.67#</td>
<td>10.68±5.08#</td>
</tr>
</tbody>
</table>

*significantly different from baseline (p<0.05)  
#significantly different from day 7 (p<0.05)

Pearsons correlation analysis revealed a positive correlation (p<0.05, r=.2682) between weight change and phagocytic activity. There was a positive correlation (p<0.05, r=.3588) between monocyte number and MHC class II expression, and between monocyte number and WBC number (p<0.05, r=.3506).
Figure 1. Mean Differences Between Days and Standard Error of the Mean Differences for MHC class II Expression
*significantly different from baseline (p<0.05)
#significantly different from day 7 (p<0.05)

Figure 2. Mean Differences Between Days and Standard Error of the Mean Differences for Phagocytic Activity
*significantly different from baseline (p<0.05)
#significantly different from day 7 (p<0.05)

Figure 3. Mean Differences Between Days and Standard Error of the Mean Differences for Fibronectin Concentration
*significantly different from baseline (p<0.05)
#significantly different from day 7 (p<0.05)
DISCUSSION

Although it is widely accepted that poor nutritional status is associated with immunosuppression and poor outcome (1,17), an ideal method or index of nutritional status has not been identified. Routinely used nutritional assessment parameters, which are considered static indices of nutritional status, include anthropometric, biochemical, and clinical measures. The disadvantages to using such measures in assessment of nutritional status include lack of sensitivity and accuracy in detecting acute dietary alterations.

Monocyte phagocytosis was sensitive to dietary alterations. Monocyte phagocytic activity decreased significantly during the starvation period. Studies involving the acute starvation of murine models have shown phagocytic function to be significantly suppressed by four days of starvation (6,7). A decrease in phagocytosis may be related to increased glucocorticoid production. The result of internal or external stress in the form of starvation is known to cause the release of glucocorticoid hormones in the body. In murine models of protein-energy malnutrition (PEM), macrophage function was significantly impaired while serum glucocorticoid levels were elevated (18). Although not measured in this study, glucocorticoids have been found to down-regulate numerous macrophage functions, including the synthesis of cytokines, phagocytosis, and cell surface expression of MHC class II (19). These combined effects may have been the cause of the decrease seen in phagocytosis. There was a positive linear correlation between phagocytosis and weight in the starvation period of this study. Body weight is an indirect marker of protein mass and energy stores. When food intake is less than nutrient loss, body energy in the form of protein, glycogen, and fat is used for fuel causing total body weight to decrease. Weight is therefore known to be influenced by acute and chronic nutrient deprivation. The positive correlation between weight and phagocytosis demonstrated that phagocytosis and body weight are similar in sensitivity to nutritional intake. During refeeding, phagocytosis continued to decrease by day 11, followed by an increase on day 14. Phagocytic activity after seven days of refeeding remained depressed as compared to baseline. Although macrophage function has been shown to be impaired in prolonged PEM (20), the effect of refeeding on macrophage function has not been studied. This study suggests that the recovery of phagocytic function may be delayed in response to nutritional repletion.

Monocyte MHC class II expression decreased significantly during starvation. In murine models, PEM is associated with impaired macrophage activation, reduced MHC class II
expression, impaired antigen presentation, and reduced responsiveness to lymphokines (8,20). Appropriate up-regulation of monocyte MHC class II expression is made possible by the lymphokine interferon gamma (IFN-γ). Although levels of IFN-γ have not been measured in models of acute starvation, it is possible that a decrease in its production may have down-regulated MHC class II expression. In rats MHC class II expression was inhibited by in vitro and in vivo administration of glucocorticoids (19). It is possible that as with phagocytosis, increased glucocorticoid levels may have affected MHC class II expression. MHC class II expression increased significantly during the early refeeding period. It is possible that the increase in MHC class II expression in the initial days of refeeding may have been a compensation mechanism to restore function after it had declined during the starvation period. The positive correlation between MHC class II and monocyte number suggests that the expression of MHC class II is somewhat dependent on monocyte number. This association is presumably related to the fact that MHC class II is expressed on monocytes. MHC class II appeared to be sensitive to acute nutritional deprivation and refeeding.

Previous studies have indicated that antibodies to human fibronectin are cross reactive to feline fibronectin, validating the use of the fibronectin kit (21). Fibronectin concentration increased significantly by day 4 followed by a drop back to baseline levels by day 7 of starvation. These results are not in agreement with those seen previously where fibronectin concentration decreases in response to starvation (10,11,12). The initial increase in fibronectin seen in this study may have been an acute-phase protein type of response. Starvation is known to cause release of glucocorticoid hormones in the body. Glucocorticoid hormones are responsible for the increase in acute-phase proteins seen in response to stress. It has been well established that fibronectin is involved as an opsonin in the reticuloendothelial phagocytosis of particulates and bacteria. It is possible that fibronectin was up-regulated in response to starvation related to its opsonin function. Many of the studies performed on fibronectin during acute starvation have been done with humans. Humans are widely recognized as a glucocorticoid resistant species, whereas other species are considered glucocorticoid sensitive (22). The results of this study may not be in agreement with previous studies in humans if the cat species is glucocorticoid sensitive. This sensitivity would have invoked a more pronounced acute phase protein response in the cats.

Fibronectin has been found to be up-regulated by both interleukin-1 (IL-1), and transforming growth factor-β (TGF-β) at the level of mRNA transcription (23). Studies have found an increase in the production of IL-1 and TGF-β in models of acute and chronic starvation.
It is possible that the levels of one or both of these acute phase proteins were elevated in the cats during starvation causing the increased transcription of mRNA for fibronectin. During refeeding, fibronectin concentration increased by 25% as compared to day 7 of starvation. Several studies support the effect of an upward trend in response to refeeding in fibronectin concentration (10,11,25). This is likely the result of an increase in the availability of substrates required to synthesize the protein.

Although a relationship between phagocytosis, MHC class II expression, and fibronectin may be hypothesized based on their interrelated functions, there were no significant correlations between these parameters. A relationship between fibronectin and phagocytic host defense is well established in that fibronectin is a strong opsonin. In this study, phagocytosis decreased while fibronectin increased during starvation. As mentioned earlier, fibronectin concentration may have increased in order to stimulate phagocytic activity as a protective mechanism in response to starvation. A similar pattern of increasing fibronectin and decreasing phagocytosis was apparent from baseline to day 4, and from day 7 to day 11. As this pattern was followed by either stabilized or increased levels of phagocytosis in both cases, it is possible that the increase in fibronectin was a mechanism to upregulate phagocytosis. Two other important opsonins involved in phagocytosis are the C3 protein and IgG. In a study where rats were starved for five days, phagocytic activity, opsonic activity, C3, and fibronectin were significantly lower, while IgG was significantly higher than fed control rats (25). Opsonic activity was fully restored when plasma was incubated with fibronectin concentrations equal to the total deficit measured after starvation. Although fibronectin is a strong opsonin of phagocytosis in vitro, it is unclear whether it has a direct effect on phagocytosis in vivo. Because of its involvement in phagocytosis, it is also possible that there was a decrease in C3 protein in this study that mediated the decrease in phagocytosis.

Because MHC class II expression is a result of ingestion via phagocytosis and processing of antigens, with decreased phagocytosis a concurrent decrease in MHC class II expression was anticipated. Although a significant relationship between MHC class II expression and phagocytosis was not found in this study, both measures decreased significantly in response to starvation. However, during early refeeding MHC class II expression increased while phagocytosis continued to decrease. These results suggest that there may be independent mediators of both phagocytosis and MHC class II expression which control their response during nutritional alterations. This response may be different depending on the condition of the animal and its environment. Feline MHC class II expression occurs irrespective of whether the
cells are resting or activated, unlike the human immune system where expression is not detected until activation (26). This may explain why MHC class II expression and phagocytosis were not correlated in the cats studied. Monocyte number decreased by day 4 of starvation followed by a significant increase on day 11 of refeeding. In vivo pharmacological doses of glucocorticoids have been shown to induce substantial decreases in the number of circulating monocytes (27). The decrease in monocyte number during starvation, although not statistically significant, may have been in response to increased glucocorticoid production. Because monocyte function was measured in this study, it is possible that the decrease in monocyte number may have played a role in the decreases seen in both phagocytosis and MHC class II during the starvation period.

Other indicators of health that were influenced by nutritional status were lymphocyte number, percent lymphocyte, and white blood cell number. All indicators decreased significantly during starvation followed by a significant increase during the refeeding period. These cells appeared to “recover” quickly in response to refeeding. As one might expect, monocyte number and WBC number were positively correlated as WBC encompasses monocytes. Albumin did not change during the 7-day starvation period, however, it did decrease significantly during refeeding. This provides evidence that albumin is a poor indicator of acute nutritional status but rather reflects changes on a delayed basis due to its long half life.

In conclusion, in this investigation, plasma fibronectin, monocyte phagocytosis and MHC class II expression, were influenced by an acute bout of starvation and subsequent refeeding. These findings indicate that the host may have depressed immunological function when taken off food for short periods of time, and that immune function is sensitive to acute dietary alterations. This may result in a greater chance of infectious complication as a result of food restriction. The combination of an acute decrease in monocyte MHC class II expression, phagocytic ability, lymphocyte number, and WBC number implies that significant immunosuppression occurred in cats withheld food for a short period. Phagocytosis, fibronectin concentration, MHC class II expression, and other immunological measures of health status were sensitive to acute alterations in nutritional intake and subsequent refeeding. Phagocytic activity and MHC class II expression were found to be reliable, functional indicators of nutritional deprivation. Fibronectin was not found to be a reliable indicator of nutritional status as it lacked consistency in its response to nutritional depletion and repletion. Because there is strong research literature support of the use and sensitivity of fibronectin to nutritional status, this parameter deserves further consideration as an indicator of nutritional status. Further research is
necessary in order to identify the mechanisms by which these immunological effects are occurring and to identify if they occur in humans under the same conditions.

ACKNOWLEDGMENTS

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REFERENCES


Chapter 4

DISCUSSION

In this animal model of acute nutritional deprivation, all 23 cats completed the study without complication. Animals were aggressively monitored daily for overall health maintenance by trained veterinary professionals. No complications were encountered, and data for each measure was successfully obtained from cats throughout the duration of the study.

Fibronectin

Decreased plasma fibronectin concentrations in humans and animals have been reported during acute starvation (Scott et al. 1982; Cheslyn-Curtis et al. 1990; Chadwick et al. 1986). These results have been widely attributed to both increased utilization and decreased synthesis of fibronectin. In this study, fibronectin concentration increased significantly by day 4 followed by a drop back to baseline levels on day 7 of starvation. Howard et al. (1984) found that in women placed on a starvation diet, fibronectin concentrations increased significantly by day 2 followed by a significant decrease below baseline on day 5. The increase of fibronectin concentration on day 2 was attributed to hemoconcentration. In this study, fluid loss from blood collection was replaced subcutaneously and signs of dehydration were monitored closely in the cats. It is possible that hemoconcentration contributed to the significant rise in fibronectin concentration, however, there were no indications or signs of dehydration seen in the cats during the study. Fibronectin is a dimer of two large polypeptide chains and is synthesized by endothelial cells, fibroblasts, and hepatocytes. It is possible that with acute deprivation of ingested protein fibronectin synthesis may decline. This may provide an explanation for the decrease in fibronectin seen between day 4 and day 7 of starvation.

The result of internal or external stress in the form of infection, trauma, environment change, or starvation is known to cause the release of glucocorticoid hormones in the body. These hormones act in immunoregulation in response to an insult experienced by the host. Glucocorticoid hormones are responsible for the increase in acute-phase proteins seen in response to stress. The initial increase in fibronectin may have been an acute-phase protein type of response. It has been well established that fibronectin is involved as an opsonin in the reticuloendothelial phagocytosis of particulates and bacteria. An opsonin is any protein that acts
to enhance the phagocytosis of bacteria by leukocytes, either by binding to the particle and the cell and thereby linking particle and cell together, or by binding to the particle alone, altering its surface properties in such a way as to increase its attachment to, and ingestion by, the phagocyte (Bodmer, 1985). As an opsonin, it is possible that fibronectin was up-regulated in response to starvation. Many of the studies performed on fibronectin have been done with humans. Humans are widely recognized as a glucocorticoid resistant species, whereas other species are considered glucocorticoid-sensitive (Woodward, 1998). The potential for cats to be a glucocorticoid sensitive species may provide an explanation for the conflicting results of this study as compared to previous studies. Glucocorticoid sensitivity would have envoked a more pronounced acute phase protein response in the cats.

Fibronectin has been found to be up-regulated by both interleukin-1 (IL-1) and transforming growth factor-β (TGF-β) at the level of mRNA transcription (Wong et al. 1989). Moriguchi et al. (1989) found a significant increase in the production of IL-1 by alveolar macrophages during 6 days of starvation in rats. In starved patients diagnosed with anorexia nervosa, TGF-β was significantly elevated (Pomeroy et al. 1994). It is possible that the levels of one or both IL-1 and TGF-β may have been elevated in the cats during early starvation resulting in the up-regulation of fibronectin synthesis. Studies measuring IL-1 and TGF-β in conjunction with fibronectin concentration during starvation have not been performed. Fibronectin concentration increased by 25% as compared to day 7 of starvation during the refeeding period in this study. Several studies support the effect of an increase in fibronectin following refeeding (Scott et al. 1982; Dillon et al. 1982; Howard et al. 1984). This is likely the result of an increase in the availability of substrates required to synthesize the protein. Although fibronectin concentration in cats was found to be sensitive to both acute nutritional deprivation and refeeding, it was found to be an inconsistent indicator of nutritional status in this study.

There was variation in fibronectin concentration among the individual cats. Variability in plasma fibronectin has also been shown in humans. Stathakis et al. (1981) found that age, sex, and the presence of various disease states had a strong influence on plasma fibronectin levels. These factors may need to be taken into consideration for each individual when evaluating fibronectin concentrations. The value of fibronectin in the assessment of nutritional status may therefore lie in the evaluation of changes in fibronectin levels among individuals rather than in group averages.
Phagocytosis

In this study, monocyte phagocytosis decreased significantly during the starvation period. This is in agreement with previous research where phagocytosis was decreased significantly in rat alveolar macrophages by day 4 of starvation (Moriguchi et al. 1989; Sakai et al. 1990). The significant decrease in phagocytosis by day 4 has been attributed to increased glucocorticoid production (Hill et al. 1995). In murine models of PEM, macrophage function was significantly impaired while serum glucocorticoid levels were elevated (Hill et al. 1995). Glucocorticoids down-regulate numerous macrophage functions, including the synthesis of cytokines, phagocytosis, and cell surface expression of MHC Class II (Snyder et al. 1982). This effect may have been a factor in the decreased macrophage phagocytic activity seen in this study. In many studies, in vivo pharmacological doses of glucocorticoids induced substantial decreases in the number of circulating monocytes (Cupps et al. 1982). The number of monocytes decreased during the starvation period of this study, followed by a significant increase during refeeding. Although the decrease seen in monocyte number was not significant, it is possible that it may have played a role in the decrease seen in monocyte phagocytosis. The degree to which glucocorticoids exert their effect on macrophages and at what point during the stress response this effect is seen, is unclear.

During the starvation period in this study there was a significant linear correlation between phagocytosis and weight. Weight is considered sensitive to acute dietary alterations, decreasing in response to starvation as body stores are used for energy in the absence of exogenous nutrient substrates. The correlation between phagocytosis and weight demonstrated the similarity in sensitivity of phagocytosis and body weight to dietary alterations. During refeeding, phagocytosis continued to decrease. Macrophage function has been shown to be impaired in prolonged PEM (Redmond et al. 1991; Welsh et al. 1996); however, the effect of refeeding on macrophage function has not been studied. This study suggests that recovery of phagocytic function may be delayed in response to nutritional repletion as values were approximately 50% baseline after 7 days of refeeding. Phagocytic ability was found to be a sensitive indicator of nutritional status during acute nutritional deprivation; however, it appeared to be less sensitive to short term nutritional repletion.

MHC Class II

In this study, MHC Class II expression decreased significantly during starvation. In murine models, protein malnutrition is associated with impaired macrophage activation, reduced
MHC class II expression, impaired antigen presentation, and reduced responsiveness to lymphokines (Reynolds et al. 1992; Conzen et al. 1988). Lymphokines or cytokines are substances produced by lymphocytes that are involved in signaling between immune cells during immune responses. Appropriate up-regulation of monocyte MHC Class II expression is made possible by the lymphokine interferon gamma (IFN-\(\gamma\)). Although IFN-\(\gamma\) production has not been measured in models of acute or chronic starvation, it is possible that a decrease in IFN-\(\gamma\) may have down-regulated MHC Class II expression in response to acute nutritional deprivation. Glucocorticoids, in response to stress, suppress the ability of IFN-\(\gamma\) to activate cells of the immune system (Brown et al. 1997).

Because MHC Class II expression on macrophages is a result of ingestion via phagocytosis and processing of antigens, with decreased phagocytosis a concurrent decrease in MHC Class II expression was anticipated. This is supported by the significant decrease seen in both phagocytosis and MHC Class II expression during the starvation period of this study. However, during the early refeeding period MHC Class II expression increased significantly while phagocytosis continued to decrease. These results suggest that phagocytic ability may not be related directly to MHC Class II expression. Rather, there may independent mediators of both phagocytosis and MHC Class II expression which control their response. This response may be different depending on the condition of the animal and its environment. It is possible that the significant increase in MHC Class II expression on day 11 may have been a compensation mechanism to restore function after it had declined during the starvation period. There was a positive correlation between monocyte number and MHC Class II expression. This relationship is likely related to the fact that MHC class II is expressed on monocytes, therefore denoting that a decrease in monocyte number will be in association with a decrease in MHC class II expression.

As mentioned previously, Synder et al. (1982) found that in rats MHC Class II expression was inhibited in vitro and in vivo with administration of glucocorticoids. It is possible that as with phagocytosis, if the glucocorticoid levels increased in the animals, it may have affected MHC Class II expression.

**Relationships Among Fibronectin, Phagocytosis, and MHC Class II Expression**

A relationship between fibronectin and phagocytic host defense is well established in that fibronectin is an opsonin. Fibronectin deficiency in trauma, septic, and surgical patients who develop multiple organ failure has been implicated in dysfunction of phagocytic activity (Saba...
et al. 1983). In vitro analysis of the effect of fibronectin supplementation on monocytes has shown a dose response relationship between fibronectin and phagocytosis; as fibronectin concentration increased phagocytic activity increased (Simpson et al. 1984; Pommier et al. 1983). As previously mentioned, studies have shown that both fibronectin and phagocytosis decreased in response to acute starvation. In this study, phagocytosis decreased while fibronectin increased during starvation. Fibronectin concentration may have increased in order to stimulate phagocytic activity as a protective mechanism in response to starvation. A similar pattern of increasing fibronectin and decreasing phagocytosis was apparent from baseline to day 4, and from day 7 to day 11. As this pattern was followed by either stabilized or increased levels of phagocytosis in both cases, it is possible that the increase in fibronectin was a mechanism to upregulate phagocytosis. Two other important opsonins involved in phagocytosis are the C3 protein and IgG. Dillon et al. (1982) studied phagocytic function, opsonic activity, C3, IgG, and fibronectin levels in rats starved for 5 days. As compared to control rats, phagocytic activity, opsonic activity, C3, and fibronectin were significantly lower, while IgG was significantly higher in the starved animals. When plasma was incubated with fibronectin at concentrations equal to the total deficit measured after starvation, opsonic activity was fully reversed. It was concluded that fibronectin was a strong opsonin of macrophages; however, it is unclear whether there was a direct effect of fibronectin on phagocytosis in vivo. Because of its involvement in phagocytosis, it is possible that in this study a decrease in C3 protein could have played a role in the decrease in phagocytosis.

Feline MHC class II expression occurs irrespective of whether the cells are resting or activated, unlike the human immune system where expression is not detected until activation (Willett and Callanan, 1995). This may help to explain why MHC class II expression and phagocytosis were not correlated.

Other Health Measures

Albumin did not change during the 7-day starvation period; however it did decrease significantly during refeeding. This is due to the fact that albumin has a long half-life and is not affected acutely but rather reflects changes in nutritional status on a delayed basis. This supports previous evidence that albumin is a poor indicator of acute nutritional status.

Lymphocyte number, percent lymphocytes, and white blood cell number decreased significantly during starvation followed by a significant increase during the refeeding period. Lymphocyte number, percent lymphocytes, and white blood cell number were responsive to refeeding as all returned to baseline values. These cells therefore appeared to “recover” quickly
in response to feeding. Studies have shown conflicting results in lymphocyte response to starvation. Many studies have found no change in lymphocyte number, white blood cell number, or percent lymphocytes during bouts of acute starvation (Wing et al. 1983; Neuvonen et al. 1984; Holm et al. 1976).

It is unclear which subset of lymphocytes, B lymphocytes or T lymphocytes, were affected by starvation. A recent advance in immunology has revealed that there are two pathways of T lymphocyte differentiation, including the intrathymic and the extrathymic pathways (Ogawa et al. 1993). After four days of starvation in mice, Ogawa et al. (1993) found that intrathymic derived T-cell number was significantly suppressed by starvation while extrathymic T-cell number was not affected. Additional experiments revealed that an elevation of glucocorticoid levels in the mice during starvation was involved in the induction of these changes. It was concluded that intrathymic T-cell differentiation pathways were sensitive to starvation while extrathymic derived T-cell differentiation pathways were resistant to starvation. In this study, the decrease in total lymphocyte number and percent may have resulted from an increase in the level of glucocorticoid production. It is unclear, however, what subset of lymphocytes and what differentiation pathways of the lymphocytes were affected.

Overall, monocyte phagocytic ability was found to be the most sensitive indicator of nutritional status as it responded to four days of nutritional deprivation; however, it lacked sensitivity to nutritional repletion. Although it did not decrease significantly until day 7 of starvation, MHC class II expression was found to be sensitive to both acute nutritional deprivation and refeeding and was a good indicator of nutritional status. Fibronectin concentration was sensitive to acute nutritional deprivation and refeeding; however, it was inconsistent in its response to acute dietary alterations. As fibronectin increased during both starvation and refeeding, it may not be a reliable indicator of nutritional status, but rather an indicator of physiological response to stress.
Chapter 5

CONCLUSIONS

Hospitalized patients commonly suffer acute periods of starvation as a consequence of surgery or disease related anorexia. Acute bouts of starvation may cause immunological dysfunction in the already compromised hospital patient. Current nutritional assessment practices include primarily static rather than functional indices and therefore lack sensitivity to acute changes in nutritional status. The purpose of this study was to determine the effects of nutritional deprivation and subsequent refeeding on specific immune function tests. In this investigation, alterations in plasma fibronectin, monocyte function, and other measures of health status were evident in response to an acute bout of seven days of starvation and subsequent refeeding.

Fibronectin concentration was sensitive to both acute nutritional deprivation and subsequent refeeding; however, it was not found to be a reliable or consistent as an indicator of nutritional status. The effectiveness of using fibronectin concentration as a nutritional assessment tool must be investigated further. Because there is strong evidence in the research literature in support of the use and sensitivity of fibronectin to changes in nutritional status, this parameter deserves further consideration as an indicator of nutritional status. Although previous studies report that it is responsive to nutritional status, conflicting results in the exact response of fibronectin to starvation in this study point to the need for further examination of the mechanisms by which fibronectin is regulated. In particular, the effect of specific factors such as interleukins, and hormones such as glucocorticoids on the regulation of fibronectin synthesis needs further consideration. Due to its variability between individuals, the efficacy of using plasma fibronectin as a nutritional assessment tool in individuals as opposed to groups also requires further investigation.

Monocyte phagocytosis and MHC class II expression were found to be sensitive to both acute starvation and refeeding and are therefore concluded to be potential functional indicators of nutritional status. Further consideration of the regulatory effects of glucocorticoids on monocyte cell function is necessary in order to determine its influence on immune cell function during starvation.

In this study, no relationships were found among fibronectin, phagocytosis, and MHC Class II expression. The role of other opsonins such as C3 protein and IgG in the regulation of
phagocytosis needs further investigation. Although fibronectin, phagocytosis, and MHC Class II expression were all independently affected by starvation, their functional relationship to one another remains unclear and requires further investigation.

Body weight, lymphocyte number, percent lymphocytes, and white blood cell number were also found to be sensitive to acute nutritional deprivation and refeeding. These measures may therefore be helpful in the overall nutritional assessment of individuals. In agreement with previous studies, albumin was not found to be sensitive to acute changes in nutritional status presumably due to its long half life.

The results of this study indicate a decreased ability of the host to mount a sufficient immune response when deprived of food for short periods of time. The combination of an acute decrease in monocyte number, MHC class II expression, phagocytic capability, lymphocyte number, and WBC number after just four days of nutritional deprivation implies a significant process of immunosuppression occurred in cats following a short period of food deprivation. The findings of this study should provide a basis for future studies involving humans. Current research is lacking on hospitalized patients who are routinely withheld food for several days at a time. Investigations are needed to determine if previously well-nourished individuals who enter hospitals and are withheld from food for short periods exhibit changes in immunological function. The implications of these changes may potentially translate into unfavorable outcome variables.

Clearly this study revealed the dependence of immune function on the continuous provision of exogenous nutrients; however, the mechanisms by which dietary intake affects immune function needs further consideration. Overall, the results of this study demonstrate the importance of considering the immune system as sensitive to acute changes in the nutritional status of the host. Since immune function is of such importance to host resistance, an understanding of its relationship to nutrient intake may aid in determining the best approach to identification of altered nutritional status.
REFERENCES


Forse RA, Shizgal HM. Serum albumin and nutritional status. JPEN 1980;4:450-454.


Appendix A

Separation & Washing of Monocytes

Purpose: To separate and purify mononuclear and porphomononuclear cell populations in fresh, whole blood.

Materials

1. Histopaque 1.119 (Sigma, St. Louis, MO)
   Histopaque 1.077 (Sigma, St. Louis, MO)
2. 7 ml EDTA Vacutainer® tube.
3. 20 gauge needles
4. 15 ml conical tubes
   50 ml conical tubes
5. Hanks Balanced Salt Solution (HBSS) (Gibco/Brl, Grand Island, NY)
6. Complete Media: Mix 87 ml RPMI 1640; 10 ml 10% fetal bovine serum (FBS); 1 ml L-glutamine; 1 ml sodium pyruvate; 1 ml pen-strep (Sigma, St. Louis, MO)
7. Hemocytometer

Separation Procedures

1. Collect whole blood sample into one 7 ml EDTA Vacutainer® tube and place on rocker immediately until use.
2. Add 3 ml of 1.119 Histopaque to a 15 ml conical tube with a 20 gauge needle and syringe.
3. Slowly layer 3 ml of 1.077 Histopaque over the previous layer with a 20 gauge needle and syringe, being careful not to disturb the layers.
4. Layer 6 ml of whole blood over histopaque layers via automatic pipette. Transport and insert tubes gently into the centrifuge.
5. Centrifuge tube at 725 x g for 30 minutes at room temp. with no brake.
6. Aspirate the mononuclear cell interface with a Pasteur pipette and put into a 50 ml conical tube labeled with cat identification #.
Washing Procedures

1. Add 15 ml HBSS to the 50 ml conical tube containing the aspirated mononuclear cell interface, vortex, centrifuge at 200 x g for 10 minutes.

2. Pour off supernatant

3. Repeat above procedure once more (steps 1 & 2)

4. Add 10 ml of complete media, vortex, centrifuge at 450 x g for 20 minutes, with brake.

5. Pour off supernatant.

6. Bring up remaining cells left in the 50 ml conical tube in 2 ml HBSS.

7. Count cells on a hemocytometer. Based on the cell count, the remaining cells should be adjusted to the appropriate concentration for the assay.
Appendix B

Measurement of Monocyte Major Histocompatibility Complex Class II Expression

Purpose:
To measure the expression of class II glycoproteins of the major histocompatibility complex (MHC). After monocytes phagocytize and process antigens, they present the antigens on the cell surface via the MHC class II molecules, which activate the T-cell response to the antigen.

Materials:
1. Hanks balanced salt solution (HBSS) - (Gibco/Brl, Grand Island, NY)
2. Paraformaldehyde (10g paraformaldehyde, 5 g FA Bacto Buffer, 400ml distilled water)
3. Monoclonal antibody - anti MHC class II for feline (TH14B), diluted 1:10 in HBSS. (VMRD, Inc., Pullman, WA)
4. Fluorescein Isothiocyanate (FITC) - conjugated Affinipure Rabbit Anti-Mouse IgG (H+L). (Jackson ImmunoResearch Laboratories, Inc., West Grove, PA)
5. 12 X 75 mm polypropylene tubes

Method:
1. Obtain monocytes from whole blood samples as described in Appendix A
2. For each cat, label the following tubes as:
   -FITC
   -MHC
3. Dilute cells to 1.0 x 10^6 per ml in HBSS.
4. To each tube labeled as above add 500 µl of the adjusted cells.
5. To the MHC tube, add 2 µl of primary MHC class II monoclonal antibody, vortex.
6. Nothing is added to the FITC tube at this time.
7. Incubate tubes at 4 °C for 30 minutes.
8. Centrifuge tubes at 200 x g for 10 minutes, with brake.
9. Pour off supernatant.
10. Resuspend pelleted cells in 500 µl of HBSS and 2 µl of FITC labeled secondary antibody in both tubes.
11. Incubate tubes for 30 minutes at 4°C.
12. Centrifuge at 200 x g for 10 minutes, with brake.
13. Pour off supernatant.
14. Resuspend cells in 500 μl of HBSS or paraformaldehyde.
15. Read on flow cytometer set to emit at 525 nm.

Determination of the expression of MHC class II molecules is measured as the mean channel fluorescence and the percent cells resulting from the subtraction of the FITC only sample from the MHC-FITC sample.
Appendix C

Monocyte Phagocytosis Assay

Purpose:
To determine the phagocytic capability of monocytes by measuring their ability to phagocytize fluorescent polystyrene beads.

Materials:
1. Krebs Ringer bicarbonate - gelatin solution (KRH): Mix 97 ml H2O; 10 ml 9% NaCl; 616 μl 1 M KCl; 600 μl 0.5 M MgSO4; 20 ml 0.1 M Hepes - TEA (pH 7.4); 0.108 g Knox unflavored gelatin
2. 10% Fetal Bovine Serum (Sigma, St. Louis, MO)
3. Polystyrene beads - Fluoresentbrite® Beads, 1 micron / FITC conjugated (Polysciences), diluted 1:10 in phosphate buffer solution (PBS) - (Sigma, St. Louis, MO)
4. PBS-gelatin-EDTA: Mix 100 ml PBS; 0.1 g Knox unflavored gelatin; 0.1 g EDTA, disodium salt
5. 12 x 75 polypropylene tubes

Method:
1. Obtain monocytes from whole blood as described in Appendix A.
2. Dilute cells to 1 x 10⁶ cells/ml in HBSS.
3. Label two tubes with cat identification #, date, and one with 4°C, and the other with 37°C.
4. Add 1 ml of adjusted cells to each tube.
5. Centrifuge tubes at 200 x g for 10 minutes, with brake.
6. Pour off supernatant.
7. Add 1 ml KRH-gelatin solution to both tubes.
8. Add 100 μl of FBS.
9. Add 20 μl of 1:10 diluted polystyrene beads.
10. Incubate both tubes for one hour at either 4°C or 37°C.
11. Add 2 ml PBS-gelatin-EDTA to each tube.
12. Centrifuge at 200 x g for 10 minutes, with brake.
13. Pour off supernatant.
14. Resuspend in 500 µl HBSS.
15. Read on flow cytometer set to emit at 525 nm.

Determination of phagocytic activity is assessed as the mean channel fluorescence and/or the percent cells resulting from the subtraction of the 4°C control sample from the 37°C sample.
Appendix D
Fibronectin Assay

Materials:

1. 2 ml plastic cuvettes.
2. Boehringer Mannheim Fibronectin Opsonic Protein Assay Kit (Boehringer Mannheim Corp., Indianapolis, IN)
3. Aprotinin protease inhibitor (Boehringer Mannheim Corp., Indianapolis, IN)
4. Capped 1.0 ml and 0.5 ml microcentrifuge tubes.
5. Phosphate Buffer Solution (Sigma, St. Louis, MO)
6. 12 x 75 mm polypropylene tubes
7. 7 ml EDTA Vacutainer® tube

Method:

1. Collect 1.5 - 2.0 ml of whole blood via venipuncture in EDTA tube.
2. Remove 1 ml whole blood from the EDTA tube in which blood was collected.
3. Place the 1 ml of blood into a 1 ml microcentrifuge tube labeled with correct cat Id #.
4. Centrifuge blood sample for 20 minutes at 1150 x g.
5. Extract off 0.25 ml of plasma from each tube and place into a small 0.5 ml tube.
   a. If sample is to be assayed immediately, skip to #6.
   b. If sample is to be assayed at a later date, add Aprotinin dilution:
      -Add 10 mg Aprotinin and 1 ml H₂O, vortex.
      -make aliquot: remove 10 µl and place in small 0.5 ml tube.
      -add 90 µl of PBS, vortex.
      -to each 0.25 ml plasma sample, add 5 µl of this aprotinin solution, vortex.
6. Freeze samples at -4°C until analysis.

Assay Procedure for Fibronectin - A turbidimetric immunoassay kit

7. Thaw samples in refrigerator. Remove samples and standard solutions from the refrigerator and place in a 37°C incubator for 10 minutes prior to performing assay.
8. Prepare reaction mixture via enclosed package directions: 1 volume antiserum is diluted with 10 volumes antiserum buffer.
9. Prepare standards by adding 1 ml antiserum buffer from step #7 and 10 µl standard to a polypropylene tube, vortex, add to 2 ml cuvette.

10. Read absorbance on spectrophotometer at 365 nm 60 seconds after addition of standard to buffer, and 10 minutes after addition of standard - returning solution to tube in between to allow vortexing prior to second measure.

11. Calculate: absorbance measurement @ 10 min. - absorbance measurement @ 60 sec. to determine the change in absorption.

12. When all standards have been completed, construct a standard curve.

13. Sample preparation: add 1 ml antiserum buffer and 10 µl sample to polypropylene tube, wait 60 seconds, vortex, transfer to cuvette and read absorbance. Transfer solution back to tube until 10 minutes after addition of sample, vortex, transfer back to cuvette, and measure absorbance.

14. Calculate absorbance change as in #10.

15. Calculate the sample fibronectin concentration from the standard curve.
### Appendix E

## Detailed Results

Table 3. Summary of Mean and Standard Deviation Values for All Measures (number of cats with complete data for each day)

<table>
<thead>
<tr>
<th>Measure</th>
<th>Baseline Day</th>
<th>Day 4</th>
<th>Day 7</th>
<th>Day 11</th>
<th>Day 14</th>
</tr>
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<tr>
<td>Weight (lb.)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
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<td>10.13</td>
<td>9.18</td>
<td>8.77</td>
<td>9.07</td>
<td>9.10</td>
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<tr>
<td>Fibronecin (μg/ml)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>n=17</td>
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<td>% MHC Class II Expression</td>
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<td>.255</td>
<td>.267</td>
<td>.534</td>
<td>.421</td>
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</table>

Table 4. Mean Differences and Standard Error of the Mean Differences for Dependent T-test Analysis

<table>
<thead>
<tr>
<th>Measure</th>
<th>Day 4 - Day 0</th>
<th>Standard Error</th>
<th>Day 7 - Day 0</th>
<th>Standard Error</th>
<th>Day 11 - Day 0</th>
<th>Standard Error</th>
<th>Day 14 - Day 0</th>
<th>Standard Error</th>
<th>Day 11 - Day 7</th>
<th>Standard Error</th>
<th>Day 14 - Day 7</th>
<th>Standard Error</th>
</tr>
</thead>
<tbody>
<tr>
<td>Weight</td>
<td>-0.9486</td>
<td>0.0663</td>
<td>-1.354</td>
<td>0.0672</td>
<td>-1.056</td>
<td>0.0812</td>
<td>-1.023</td>
<td>0.0891</td>
<td>0.2978</td>
<td>0.026</td>
<td>0.3313</td>
<td></td>
</tr>
<tr>
<td>MHC class II expression</td>
<td>-3.94</td>
<td>4.67</td>
<td>-7.675</td>
<td>2.97</td>
<td>10.6</td>
<td>5.47</td>
<td>-4.527</td>
<td>4.69</td>
<td>20.78</td>
<td>4.946</td>
<td>5.04</td>
<td></td>
</tr>
<tr>
<td>Phagocytosis</td>
<td>-4.013</td>
<td>1.419</td>
<td>-4.183</td>
<td>1.616</td>
<td>-5.778</td>
<td>1.914</td>
<td>-4.686</td>
<td>1.739</td>
<td>-1.595</td>
<td>0.9533</td>
<td>-0.0504</td>
<td></td>
</tr>
<tr>
<td>% Lymphocytes</td>
<td>-2.857</td>
<td>2.763</td>
<td>-5.409</td>
<td>2.183</td>
<td>-1.863</td>
<td>2.709</td>
<td>-1.272</td>
<td>2.885</td>
<td>3.391</td>
<td>1.988</td>
<td>4.13</td>
<td></td>
</tr>
<tr>
<td>No. Lymphocytes (x10^3/μl)</td>
<td>-0.5596</td>
<td>0.2278</td>
<td>-0.4323</td>
<td>0.2343</td>
<td>0.3027</td>
<td>0.2666</td>
<td>0.0312</td>
<td>0.2371</td>
<td>0.7251</td>
<td>0.2323</td>
<td>0.4685</td>
<td></td>
</tr>
<tr>
<td>No. WBC</td>
<td>-1.866</td>
<td>0.8691</td>
<td>-0.5545</td>
<td>0.6536</td>
<td>0.2182</td>
<td>1.037</td>
<td>-0.2681</td>
<td>1.044</td>
<td>0.9391</td>
<td>0.537</td>
<td>0.3391</td>
<td></td>
</tr>
<tr>
<td>Albumin</td>
<td>0.0476</td>
<td>0.0225</td>
<td>0.0273</td>
<td>0.0229</td>
<td>-0.0818</td>
<td>0.0204</td>
<td>-0.955</td>
<td>0.0222</td>
<td>-0.957</td>
<td>0.0239</td>
<td>-0.1</td>
<td></td>
</tr>
<tr>
<td>Monocyte No. (x10^6/μl)</td>
<td>-0.0803</td>
<td>0.07386</td>
<td>-0.06276</td>
<td>0.0711</td>
<td>0.2167</td>
<td>0.1692</td>
<td>-0.0016</td>
<td>0.0852</td>
<td>0.2666</td>
<td>0.117</td>
<td>0.1535</td>
<td></td>
</tr>
</tbody>
</table>
Table 5. Dependent T-test Analysis to Test the Difference Between Given Days (p-values)

<table>
<thead>
<tr>
<th>Measurement Variable</th>
<th>Day 4 minus Baseline</th>
<th>Day 7 minus Baseline</th>
<th>Day 11 minus Baseline</th>
<th>Day 14 minus Baseline</th>
<th>Day 11 minus Day 7</th>
<th>Day 14 minus Day 7</th>
</tr>
</thead>
<tbody>
<tr>
<td>Weight</td>
<td>0.0000</td>
<td>0.0000</td>
<td>0.0000</td>
<td>0.0000</td>
<td>0.0000</td>
<td>0.0000</td>
</tr>
<tr>
<td>Fibronectin</td>
<td>0.04034</td>
<td>0.82036</td>
<td>0.05568</td>
<td>0.02165</td>
<td>0.0000</td>
<td>0.0000</td>
</tr>
<tr>
<td>% MHC Class II</td>
<td>0.40981</td>
<td>0.01828</td>
<td>0.06559</td>
<td>0.34553</td>
<td>0.0000</td>
<td>0.0000</td>
</tr>
<tr>
<td>% Phagocytosis</td>
<td>0.0101</td>
<td>0.01681</td>
<td>0.00632</td>
<td>0.01325</td>
<td>0.12943</td>
<td>0.94219</td>
</tr>
<tr>
<td>% Lymphocytes</td>
<td>0.31346</td>
<td>0.02179</td>
<td>0.49903</td>
<td>0.66365</td>
<td>0.0000</td>
<td>0.0000</td>
</tr>
<tr>
<td># Lymphocytes</td>
<td>0.0233</td>
<td>0.07923</td>
<td>0.26888</td>
<td>0.89647</td>
<td>0.0000</td>
<td>0.0000</td>
</tr>
<tr>
<td># WBC</td>
<td>0.04417</td>
<td>0.40575</td>
<td>0.83546</td>
<td>0.79969</td>
<td>0.00023</td>
<td>0.00214</td>
</tr>
<tr>
<td>Albumin</td>
<td>0.04693</td>
<td>0.24824</td>
<td>0.00064</td>
<td>0.00033</td>
<td>0.0000</td>
<td>0.0000</td>
</tr>
<tr>
<td>Monocyte #</td>
<td>0.2897</td>
<td>0.3878</td>
<td>0.2149</td>
<td>0.985</td>
<td>0.0328</td>
<td>0.1067</td>
</tr>
</tbody>
</table>

Table 6. Pearson Correlation Analysis of the Relationships Between Weight and Immune Function Tests

<table>
<thead>
<tr>
<th>Measurement</th>
<th>Pearson's r</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fibronectin</td>
<td>0.05668</td>
<td>0.6064</td>
</tr>
<tr>
<td>Phagocytosis</td>
<td>0.2682</td>
<td>0.0039</td>
</tr>
<tr>
<td>MHC class II</td>
<td>0.16783</td>
<td>0.0825</td>
</tr>
</tbody>
</table>

Table 7. Pearson Correlation Analysis of the Relationships Between Monocyte Number and Immune Function Tests, and Monocyte Number and Other Measures of Health Status

<table>
<thead>
<tr>
<th>Measurement</th>
<th>Pearson's r</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fibronectin</td>
<td>-0.0902</td>
<td>0.4116</td>
</tr>
<tr>
<td>Phagocytosis</td>
<td>-0.0036</td>
<td>0.97</td>
</tr>
<tr>
<td>MHC class II</td>
<td>0.3588</td>
<td>0.0002</td>
</tr>
<tr>
<td>% Lymph.</td>
<td>-0.1656</td>
<td>0.081</td>
</tr>
<tr>
<td>Lymph #</td>
<td>0.13474</td>
<td>0.1567</td>
</tr>
<tr>
<td>WBC #</td>
<td>0.3505</td>
<td>0.0002</td>
</tr>
</tbody>
</table>
Figure 4. Fibronectin Standard Curve (r=0.993)
Jeannine Cobbett Simon was born on November 29, 1971 in Rochester, New York. She attended undergraduate school at Virginia Polytechnic Institute and State University where she earned her Bachelors degree in Human Nutrition and Foods. In May of 1995, Jeannine married her soulmate Stephen G. Simon. She then competed her dietetic internship at Virginia Tech the following year and continued on to pursue her graduate degree in human nutrition at Virginia Tech. In August of 1997, Jeannine was offered a full-time position as Trauma Dietitian at a local Level I Trauma Center and after much soul searching, decided to take the job and complete her master’s degree at the same time. Upon completion of her master’s degree in human nutrition in May of 1998, she plans to continue her job as a clinical dietitian and eventually pursue her PhD in nutrition. Her ultimate goal is to teach and do research in the area of clinical nutrition and nutritional immunology.