Acute Starvation and Subsequent Refeeding Affect Lymphocyte Subsets and Proliferation in Cats

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ABSTRACT Although the early identification of patients with suboptimal nutritional status can allow the implementation of nutritional intervention to enhance the ability of the body to fight infection and disease, currently no definitive test of nutritional status exists. Therefore, this study was conducted to identify possible functional indicators of acute nutritional deprivation. The effects of total nutritional deprivation and subsequent refeeding on lymphocyte functions and subpopulations were examined in 23 healthy cats. Peripheral blood samples were analyzed at various times during food deprivation and refeeding periods. During the food deprivation period, decreases were observed in leukocyte number (P < 0.05), lymphocyte number (P < 0.05), percentage of CD4+ cells [before stimulation with concanavalin-A (Con-A); P < 0.05] and the CD4/CD8 ratio (before stimulation with Con-A; P < 0.01) compared with d 0. Increases were observed in the percentage of CD8+ cells [before (P < 0.05) and after (P < 0.01) stimulation with Con-A] and in intracellular calcium (P < 0.01) during acute starvation. During the refeeding period, increases were observed in the percentage of CD4+ cells (before and after stimulation with Con-A; P < 0.01), the percentage of CD8+ cells (before stimulation with Con-A; P < 0.05) and lymphocyte number (P < 0.05) compared with d 7. Lymphocyte proliferative capacity tended to decrease (P = 0.07) during starvation and increased (P < 0.01) during the refeeding period. These findings suggest that a 7-d starvation period had immunosuppressive effects on cats and that these effects were not completely normalized during 7 d of refeeding. CD4+/CD8+ subset alterations and CD4/CD8 ratio in conjunction with lymphocyte proliferation may be useful as indices of nutritional status. J. Nutr. 130: 2444–2449, 2000.

KEY WORDS: • CD4 • CD8 • starvation • lymphocytes • calcium • cats

Patients often are malnourished as well as critically ill when admitted to a hospital. Whether a critically ill patient requires surgery or treatment of a disease or is posttraumatic or septic, an assessment of the nutritional status is important to identify the nutritional risk (Manning and Shenkin 1995). The incidence of sepsis, prolonged ventilation and increased mortality rates has been associated with malnutrition (Reinhardt et al. 1980). The primary goal of nutritional status assessment is to identify nutrient deficiencies. In doing so, steps can be taken to improve the overall nutritional status, which would enhance the capability of the body to fight infection or illness. Nutritional assessment can be defined as a systematic method of gathering data, classifying the degree of malnutrition and instituting the appropriate treatment and intervention techniques (Gilbride et al. 1984).

Current methods of nutritional status assessment include anthropometric, biochemical, dietary and clinical evaluations. No definitive test of nutritional status exists due to the complexity of the human diet and the multiple effects that nutrients have on various tissues, organs and physiological functions (Manning and Shenkin 1995). In addition, non-nutritional aspects of the response to illness, such as fever, infections and drugs, may affect assessment tests (Blackburn and Thornton 1979). Tests of immunological functions can be indicative of nutritional status, because many are sensitive to overall nutritional status as opposed to deficiencies of individual nutrients (Puri and Chandra 1985). This is appropriate because it is rare for a patient to be deficient in only one nutrient. The purpose of the present study was to identify immune system indicators associated with acute nutrient deprivation in a feline model. Specifically, the identification and quantification of cluster of differentiation CD4 and CD8 markers in T-lymphocytes, lymphocyte proliferation and intracellular calcium concentration were examined in cats before, during and after a 7-d period of acute nutrient deprivation. A feline model for acute starvation was chosen because cats are less tolerant of energy and protein deficiencies due to their general inability to regulate enzymes and to use alterna-
TABLE 1
Felid diet composition

<table>
<thead>
<tr>
<th>Nutrient</th>
<th>As fed, g/418 kJ</th>
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</thead>
<tbody>
<tr>
<td>Protein</td>
<td>11.6</td>
</tr>
<tr>
<td>Fat</td>
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<tr>
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<td>Fiber</td>
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<td>Phosphorus</td>
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<td>Chloride</td>
<td>0.28</td>
</tr>
<tr>
<td>Magnesium</td>
<td>0.017</td>
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</table>

**MATERIALS AND METHODS**

**Animals and experimental design.** Twenty-three (4–7 y old) healthy, neutered adult domestic shorthaired cats weighing 3–6 kg were used for this study. These cats were part of an established research colony at the Virginia-Maryland Regional College of Veterinary Medicine (VMRCVM). The study protocol was approved by the Virginia Tech Animal Care Committee. Cats were randomly chosen in groups of eight, eight and seven to undergo the food deprivation and refeeding study, because smaller groups of cats were easier to monitor during the 14-d period. Each cat acted as its own control, and each group was separately housed in a climate- and environmentally controlled room equipped with individual steel cages (61 cm × 61 cm) during the study period. During the 14-d period, food was withheld on days 0–7 and then subsequently fed (Table 1) to meet daily energy requirements on days 7–14. Fresh water was available at all times throughout the study period, and daily water intake was monitored.

Whole blood was drawn via jugular venipuncture from each cat on days 0, 4, 7, 11 and 14 into one 7-ml and one 3-ml EDTA Vacutainer tube for analysis of immune function tests and complete blood cell count with differential, respectively. Blood was also collected into a 3-ml Vacutainer tube that contained sodium heparin for serum chemistry analysis. Fluid loss from blood collection was replaced subcutaneously with ~30 mL of lactated Ringer’s solution after each sampling period. General attitude, health and body condition were assessed each morning and evening by monitoring temperature, respiration, pulse, urination, defecation, capillary refill time and alertness. Hydration status was determined daily by checking the dryness of the mucous membranes and skin elasticity. Body weight was measured and recorded at the same time of day on days 0, 4, 7, 11 and 14. In addition, total protein, albumin, blood urea nitrogen, alkaline phosphatase, hemoglobin and hematocrit were determined (Olympus AV400 Analyzer, Dallas, TX) on days 0, 4, 7, 11 and 14 to further assess the health status of the cats. Peripheral blood differential counts were performed in the clinical pathology laboratory at VMRCVM on the same days (0, 4, 7, 11 and 14).

**Cell isolation.** To maintain sterile conditions for lymphocyte (cell culture) proliferation, cell separation was performed under a laminar flow hood. Peripheral blood mononuclear cells (PBMC), consisting of lymphocytes and monocytes, and polymorphonuclear cells (PMNC) were isolated according to a double density Ficoll separation method (Toth et al. 1992) with centrifugation. Briefly, 3 mL of Histopaque 1.119 (Sigma Chemical, St. Louis, MO) was added to 10 mL of adjusted cells (109 cells/mL) and layered over the 1.119 layer. Next, 6 mL of whole blood was layered over the 1.077 layer with an automatic pipette. The conical tube was centrifuged at 700 × g for 20 min with no brake at room temperature. After centrifugation, separation of the cells led to two opaque interfaces consisting of PBMC and PMNC. The top layer (PBMC) of each sample was aspirated with a Pasteur pipette and transferred to a 50-ml conical centrifuge tube for washing. Isolated cells were washed twice with Hank’s Balanced Salt Solution (HBSS; Gibco Laboratories, Burlington, Ontario, Canada) and centrifuged at 200 × g for 10 min. Cells were resuspended in 10 mL of complete medium [RPMI 1640, fetal bovine serum (FBS), L-glutamine, sodium pyruvate and penicillin-streptomycin; Sigma Chemical] and centrifuged at 400 × g for 20 min with the brake on to remove platelets. Cells were again suspended in 2–4 mL of HBSS for cell counting with a hemocytometer and adjusted to 1 × 108 cells/L with HBSS for time 0 [before stimulation with concanavalin A (Con-A)] CD4/CD8 quantification, to 3 × 107 cells/L for proliferation assay and to 2 × 106 cells/L for time 72 h (after stimulation with Con-A) CD4/CD8 quantification.

**Immunophenotyping.** T-lymphocytes were identified through the detection of their surface markers with flow cytometric analysis according to the method described by Ackley et al. (1990). Briefly, 250 μL of a 1:25 dilution of primary mouse anti-cat unlabeled purified CD4 or CD8 monoclonal antibody (Southern Biotechnology, Birmingham, AL) was added to 500 μL of adjusted cells (1 × 109 cells/L). Each tube was incubated for 30 min at 4°C and centrifuged for 10 min at room temperature at 200 × g. The supernatants were discarded, and the pellets were resuspended in 500 μL of HBSS. Two microliters of fluorescein isothiocyanate (FITC)-conjugated rabbit anti-mouse IgG (H + L) secondary antibody (Southern Biotechnolog-y, Inc., Birmingham, AL) was added to each tube and incubated for 30 min at 4°C. Samples were centrifuged at room temperature at 200 × g for 10 min. Cell pellets were resuspended in 500 μL of paraformaldehyde (2.0%) and analyzed on the flow cytometer. CD4 and CD8 expressions of PBMC were calculated by subtracting the values of the negative control (FITC) sample from the value obtained from the sample that had been incubated with both primary (CD4 or CD8) and secondary (FITC) antibodies.

**Lymphocyte proliferation assays.** One hundred microliters of mononuclear cells (3.0 × 106 cells/L) in complete medium was cultured in triplicate in two sterile 96-well round-bottom plates (Corning Glassworks, Corning, NY). One hundred microliters of either complete medium alone or Con-A (a stimulus for lymphocyte proliferation) (5 μg/mL; Sigma Chemical) was added. The plates were incubated in a humidified incubator at 37°C in 5% CO2 for 72 h. In one plate, lymphocyte proliferation was determined according to the Alamar Blue staining methods of Gogal et al. (1997). After 48 h, 20 μL of Alamar Blue (Accumed International, Westlake, OH) was added to all wells in the plate and returned to the incubator. The proliferation of cultures was determined 24 h later by measuring absorbance at 570 and 600 nm in a microplate reader (Molecular Devices, Menlo Park, CA). When Alamar Blue is added to cells, it is in an oxidized (blue color) form, and as the cells proliferate, the dye is reduced (red color). The absorbance at 570 and 600 nm determines the optical density (OD) of reduced and oxidized, forms of Alamar Blue. The subtraction of OD at 600 nm from OD at 570 nm accurately determines the true absorbance (specific absorbance), which reflects proliferation. The specific absorbance of unstimulated cells (medium alone) was subtracted from the specific absorbance of the cells incubated with Con-A to yield a Δ-specific absorbance. The mean Δ-specific absorbance of triplicate determinations was calculated.

To determine CD4+ / CD8+ quantification of lymphocytes at 72 h, cells from the second plate were aspirated and resuspended in 500 μL of HBSS. T-lymphocyte subsets were quantified according to the flow cytometric methods of Ackley et al. (1990). Data for CD4/8 subset immunophenotyping are reported as prestimulation (0 h) and poststimulation (72 h) with Con-A.

**Calcium flux.** Two fluorescent probes, Fluo-3 and SNARF-1 (Molecular Probes, Eugene, OR), were dissolved in dimethylsulfoxide (Sigma Chemical) at concentrations of 1 and 2 μmol/L for stock solutions. A 1:10 dilution of Fluo-3 and SNARF-1 stocks in RPMI...
1640 and 1% FBS (Sigma Chemical) was made and used as working solutions to load the cells for flow cytometric analysis. PBMC were resuspended to a concentration of 1.0 × 10⁹ cells/L in RPMI 1640 and 1% FBS. Fluo-3 and SNARF-1 were loaded into the cells according to the procedure previously described by Rijkers et al. (1990) with modifications. PBMC were incubated with 10 µL of each Fluoro-3 and SNARF-1 working solution and incubated for 30 min in a 37°C water bath. Cells were washed twice in RPMI plus 1% FBS, centrifuged at 200 × g for 10 min and resuspended in calcium (Ca²⁺)-free HBSS at a final concentration of 1 × 10⁹ cells/L. Cells were analyzed on a flow cytometer by measuring the Fluo-3/SNARF-1 ratios over time. Data were collected for a total of 120 s. Intracellular Ca²⁺ flux was expressed as a mean ratio (Fluo-3 ratio of activated versus resting population).

Statistical analysis of data was carried out with a multiple dependent t test to determine significant differences between baseline values and each subsequent measure, and analysis between d 7 and each subsequent measure was carried out with SAS Statistical Software (1996). Differences with values of P < 0.05 were considered significant.

RESULTS

Weight and albumin changes. The cats tolerated the starvation period without incident. Measures of health, including total protein, alkaline phosphatase and blood urea nitrogen, remained within normal limits in all cats as reported by workers at the Clinical Pathology Laboratory. All cats lost weight (P < 0.0001) throughout the food deprivation period and gained weight (P < 0.0001) during the refeeding period (Table 2). Compared with day 0 values, albumin levels (P < 0.05) increased on day 4 of the food deprivation treatment and decreased (P < 0.05) on days 11 and 14 compared with day 7.

Effects of starvation on peripheral blood leukocytes. Blood leukocyte numbers decreased (P < 0.05) on day 4 compared with day 0 (Table 2). There were no significant changes in blood leukocyte numbers during the refeeding period. The total lymphocyte number decreased (P < 0.05) during the starvation period and increased (P < 0.05) at days 11 and 14 of the refeeding period compared with day 7. The percentage of lymphocytes decreased (P < 0.01) throughout the latter part (day 7) of the food deprivation period and tended to increase (P = 0.09) throughout the refeeding period.

Changes in lymphocyte cell membrane surface markers. The percentage of CD⁴⁺ lymphocytes decreased (P < 0.05) on day 4 of the food deprivation period compared with day 0 (Fig. 1). An increase (P < 0.01) in the proportion of CD⁴⁺ cells was observed on day 11 compared with day 7. There was no significant change in percentage of CD⁴⁺ cells on day 14. An increase in the percentage of CD⁸⁺ cells was observed on day 7 (P < 0.05) compared with day 0 and on day 11 compared with day 7.

CD⁴⁺/CD⁸⁺ ratios were calculated from the percentage of cells that expressed specific lymphocyte markers before stimulation with Con-A after initiation of the starvation and refeeding treatments. The prestimulated CD⁴⁺/CD⁸⁺ ratios at time 0 were lower on days 4, 7, 11 and 14 (P < 0.01) compared with day 0 (Fig. 2).

Lymphocyte proliferation in response to Con-A stimulation. The proliferative capacity of lymphocytes in culture tended to decrease (P = 0.07) during the 7-d food deprivation period and subsequently increased (P = 0.01) during refeeding. The Δ-absorbance values as measured for days 0, 4, 7, 11, and 14 were 0.328 ± 0.06, 0.291 ± 0.06, 0.235 ± 0.04, 0.307 ± 0.04 and 0.300 ± 0.05, respectively.

A nonsignificant decrease (P = 0.10) in the percentage of cells expressing CD⁴⁺ marker after stimulation with Con-A was observed throughout the starvation period. The percentage of CD⁴⁺ cells was greater (P < 0.01) on day 14 than on day 7 (Fig. 3). The percentage of cells expressing CD⁸⁺ cell markers after proliferation was greater on day 4 (P < 0.01) than on day 0. The percentage of CD⁸⁺ cell expression tended to decrease (P = 0.10) for the remainder of the food deprivation period and into the early refeeding period. By the end of the refeeding period (day 14), CD⁸⁺ cell expression was greater than on day 0 (P < 0.01).

No significant changes were observed in the CD⁴⁺/CD⁸⁺ ratios.

FIGURE 1 Percent expression of CD⁴⁺ and CD⁸⁺ lymphocyte cell membrane markers before stimulation with concanavalin-A in cats starved for 7 d and refeed for 7 d. Percent expression of CD⁴⁺ and CD⁸⁺ markers was detected by flow cytometric methods. Cells (1.0 × 10⁹ cells/L) were stained with fluorescein isothiocyanate-conjugated rabbit anti-mouse IgG secondary antibody and either anti-feline CD4 monoclonal antibody, anti-feline CD8 monoclonal antibody or no antibody (negative control). Values are in means ± SEM (n = 23). *Different from d 0 (P < 0.05). **Different from d 7 (P < 0.01). ***Different from d 7 (P < 0.05).

### TABLE 2

<table>
<thead>
<tr>
<th></th>
<th>Starvation</th>
<th>Refeeding</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>Day 0</td>
<td>Day 4</td>
</tr>
<tr>
<td>Albumin, g/L</td>
<td>30.5 ± 0.3</td>
<td>30.9 ± 0.4</td>
</tr>
<tr>
<td>Weight, kg</td>
<td>4.60 ± 0.16</td>
<td>4.47 ± 0.03</td>
</tr>
<tr>
<td>Leukocytes, ×10⁹/L</td>
<td>11.0 ± 1.1</td>
<td>8.2 ± 0.99</td>
</tr>
<tr>
<td>Lymphocytes, %</td>
<td>30.5 ± 3.0</td>
<td>27.5 ± 2.8</td>
</tr>
</tbody>
</table>

1 Values are mean ± SEM (n = 23).
2 Means in columns with common superscripts indicate the following: a different from day 0 (P < 0.05); b different from day 7 (P < 0.05); c different from day 0 (P < 0.0001); d different from day 7 (P < 0.0001); e different from day 0 (P < 0.01).
Depressed CD4/CD8 ratios are associated with decreased immunity. In humans, a CD4/CD8 ratio of <1.5 has been associated with immunosuppression (Chailleux et al. 1985). In our study, the CD4/CD8 ratio (~1.0) during acute starvation was below normal feline ratios (1.2–2.6) (Dean et al. 1991, Komaki et al. 1997). The change in the CD4/CD8 ratio in our study was attributed to an increase in the percentage of CD8 cells, as well as a decrease in the percentage of CD4+ cells. The increase in the CD8+ cell population in our study was consistent with other findings (Nuevonin and Salo 1984, Ogawa et al. 1993). The route of circulation may in part explain an increase in CD8+ cells. CD8+ cells migrate from the thymus to the spleen, where nonspecific CD8+ cells proliferate (Garre et al. 1986). Recirculating cells are limited in number and are short lived. This along with the facts that acute starvation acts initially on the proliferation of CD8+ cells in the thymus (Malave et al. 1980) and that CD8+ cells further proliferate and differentiate in the spleen may explain why the proportion of CD8+ cells may remain normal (Chandra 1983) or become elevated in circulating blood.

A decrease in percent CD4+ cells was seen by day 4, followed by an increase on day 11. The CD4+ cell population in the present study decreased significantly (P < 0.05) during the starvation period, which parallels other investigations (Barlough et al. 1991, Komaki et al. 1997). The development and route of circulation of CD4+ cells may influence their susceptibility to change. When CD4+ cells migrate from the thymus to the lymph nodes, they are more mature than CD8+ cells and therefore proliferate less than CD8+ cells. In contrast, to CD8+ cells, CD4+ cells recirculate and are long lived. The secondary effects of malnutrition affect thymus-derived cells (Garre et al. 1986). During the adaptive phase of undernutrition (24–48 h), differentiation of cells is impaired. This may explain why the proportion of CD4+ cells initially decreased in the circulating blood in our study. As the nutrient deprivation period continued, the proportion of CD4+ cells was unchanged, whereas the proportion of CD8+ cells increased (Fig. 1).

In humans, many cell types produce interleukin-1 (IL-1) in response to damage, infection or antigens. IL-1 positively influences CD4+ cell proliferation, IL-2 receptor expression, and cytokine production (Roitt et al. 1996). In our study, food deprivation for >4 d may have stimulated IL-1 production in the cats, which in turn stimulated CD4+ proliferation and helped to prevent a further decline in the percentage of CD4+ cells, as observed at day 7. Stimulated CD4+ cells secrete cytokines, which are involved in cell-mediated immunity responses. IL-10 does not appear with the initial stimulation of

### DISCUSSION

Acute starvation and refeeding in the present study were defined as 7 d without and with food, respectively. The results demonstrated that immunosuppression occurred during periods of acute starvation and that refeeding counteracted these effects. These conclusions are based on the decrease in total lymphocyte number, percentage and proliferative capacity of lymphocytes and the decrease in the lymphocyte CD4/CD8 ratio that were observed during the starvation period and the subsequent increase or stabilization in these variables after refeeding.

In this study, a decrease in percent lymphocytes, as determined by differential counts, occurred throughout the food deprivation treatment and tended to increase during refeeding. Our findings are consistent with those of other studies (Dhabher et al. 1995, Komaki et al. 1997) that also examined starvation and refeeding treatments in animals. Similar findings were also reported in humans who consumed a very low energy diet for 6 weeks followed by a 1- to 2-wk refeeding period (Field et al. 1991). The effects that a decrease in total lymphocytes has on immunodeficiency are still unknown at this time.

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### FIGURE 2

CD4/CD8 ratio in peripheral blood mononuclear cells of cats starved for 7 d and refeed for 7 d. Values are reported in means ± SEM (n = 23). *Different from d 0 (P < 0.01). No significant differences occurred in CD4/CD8 ratios in poststimulated cells (cells incubated with concanavalin-A for 72 h); data are not reflected in this figure.

### FIGURE 3

Percent expression of CD4+ and CD8+ lymphocyte cell membrane markers after stimulation with concanavalin-A in cats starved for 7 d and refeed for 7 d. Lymphocytes (2.0 × 10^9 cells/L) were cultured for 72 h with concanavalin-A, and percent expression of CD4+ and CD8+ cells was detected by flow cytometric methods. Cells were stained with fluorescein isothiocyanate-conjugated rabbit anti-mouse IgG secondary antibody and either anti-feline CD4 monoclonal antibody, anti-feline CD8 monoclonal antibody or no antibody (negative control). Values are reported in means ± SEM (n = 23). *Different from d 0 (P < 0.01). **Different from d 7 (P < 0.01).
CD4+ cells but does after repeated in vitro stimulation, and this may allow for the down-regulation of CD4+ production (Palmer and van Seventer 1997). The secretion of IL-10 by CD4+ cells may have prevented a continued increase in percent CD4+ cells from day 11 to day 14 of the refeeding period (Fig. 1). An evaluation of IL concentrations such as IL-1 and IL-10, as well as IL-2 receptor expression, may aid in the explanation of the alterations in CD4+ expression. Unfortunately, at the time of this study, the technology to examine these cytokines in cats was unavailable.

In retrospect, the measurement of corticosterone would have contributed greatly to this study, because it is reported that malnutrition is associated with increased secretion of corticosterone, a major corticosteroid produced in mammals (Stinner 1983, Winick and Nobel 1966). Starvation-induced stress has been shown to increase corticosteroid production in humans, and an inverse correlation between serum cortisol (a corticosteroid) and the proportion of CD4+ cell subsets during fasting has been reported in humans (Komaki et al. 1997). Lymphocytes express receptors for corticosteroid, and therefore cortisol affects circulating lymphocytes in humans (Fauci et al. 1976). Serum cortisol can therefore directly influence lymphocyte and lymphocyte proliferation. An increase in serum cortisol during food deprivation could be responsible for substantial alterations in immunosuppression (Komaki et al. 1997). Initial decreases in CD4+ cells in our study may have resulted from increased secretion of cortisol by cats. Corticosteroids act as a major feedback mechanism on immune responses. An examination of corticosteroid concentration may provide additional information for the determination of changes in lymphocyte subset populations.

Lymphocytes can respond to corticotropin-releasing factor to generate their own adrenocorticotropic hormone. Lymphocyte-secreted adrenocorticotropic hormone in turn induces corticosteroid release (Roitt et al. 1996) and may cause inhibition of lymphocyte proliferation. Increased corticosteroid secretion had a lympholytic effect in response to 72-h starvation in mice (Wing et al. 1987). We also observed a decrease in total lymphocytes in response to starvation. Although we did not measure corticosterone secretion, this is a possible mechanism in the cat for alterations in immune responses. Corticosteroids may explain the reductions in lymphocyte proliferation that occurred during the 7-d starvation period.

Con-A is a plant lectin used to study lymphocytes as both CD4+ and CD8+ T-cell populations respond to this mitogen (Reinherr and Schlossman 1980). The in vitro mitogen-response assay detects defects in some aspects of mononuclear and lymphocyte cell function (Field et al. 1991). In our study, lymphocyte proliferation decreased throughout the food deprivation period and increased during refeeding. Lymphocyte proliferative capacity appeared consistent with the observed in vitro changes in the numbers of CD4+ cells during food deprivation and refeeding. Dowd et al. (1986) implied that T-cell subsets stimulated by Con-A are differentially affected in a variety of clinical conditions, such as altered nutritional states. Our study supports these findings because we observed an increase in the percentage of CD8+ cells compared with day 0, whereas the percentage of CD4+ cells decreased. Con-A-stimulated T-cell subsets may be differentially affected during acute nutritional deprivation.

Altered nutritional states, such as food deprivation, decrease the availability of nutrients needed for cell proliferation. A decrease in mitogen-induced lymphocyte proliferation was observed in rats fed a protein-deficient diet (Mengberi et al. 1992) and in hospitalized humans with low serum zinc and transferrin levels (Dowd et al. 1986). A decrease in mitogenic response suggests a decrease in the rate of DNA synthesis. Mechanisms for this decrease in mitogen response are unclear (Field et al. 1991); however, low serum zinc levels have been shown to depress lymphocyte proliferation, suggesting that it is required for optimal lymphocyte transformation (Dowd et al. 1986, Messer et al. 1982).

A decrease in dietary zinc associated with the food deprivation period may have been in part responsible for the decrease in lymphocyte proliferation observed in our study cats. Zinc has also been shown to regulate transcription through its interaction with metallothionein as well as to stimulate the production of interferon-γ and IL-2, which are required for lymphocyte proliferation and differentiation (Hannigan 1994). In rats fed a zinc-deficient diet, T-lymphocyte proliferative capacity was decreased (James et al. 1987). Similar results occurred in our food deprivation study. Decreased cellular zinc concentrations resulting in altered metallothionein, interferon-γ and IL-2 concentrations may explain the results in the present study.

Altered lymphocyte proliferation observed in our study may also have been associated with increases in the CD8+ regulatory cell population. Neuvonen and Salo (1984) reported that during acute starvation, increased subsets of regulatory cells contributed to a decrease in lymphocyte proliferative response. The depression in lymphocyte proliferation observed during the food deprivation period in our study may also have been due to a reduction in total circulating T cells, which has been previously reported (Chandra 1977, Salimonu et al. 1982). Although the mechanism is not clear, the decrease in lymphocyte proliferation exhibited by our study cats may be linked to T-cell number, altered expression of CD4+ and CD8+ subsets, altered cellular zinc concentrations or a combination.

Intracellular Ca2+ is determined by stimulating the lymphocytes with ionomycin to release intracellular Ca2+ stores. The amount of intracellular Ca2+ is indicative of the activation state of the cell. One of the early responses of T-cell receptor stimulation is a rise in [Ca2+], (Verhueneg et al. 1997). In the present study, [Ca2+]i increased through day 11 and decreased on day 14. We interpret the changes in [Ca2+]i stores as reflecting alterations in expression of the CD8+ subset population during starvation and refeeding. The literature indicates a relationship among [Ca2+]i, protein kinase Cz and IL-2 with regard to cell activation and proliferation (Nishizuka 1984, Roitt et al. 1996, Verheugen et al. 1997), suggesting that changes in [Ca2+]i, should correlate closely with cell function and nutrient status. However, data derived from cats in our study did not suggest such a relationship in that [Ca2+]i changes did not mirror lymphocyte proliferative activity. Additional studies on this topic may help to better determine the value of [Ca2+]i changes as an indicator of nutrient status.

In conclusion, our data show that immunosuppression can result in cats that have undergone 7 d of acute starvation. Immunosuppression began to be alleviated with refeeding. The decreases in lymphocyte number, proliferation and CD4/CD8 ratio are all indicative of immunosuppression; the use of these variables as measures of nutritional status is hopeful for both human and veterinary patients. The incidence of sepsis (Chandra 1983), prolonged ventilation and increased mortality rates has been associated with malnutrition in the critically ill (Reinhart et al. 1980). Patients who are immunosuppressed as a result of malnutrition must be identified so nutritional intervention can be implemented to enhance the capability of the body to fight infection and disease. The many complex interactions between starvation and immunity offer a
great challenge to researchers. A greater understanding of this area may help decrease patient mortality rates.

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