Effect of feeding malnourished patients for 1 mo on mitochondrial complex I activity and nutritional assessment measurements¹⁻³

Francoise Briet, Clare Twomey, and Khursheed N Jeejeebhoy

ABSTRACT
Background: We showed previously that the activity of complex I (the first enzyme of the electron transport chain) in peripheral blood mononuclear cells decreases with malnutrition and increases to a subnormal value after 1 wk of refeeding, but the traditional markers of nutritional status do not do so.
Objective: The aim of this study was to ascertain whether a period of nutritional intervention longer than 1 wk would normalize complex I activity and traditional markers of nutritional status.
Design: Fifteen malnourished patients (7 women and 8 men) with ≥ 10% body weight loss over the previous 6 mo were studied on the day of their admission to hospital and 7, 14 and 30 d after the beginning of nutritional support. Complex I activity in peripheral blood mononuclear cells, weight, height, body composition, body water compartments, dietary intake, and serum albumin concentrations were measured on each occasion. The results before and during nutritional intervention were compared with values obtained in 30 healthy volunteers (17 women and 13 men).
Results: Complex I activity increased significantly after the first week of refeeding (P<0.001) and reached a normal value after 1 mo of nutritional supplementation. Among the classic markers of nutritional status, only the ratio of extracellular water to intracellular water tended to decrease over the refeeding period.
Conclusion: Complex I activity increases rapidly and is normalized by refeeding at a time when other markers of nutritional status do not change significantly. Am J Clin Nutr 2004;79:787–94.

KEY WORDS Malnutrition, refeeding, peripheral blood mononuclear cells, mitochondria, complex I

INTRODUCTION
Malnutrition is the result of a disturbance in the equilibrium between dietary intake and nutrient needs (1). The sequential changes of ongoing malnutrition are altered cellular metabolism, impaired physiologic function, and finally, loss of body tissues (2). Concurrent stress, such as trauma, sepsis, inflammation, and burns, accelerates the loss of function and tissue mass (3).

Previously, we showed that activity of complex I [NADH dehydrogenase (ubiquinone), the first enzyme of the electron transport chain] in mitochondria decreases in proportion to the degree of malnutrition evaluated by changes in body composition in humans (4). Moreover, complex I activity in peripheral blood mononuclear cells (PBMCs) responds rapidly to a 7-d period of nutritional support but does not reach a normal value (4, 5). Although complex I activity increases significantly after 7 d of refeeding, other markers of nutritional status, including albumin, body mass index (BMI; in kg/m²), body composition, and body water compartments (ie, total body water), do not change significantly over this short period (4, 5). Nevertheless, it is not known whether a longer period of feeding will normalize complex I activity and the markers of nutritional status in malnourished patients.

SUBJECTS AND METHODS
Subjects
Because rapid weight loss is a predictor of nutrition-related complications (6, 7), we selected patients who had lost 10% of their usual (ie, premorbid) body weight within the previous 6 mo and who did not receive any prior nutritional intervention. Patients with neurologic disease, bone marrow disorder, renal dysfunction, sepsis, or metabolic disease (eg, diabetes) were excluded from the study. Fifteen malnourished patients (MPs) corresponding to these conditions [7 women, 8 men; x (± SD) age: 50±15 y] were recruited after their admission to the Gastroenterology Service of St Michael’s Hospital. All patients received medical treatment during the study. Patient characteristics are shown in Table 1. It was not possible to measure body composition in a patient who had ascites. Thirty healthy volunteers (HVs; 17 women, 13 men; x age: 45±14 y) participated in this study as control subjects. They were recruited from the staff of St Michael’s Hospital.

Refeeding study
All patients were investigated 7 and 14 d after nutritional support had commenced, and 10 of the MPs were investigated 30 days after nutritional support had commenced. All measurements were performed after an overnight fast. Before nutritional intervention, 5 MPs had very little energy intake (=0 MJ/d) during the previous 3 d. Nutritional support was administered as parenteral nutrition (n = 11), enteral nutrition (n = 2), or nutritional supplements (n = 2). In conjunction with the medical

¹ From the Department of Medicine, University of Toronto, Toronto.
² Supported by MRC grant MT-10885. Ubiquinone used for the complex I activity assay was a gift from Go Ichien (Eassai Chemical Co, Tokyo).
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Received June 25, 2003.
Accepted for publication October 28, 2003.
Weight loss (% of usual weight) for individual patients. The Ethics Committee of St Michael’s Hospital approved the study protocol, and all subjects gave written informed consent before the start of the study.

All HVs were instructed by the dietitian in keeping food records and were asked not to change their habitual food intakes. The participants were asked to record, in as much detail as possible, all food and beverages consumed over a 3-d period, including at least one weekend day.

Intakes of energy and macronutrients were calculated from the data from food records for the MPs and HVs with the use of a computer program based on food tables. These calculations were then analyzed with the use of the NUTRIWATCH nutrient analysis program for WINDOWS (version 320; Elizabeth Warwick, Cornwall, Canada), which was based on the 1997 Canadian Nutrient File.

### Peripheral blood mononuclear cell analyses

For PBMC isolation, a venous blood sample (24 mL) was obtained from each subject and delivered to the laboratory within 1 h. PBMCs were isolated by density gradient centrifugation (10), and all procedures were performed at ambient room temperature (20–25 °C). After gentle agitation, an 8-mL aliquot was layered onto a Percoll-saline solution. The Percoll-saline (Percoll; Amersham Pharmacia Biotech, Baie d’Urfé, Canada) was prepared as described by the supplier [56.25% Percoll (by wt), 37.50% Hanks' balanced salt solution (by vol), and 0.50% NaCl at a final density of 1.06–1.08 kg/L (wt by vol)]. The layered blood and Percoll solution was centrifuged at 1600 × g for 30 min. The mononuclear cells were collected at the interface, washed 3 times with potassium phosphate buffer (20 mmol/L, pH 7.2), and centrifuged at 480 × g for 10 min as described previously (11). The cell pellet was resuspended in 200 μL potassium phosphate buffer (20 mmol/L, pH 7.2), sonicated for 15 s (3 bursts of 5 s each) at 300 W on ice, and stored at −70 °C (11).

The protein concentration of the cell suspension was measured by using the biuret method. The mononuclear cell suspension was diluted with potassium phosphate buffer (20 mmol/L, pH 7.2) to a protein concentration of 5 g/L, and the samples were frozen and thawed 3 times to disrupt the mitochondrial membrane.

Enzyme activity was measured spectrophotometrically under conditions of maximal reaction velocity at an optimal pH and at room temperature as described below. All assays were performed in duplicate in a final volume of 1 mL by using a double-beam spectrophotometer (Spectrophotometer DU Series 600; Beckman Instruments, Fullerton, CA).

Complex I activity was measured by following the oxidation of NADH, calculated from the slope of the change of optic density at 340 nm (12). Briefly, a mononuclear cell suspension was added to a buffer containing potassium phosphate buffer (25 mmol/L, pH 7.2), 5 mmol MgCl₂/L, 2 mmol KCN/L, 2.5 g bovine serum albumin/L (fraction V), 2 mg antimycin A/L, 0.13 mmol NADH/L, and 65 μmol ubiquinone 1/L. The NADH–ubiquinone oxidoreductase activity was measured for 4 min. Then, 2 mg rotenone/L was added, and the activity was measured for an additional 3 min. The specific complex I activity corresponds to

#### Table 1

Physical and clinical characteristics of malnourished patients (*n* = 15)

<table>
<thead>
<tr>
<th>Diagnosis</th>
<th>Patients</th>
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<tbody>
<tr>
<td>Crohn disease</td>
<td>4</td>
</tr>
<tr>
<td>Crohn disease with celiac disease</td>
<td>1</td>
</tr>
<tr>
<td>Short bowel syndrome</td>
<td>2</td>
</tr>
<tr>
<td>Depression-anorexia</td>
<td>1</td>
</tr>
<tr>
<td>Intestinal obstruction</td>
<td>1</td>
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<tr>
<td>Intestinal lymphangiectasia</td>
<td>1</td>
</tr>
<tr>
<td>Gastric adenocarcinoma</td>
<td>3</td>
</tr>
<tr>
<td>Colon adenocarcinoma</td>
<td>1</td>
</tr>
<tr>
<td>Pancreatic adenocarcinoma</td>
<td>1</td>
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</tbody>
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<table>
<thead>
<tr>
<th>BMI (kg/m²)</th>
<th><em>n</em></th>
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<tbody>
<tr>
<td>≤ 14</td>
<td>5</td>
</tr>
<tr>
<td>15–18</td>
<td>5</td>
</tr>
<tr>
<td>19–25</td>
<td>3</td>
</tr>
<tr>
<td>≥ 26</td>
<td>2</td>
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</tbody>
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<table>
<thead>
<tr>
<th>Weight loss (% of usual weight)</th>
<th><em>n</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>10–19</td>
<td>5</td>
</tr>
<tr>
<td>20–29</td>
<td>7</td>
</tr>
<tr>
<td>≥ 30</td>
<td>2</td>
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</table>

<table>
<thead>
<tr>
<th>Duration of weight loss (mo)</th>
<th><em>n</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>≤ 5</td>
<td>7</td>
</tr>
<tr>
<td>6–11</td>
<td>6</td>
</tr>
<tr>
<td>≥ 12</td>
<td>2</td>
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</table>

1. Patient with ascites in whom it was not possible to estimate the percentage of usual weight that was lost at the time of the study or the duration of weight loss.
the rotenone-sensitive NADH–ubiquinone oxidoreductase activity expressed as nmol · min⁻¹ · mg PBMC protein⁻¹ (11).

Statistical analysis
All results are presented as means ± SDs. The nonparametric Mann-Whitney U test was used to compare data between MPs (before and during refeeding) and HVs. The nonparametric Friedman test (equivalent to repeated-measures analysis of variance) was used to compare data from MPs before and during refeeding. For multiple pairwise comparisons, P values obtained after the Mann-Whitney U and Friedman tests underwent Bonferroni-Dunn correction. Spearman’s correlation was used to ascertain the relation between complex I activity, serum albumin concentration, and other measurements. STATISTICA software for WINDOWS (version 5.0; Statsoft, Tulsa, OK) was used for the statistical analyses.

RESULTS

Comparison of body measures, nutrition, and complex I activity in malnourished patients and healthy volunteers

Before any nutritional intervention, body weight and BMI were significantly lower in MPs than in age-matched HVs (Figure 1). The lower BMI in MPs was due to the fact that fat mass (12.1 ± 8.7 and 26.8 ± 8.1 kg; P < 0.0001) and FFM (35.8 ± 10.3 and 47.8 ± 13.1 kg; P < 0.002) were significantly lower in MPs than in HVs, respectively. In contrast, percentage of FFM and percentage of fat mass did not differ significantly between MPs and HVs (Figure 1). Measured TBW and ICW were significantly lower in MPs than in HVs (Figure 2). The ECW also tended to be lower in MPs than in HVs, but the difference was not significant (P = 0.065). Furthermore, the ratio of ECW to ICW was significantly higher in MPs than in HVs (Figure 2). The lower ICW and the higher ECW:ICW with a nonsignificant increase in ECW in MPs are all consistent with a reduction in FFM.

The energy, protein, and carbohydrate intakes were significantly (∼60%) lower in MPs than in HVs (Figure 3). In contrast, the fat intake did not differ significantly between MPs and HVs (Figure 3). The mean serum albumin concentration in MPs was significantly below normal (25 ± 8 g/L; normal range: 25–50 g/L). Most of the patients with a low albumin concentration had pathologic conditions such as inflammation and cancer, which could contribute to hypoalbuminemia. Correspondingly, complex I activity was significantly (∼43%) lower in MPs than in HVs (Figure 4).

Effects of 1 mo of refeeding on nutritional assessment measurements in malnourished patients

Over the 1-mo refeeding period, the nutritional assessment measurements did not change significantly in MPs (Figures 1 and 2). Only the ECW:ICW tended to decrease over the refeeding period, but the difference was not significant. Body weight, BMI, and ICW remained significantly lower in MPs than in HVs during the refeeding period (Figures 1 and 2).

Also during the refeeding period, the MPs received substantial amounts of energy and macronutrients, and the daily intake reached HV values after 2 wk of refeeding, as shown in Figure 3. Over this period, PBMC complex I activity in MPs increased progressively, and it equaled that in the HVs by the end of the
refeeding period (Figure 4). In contrast, serum albumin concentrations did not change significantly (day 0: 25 ± 8; day 7: 23 ± 7; day 14: 22 ± 7; and day 30: 28 ± 8 g/L).

Correlation between changes in complex I activity and the different measurements over the 1-mo refeeding period

The rate of change of a responsive measurement—namely, complex I activity—is likely to be maximal during the initial phase of greater nutrient intake. As complex I activity approaches a normal value, the rate of change is likely to plateau. During the first week of refeeding, as would be expected, the change in complex I activity correlated significantly with the mean energy, protein, fat, and carbohydrate intakes (Figure 5). During the second week, the change in complex I activity correlated only with the mean protein intake (Figure 6). By the fourth week, complex I activity had reached normal levels and was therefore unlikely to change. Correspondingly, at the fourth week (as expected), the correlation between nutrient intake and complex I activity was no longer significant (P = 0.15; data not shown). It is interesting that there was no correlation between the change in complex I activity and the change in any other marker of nutritional status over the refeeding period. However, it should be recognized that the traditional markers of malnutrition are altered by disease states, and some are altered only by advanced nutritional deprivation. Therefore, a lack of correlation, although interesting, is not altogether unexpected.

Correlation between change in serum albumin concentrations over the 1-mo refeeding period and changes in total body water

Before the refeeding intervention, serum albumin concentrations were inversely correlated with percentage of ECW and ECW:ICW (Figure 7), which suggested that the concentrations were reduced by dilution with ECW. The explanation that reduced albumin concentrations were due to dilution was supported by the change in serum albumin concentrations during the first 2 wk of refeeding. After 1 wk of refeeding (day 0–day 7), the change in serum albumin concentrations was inversely correlated with a change in percentage of ECW (Figure 8). Similar results were observed after 2 wk of refeeding (day 0–day 14; Figure 9A). Moreover, the change in serum albumin concentrations correlated with the change in TBW after 2 wk of refeeding (Figure 9B). After 1 mo of refeeding (day 0–day 30), similar correlations were observed, but, because of the small number of patients, they were not significant (data not shown).

DISCUSSION

The goal of the present study was to ascertain whether feeding malnourished patients for 4 wk normalizes complex I activity and markers of nutritional status (ie, body composition, body water compartments, and serum albumin concentrations). The small sample size is to be expected from the fact that hospital patients...
currently are discharged quickly, and thus it is difficult to study patients for 30 days in the hospital. The small sample size and the heterogeneity of the patients reduced power and increased the possibility of a type II error. On the other hand, close observation in the hospital allows a careful evaluation of nutrient intakes and disease states, which makes the data more accurate.

Despite the small sample size, PBMC complex I activity increased in proportion to the intakes of energy and macronutrients (Figures 5 and 6). Furthermore, a rapid improvement in and normalization of PBMC complex I activity were observed over the refeeding period (Figure 4). In contrast, body composition, fluid distribution, and insulin concentration failed to show a significant improvement after 1 mo of nutritional intervention.

**Significance of normalization of complex I activity after 1 mo of refeeding**

Our previous animal study (11) found that a decrease in PBMC complex I activity is associated with similar changes in muscles. Therefore, it is likely that the observed changes in PBMCs in human subjects reflect reduced muscle mitochondrial complex I activity. In addition, our preliminary results in humans showed that this loss of function (PBMC complex I activity) can occur independently of wasting and can rapidly improve independently of the repletion of body stores (4). In that earlier work, we showed that the increase in complex I activity was directly related to the quantity of energy and protein received during the first 2 wk of refeeding. Moreover, the normalization of complex I activity to control value after 1 mo, a time at which other nutritional assessment measurements were still unchanged, is a very interesting finding. It is well known that refeeding MPs seems to improve their muscle function first, whereas their muscle mass is nearly unchanged (2, 13). In the same way, our results would suggest that the restoration of mitochondrial function in PBMCs occurred after refeeding. Moreover, our results point out the inadequacies of the classic markers as indicators of "nutritional status" during a short period of refeeding of persons with malnutrition complicated by active inflammatory stress.

**FIGURE 3.** Mean (± SD) energy (MJ/kg body water (BW)) and macronutrient (protein, fat, and carbohydrate; g/kg BW) intakes in healthy volunteers (HVs) and in malnourished patients before (day 0; D0) and during (D7, D14, and D30) 1 mo of refeeding. * Significantly different from the age-matched HV group, \( P < 0.002 \) (Mann-Whitney U test and Bonferroni correction). ** Significantly different from D0 in malnourished patients, \( P < 0.003 \) (Friedman test and Bonferroni correction).

**FIGURE 4.** Mean (± SD) complex I activity in peripheral blood mononuclear cells (PBMCs) in healthy volunteers (HVs) and in malnourished patients before (day 0; D0) and during (D7, D14, and D30) 1 mo of refeeding. * Significantly different from the age-matched HV group (Mann-Whitney U test and Bonferroni correction): \( ^* P < 0.0001 \), \( ^{**} P < 0.05 \). † Significantly different from D0 in malnourished patients (Friedman test and Bonferroni correction): \( ^{†} P < 0.001 \), \( ^{‡} P < 0.02 \).
Effect of malnutrition and refeeding on body water compartment measurements

In pathophysiologic states such as trauma and malnutrition, the transmembrane potential across cells and the electrolyte status change, and, therefore, fluid shifts between the intracellular and extracellular compartments (14). In healthy persons, the hydration of FFM and the ECW:TBW are tightly regulated. Measurement of ECW and ICW may give important information about metabolic changes in MPs.

An expansion of ECW, which is a consequence of chronic inflammation (15), malnutrition (16), or both, can be associated with cell shrinkage (17). We were not surprised to observe tissue depletion and changes in fluid distribution in the MPs (Figures 1 and 2). Because the ICW is a constant fraction of the body cell mass (17), the increased ECW:ICW observed in the current study may be predominantly the result of ECW expansion, which is consistent with both a lack of nutrition and the cytokine-mediated inflammatory response (18). Moreover, the reduced ICW observed in the MPs suggests that body cell mass, the metabolically active part of the human body, is less than that in the HVs.

Refeeding will eventually increase TBW and ICW and reduce

FIGURE 5. Relation between changes in complex I activity in peripheral blood mononuclear cells (PBMCs) and changes in mean energy and macronutrient intakes during the first week of refeeding in malnourished patients.

FIGURE 6. Relation between changes in complex I activity in peripheral blood mononuclear cells (PBMCs) and changes in mean protein intake during the second week of refeeding in malnourished patients.

FIGURE 7. Relations of percentage of extracellular water (ECW) and the ratio of ECW to intracellular water (ICW) with the serum albumin concentration in malnourished patients before the nutritional intervention.
ECW, and monitoring these values may give an indication of the effectiveness of the nutritional support (19). Our findings showed that there were no changes in ICW or ECW over the 4 wk of refeeding. The ECW:ICW tended to decrease, but relatively little, because, even after 4 wk, it did not differ significantly from the value observed in MPs before refeeding (Figure 2). On admission to the study, all patients had clinical evidence of fluid status abnormalities (ie, dehydration, edema, or current intravenous therapy) that, coupled with the alteration of fluid status during illness, would account for the insensitivity of fluid distribution as a marker of nutritional status. Furthermore, distortion of fluid distribution is due to factors other than malnutrition, and these factors must be stabilized before the effects of refeeding can become apparent. The need for the stability of other factors is illustrated by the nonsignificant changes in fluid distribution observed during the refeeding period in the MPs. On the other hand, complex I activity responded rapidly and did not seem to be affected by the whole-body fluid status.

**Effect of malnutrition and refeeding on serum albumin concentration**

The routine measurement of serum albumin concentrations in blood samples from hospitalized patients is still considered a quick and simple way to ascertain a person’s nutritional status. Nevertheless, the mechanisms of hypoalbuminemia have been the subject of considerable debate (20). The causes are multiple and are related to poor nutrition, inflammation, and comorbid disease (20–23). It appears that hypoalbuminemia is a predictor of increased complications and underlying disease, rather than an index of pure malnutrition.

Before any nutritional intervention, the serum albumin concentration was low in 12 of the 15 MPs. Most of these 12 MPs, besides being malnourished, overhydrated, or both, had been given diagnoses of inflammation, cancer, or both. These conditions are known to reduce albumin concentrations (20, 22, 23). Nevertheless, serum albumin in the MPs was inversely related to percentage of ECW and ECW:ICW, which suggested that the concentrations were calculated by the degree of dilution by ECW. During the refeeding period, the serum albumin concentration did not change significantly, which can be explained by the fact that most of the MPs continued to experience active inflammatory stress. However, the changes in serum albumin correlated with the changes in percentage of ECW over the first 2 wk of refeeding (Figures 8 and 9), which made hypoalbuminemia a questionable marker of malnutrition per se.

**Conclusion**

Our results lead us to question the value of defining malnutrition on the basis of loss of lean body mass and low serum albumin concentrations and of defining recovery from malnutrition as repletion of lean body mass and an increase in serum albumin concentrations. Randomized controlled trials have shown that, despite modest gains in nitrogen, nutritional support improves outcome when outcome is defined as fewer complications, lower mortality, and shorter hospital stays (24–27). These data suggest that a reversal of the adverse effects of malnutrition is not based on improvements in the traditional measurements of nutrition assessment, such as a gain in body nitrogen or a demonstrable increase in muscle mass or plasma proteins. Improvement in function seems to be more sensitive to nutritional support than are the traditional assessment markers, and our study suggests that function (complex I activity) is restored before lean tissue is regained. Moreover, our study highlights the inadequacies of the currently available methods of evaluating recovery from malnutrition in clinical practice. We studied several markers of nutritional status, body composition, plasma proteins, and fluid distribution, none of which were sensitive enough to respond to the increase in nutrient intake. Complex I activity, on the other hand, has been shown to respond rapidly, and we can infer from this study that it is not influenced by fluid status or inflammatory stress. However, because of this study’s limitations (ie, its limited sample size and patient heterogeneity), a large study in patients with pure malnutrition would be required to ascertain the potential of complex I activity to predict clinical outcome.

We are especially grateful to all the patients and healthy control subjects who participated in the study. We acknowledge all those involved in con-
REFERENCES