Higher skeletal muscle protein synthesis and lower breakdown after chemotherapy in cachectic mice

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WASTING OF SKELETAL MUSCLE mass is a hallmark of cancer cachexia, where the depletion of skeletal muscle mass may be as high as 75% (20). This wasting results in weakness and higher mortality compared with patients without wasting (13, 23). Chemotherapy, which is widely used in the treatment of cancer, unfortunately has a number of nonspecific cytotoxic effects to the host. There is recent evidence to suggest that chemotherapy itself may directly contribute to cancer cachexia, further confounding the treatment of this disease and subsequent recovery by the patient (14). The precise interaction between cancer cachexia and chemotherapy on skeletal muscle mass and protein metabolism has not been addressed. The cytotoxic effects of chemotherapy may cause further skeletal muscle wasting and impede the longer-term ability of skeletal muscle to recover; these possibilities need to be addressed. Furthermore there are no studies that have investigated the processes that might mediate recovery after chemotherapy.

Knowledge of the impact of cancer cachexia and chemotherapy on skeletal muscle protein metabolism is critical to developing treatments to promote recovery of skeletal muscle mass after cancer treatment. In doing so, the potential exists to reduce morbidity, to improve the ability to withstand any subsequent cancer treatments, and to return the individual to an improved physical state. Clearly more research is required on the mechanisms by which chemotherapeutic agents acutely affect skeletal muscle protein turnover and the longer-term ability of skeletal muscle to generate an effective anabolic response to affect restoration of skeletal muscle mass.

The objective of the present research was to measure protein mass and the in vivo rates of protein synthesis and degradation in skeletal muscle during cancer cachexia and chemotherapy and the subsequent recovery from cancer cachexia. We achieved our objective using colon 26 adenocarcinoma (C26) in mice, which results in cachexia characteristic to the human condition, with a relatively small tumor burden (8, 25). To study the recovery of skeletal muscle mass from cancer cachexia, we used cytemustine, a recently developed nitrosourea that cures C26 with 100% efficacy (4, 16).
MATERIALS AND METHODS

Animals, housing, and diet. All animal studies were completed in accordance with the guidelines of the Canadian Council of Animal Care. Male BALB/cByJ mice (20 g, 4–5 wk old, Jackson Laboratories, Bar Harbor, ME) were housed in individual cages and maintained at 22–23°C on a 12:12-h light-dark cycle commencing at 0800. Mice were given at least 4 days to adjust to their new environment and diet before treatments were imposed. Mice were given free access to water and food (standard rodent chow, UBC Animal Care Centre), with the exception of pair-fed mice.

Tumor model, inoculation, and transplantation. To study the effect of cancer cachexia and chemotherapy on skeletal muscle, an appropriate model must be used to represent the condition observed in human cancer patients. The tumor must be able to induce cachexia and the associated physiological abnormalities while maintaining a reasonably small tumor burden (3). The murine C626 meets these requirements (8, 22, 25). This model results in extensive losses in adipose and skeletal muscle tissue weight, which are not caused by decreased food intake (9, 22, 25, 26).

Stock cells of C26 were used to generate the first tumor passage. Cells were injected subcutaneously into the upper dorsal region of the mouse, developing into a solid tumor at the site of injection. Subsequent tumor passages were generated by serial transplantation from solid tumors. A volume of 0.1 ml of filtered tumor homogenate (0.5 g/ml saline) of filtrate was injected subcutaneously into recipient mice. Mice from passages three and four were used for the experiments described below. All tumor-bearing mice used in experiments showed evidence of wasting characteristic of this model. A detailed description of these characteristics is provided in Samuels et al. (22).

Chemotherapeutic agent and regimen. To study the recovery of skeletal muscle mass from cancer cachexia, a chemotherapeutic drug that cures the cancer is required. Cystemustine, N-(2-chloroethyl)-N-[2-(methylsulfonyl)ethyl]-N-nitrosourea (C2H2C6ClN3O4S), a member of the chloroethylnitrosourea family of alkylating antineoplastic agents, has been shown to cure C26 with a 100% efficacy (4, 16). Cystemustine, similar to the other chloroethylnitrosoureas, is a bialkylating agent acting mainly through DNA interstrand cross-links to block tumor cell growth and ultimately induce cellular death (11). Cystemustine also has a degree of relevance to human cancer patients, as phase II clinical trials have been completed (e.g., 30) and current research is being focused on increasing its antineoplastic activity (6).

A solution of cystemustine was made 30 min before use with 1 mg/ml sterile, nonpyrogenic saline. A single intraperitoneal injection of cystemustine was given at a dose of 20 mg/kg body wt between 1015 and 1045. The timing of injection was important, as cystemustine is highly chronotoxic (17). This dose cures C26 in mice (4). Details of tumor regression are given in Samuels et al. (22).

Experimental treatments. There were four principal treatment groups: 1) healthy mice, 2) healthy mice treated with cystemustine, 3) tumor-bearing mice, and 4) tumor-bearing mice treated with cystemustine. Mice were randomly designated to one of the four treatment groups. Tumors were transplanted into mice in the tumor-bearing groups, whereas mice in the healthy groups were sham injected. About 4 days after the onset of cachexia, chemotherapy was administered to one of the tumor-bearing groups of mice and one healthy group. All other mice were injected with vehicle alone. An additional group of mice (healthy pair-fed mice) was used to control for any possible effects of reduced food intake by tumor-bearing mice resulting from chemotherapy. Healthy mice were initially fed the food intake of tumor-bearing mice. Once the chemotherapeutic agent was administered, healthy pair-fed mice received the food intake of tumor-bearing mice treated with cystemustine. Pair-fed mice received their allotted food between 1000–1100 and 1700–1800. Pair-fed mice were sham injected on the day of tumor transplantation and chemotherapy.

Experimental time points. A group of tumor-bearing and healthy (ad libitum fed) mice were killed on day −2 (2 days before chemotherapy) to determine the acute mechanisms responsible for the initiation of skeletal muscle wasting. Chemotherapy was administered on day 0 (−4 days after the onset of cachexia). The onset of cachexia was determined on the basis of body weight loss. The cachexia causes a sudden and sustained decrease in body weight, starting at about day −4. We defined the onset of cachexia as the first day in which body mass of cachectic mice was significantly lower than that of healthy mice. Healthy mice, tumor-bearing mice, and tumor-bearing mice treated with cystemustine were killed on days 2 and 11 after chemotherapy. Days 2 and 4 were used to determine the acute effects of chemotherapy and the mechanisms initiating recovery, respectively. By day 11, the tumor had fully regressed and therefore served as a good indicator of longer-term recovery.

Healthy mice treated with cystemustine were killed on days 2 and 4 only. Chemotherapy caused a slight body weight loss, but recovery of body mass was complete by day 4 (22). Thus we did not include a group of healthy mice treated with cystemustine on day 11. Indeed, in the present experiment, skeletal muscle phenylalanine mass, muscle mass, and protein synthesis were not different between healthy mice treated with cystemustine and healthy mice on days 2 and 4.

Healthy mice, pair-fed to the intake of tumor-bearing mice, were killed on days 2 and 4 only. The effect on food intake in this model is minor (9, 22, 25, 26). In the present experiment (22), food intake was only slightly decreased (~20%) in tumor-bearing mice in the early stages of cachexia and it was not different from healthy ad libitum-fed mice by day 1 (22). Also body weight of pair-fed mice was never different from ad libitum-fed mice (22). Skeletal muscle mass, skeletal muscle phenylalanine mass, and rate of protein synthesis were not different between pair-fed and healthy mice given free access to food on days 2 and 4. Thus we did not include a pair-fed group on day 11. Because of the lack of effect of food intake, we used healthy mice that had free access to food to make comparisons with the other treatment groups.

Protocol used on experimental days. Protein synthesis was measured in vivo using the flooding dose method as described previously (10, 18). Between 1100 and 1400, mice were injected intraperitoneally with 150 μmol phenylalanine/100 g body wt with a specific radioactivity of 0.6 μCi·1·26[3H]phenylalanine/μmol (Amersham Canada Limited, Oakville, Canada) in 1.25 ml saline/100 g body wt. After precisely 15 min from time of injection, each mouse was killed by cervical dislocation, and the right and left gastrocnemius muscles were rapidly excised, blotted, weighed, frozen in liquid nitrogen, and stored at −70°C until analyzed.

In vivo rate of protein synthesis and degradation. The flooding dose method is considered to be the method of choice for measuring protein synthesis in vivo in small animals; Davis et al. (5) recently showed that the aminoaeryl-tRNA pool, the true precursor pool, equilibrates with the tissue and blood free amino acid pools in skeletal muscle when a flooding dose of phenylalanine is used. To estimate protein synthesis and degradation, muscle samples were analyzed in a manner similar to that reported by McAllister et al. (18) and...
Garlick et al. (10). The specific radioactivity of free (SA) tissue and protein-bound (SB) phenylalanine was measured in each tissue. The fractional rate of protein synthesis (k_{syn}; %/day) was calculated as: k_{syn} = (S_P × 100)/(S_A × t) × 100, where t is the time between injection and slaughter (in days). The fractional growth rate (k_{growth}) for each tissue was determined for each mouse as the average fractional growth rate over 24–48 h immediately preceding the measurement of protein synthesis (1, 21). The absolute rate of protein synthesis was calculated by multiplying the fractional rate by the phenylalanine mass of that tissue (21).

Fractional degradation (k_{deg}; %/day) was calculated by subtracting k_{growth} from k_{syn}. The absolute rate of protein degradation was calculated by multiplying the fractional rates by the phenylalanine mass of that tissue (21). Rates of protein degradation determined in vivo are indirect measures, i.e., they are determined as the difference between synthesis and growth. Thus there are more inherent errors associated with the determination of degradation so results must be interpreted with additional caution. Protein degradation could not be estimated on day 2 in chemotherapy-treated healthy and tumor-bearing mice, because the rate of growth was not linear at that time, a criterion that is necessary for the estimation of protein degradation.

Statistics. The experiment was completed in two replicates, and all measurements were combined. The total sample size for any one time point and treatment group ranged from 5 to 10 mice. The effects of treatment, day, and the treatment × day interaction were tested using a 2-way ANOVA (23). When a treatment × day interaction occurred, differences between individual means were assessed using t-tests (23). In the present experiment, there was a significant treatment × day interaction present for all parameters tested. Variability was expressed as means ± SE. Differences were considered significant at P < 0.05.

RESULTS

Characterization of the experimental model. A detailed characterization of the tumor and effect of the tumor and chemotherapy on body weight and food intake is provided in Samuels et al. (22). Briefly, rapid body weight loss (~20%) occurred between days −3 and 2. Between days 2 and 11, body weight loss was more gradual; by day 11, it was 30% lower than that of healthy mice. In chemotherapy-treated tumor-bearing mice, body weight was reduced 7% compared with untreated tumor-bearing mice 1 day after treatment. By day 2, body weight of treated mice started to increase, and mice were well into a state of recovery by day 4. By day 11, tumor regression was complete with only traces of tumoral tissue present in a few mice; body weights of cured mice were 5% lower than those of healthy mice. In healthy mice treated with cycmustine, body weight was lower compared with healthy controls on day 2, but was normal by day 3. Cancer cachexia and chemotherapy had a minor effect on food intake.

Skeletal muscle phenylalanine mass and wet weight. At the onset of cachexia (day −2), skeletal muscle phenylalanine mass of tumor-bearing mice was not different from healthy mice (Fig. 1; P > 0.05). However, evidence of wasting was present by day 2, as skeletal muscle phenylalanine mass of tumor-bearing mice was 28% lower than in healthy mice (P < 0.05) and 32% lower (P < 0.05) compared with tumor-bearing mice on day −2. This wasting was not the result of decreased food intake, as pair-fed controls showed no evidence of wasting compared with healthy mice (2.15 ± 0.10 vs. 2.06 ± 0.08 mg wet wt, respectively, on day 2, P > 0.05). Skeletal muscle phenylalanine mass continued to decline in tumor-bearing mice; by the end of the experiment on day 11, it was 43% lower than in healthy mice (P < 0.05) and 42% lower compared with tumor-bearing mice on day −2 (P < 0.05).

There was no evidence of acute toxicity from chemotherapy itself, because skeletal muscle phenylalanine mass in healthy mice treated with cycmustine on days 2 and 4 was not different from healthy mice (P > 0.05; Fig. 1). Skeletal muscle phenylalanine mass was also not different between days 2 and 4 (P > 0.05). By day 4, skeletal muscle phenylalanine mass appeared to begin to recover (Fig. 1). At the end of the study on day 11, skeletal muscle phenylalanine mass in treated tumor-bearing mice had climbed 20% from what it had been on day 2 (P < 0.05) and was 51% higher (P < 0.05) than in untreated tumor-bearing mice; however, it remained 11% lower (P < 0.05) than in healthy mice.

Results when analyzed on the basis of skeletal muscle wet weight were not different from those for phenylalanine mass (data not shown). Also the ratio of phenylalanine mass to wet weight was not different (P > 0.05) among treatments (data not shown).

Mechanisms of skeletal muscle wasting. At the onset of cachexia (day −2), before any losses in protein mass were detected (Fig. 1), the fractional and absolute rates of protein synthesis were lower (~38%; P < 0.05) and degradation higher (+125%; P < 0.05) in skeletal muscle of tumor-bearing compared with healthy mice (Figs. 2 and 3). During the protein wasting phase, between
days 2 and 11, the fractional (approximately −54%) and absolute (−69%) rates of protein synthesis were chronically lower in tumor-bearing than in healthy mice (P < 0.05). The fractional and absolute rates of protein synthesis in tumor-bearing mice were lower on days 2, 4, and 11 compared with day −2 (P < 0.05), but were not different from each other (P > 0.05). The fractional (+67%) and absolute (+22%) rates of protein degradation remained higher on day 2 in tumor-bearing mice compared with healthy mice, although they were lower than they had been on day −2 (P < 0.05). By day 11, the fractional and absolute rates of protein degradation in tumor-bearing mice were not different from healthy mice; as a consequence of the lowered muscle phenylalanine mass in tumor-bearing mice, the absolute rate of protein degradation was lower than it had been on day 4 (P < 0.05). These results are not the product of decreased food intake in tumor-bearing mice; the small reduction in food intake in tumor-bearing mice that occurred before day 1 had no effect on protein synthesis in pair-fed compared with healthy mice (4.7 ± 0.3 vs. 5.0 ± 0.18 %/day, respectively, day 2, P > 0.05). These data clearly demonstrate that skeletal muscle wasting was mediated initially by decreased protein synthesis and increased protein degradation but that chronic wasting was mediated by decreased synthesis.

Acute effect of chemotherapy on skeletal muscle protein turnover. Two days after chemotherapy, the fractional and absolute rates of protein synthesis were not lower in treated compared with untreated tumor-bearing mice (Fig. 2). This suggested that chemotherapy had no negative effects on protein synthesis. We con-
considered that the chemotherapy might have a negative effect on protein synthesis on day 1. We therefore measured protein synthesis on day 1 in the second replicate of the experiment. One day after chemotherapy, the fractional rate of protein synthesis in treated tumor-bearing mice was 66% lower than in healthy mice ($P < 0.05$) (data not shown) and was ~24% lower than in untreated tumor-bearing mice on day 2, although this difference was not statistically significant (data not shown). Protein degradation could not be estimated on day 2 in treated mice because their growth rate was not linear, a criterion for this method.

In healthy mice, chemotherapy decreased the fractional and absolute rates of protein synthesis by 11% compared with healthy untreated mice on days 2 and 4, but this difference was not statistically significant (Fig. 2). The fractional and absolute rates of protein degradation were 30% lower on day 4, but these differences were not statistically significant (Fig. 3). Again, protein degradation could not be estimated on day 2 in treated mice because their body growth rate was not linear. These data suggest that the chemotherapy acutely depressed protein synthesis in both healthy and tumor-bearing mice but the effect was not permanent. Chemotherapy clearly did not increase protein degradation.

**Mechanism of skeletal muscle recovery in cured mice.** Protein synthesis in treated tumor-bearing mice began to increase on day 2 (Fig. 2). The fractional and absolute rates of protein synthesis were ~34% higher on day 2 ($P > 0.05$) in treated compared with untreated tumor-bearing mice. On day 4, protein synthesis was ~110% higher compared with untreated tumor-bearing mice and ~60% higher compared with treated mice on day 2 ($P < 0.05$). Protein synthesis approached healthy levels by day 4. The fractional and absolute rates of protein degradation were ~85% lower in treated tumor-bearing mice compared with healthy mice on day 4 (Fig. 3). The profound decrease in protein degradation and the increase in protein synthesis on day 4 corresponds to the day hypertrophy of muscle mass was first detected. These data clearly indicate that recovery of skeletal muscle protein mass was initiated by decreased protein degradation and the restoration of protein synthesis.

By day 11, the fractional (+73%) and absolute (+46%) rates of protein synthesis were higher ($P < 0.05$) in cured compared with healthy mice and were higher ($P < 0.05$) than they had been on day 4. The fractional (+57%; $P < 0.05$) and absolute (+33%; $P > 0.05$) rates of protein degradation were higher in cured compared with healthy mice; degradation was about eight times higher than on day 4 ($P < 0.05$). These data clearly show that longer-term recovery is mediated solely by increased protein synthesis.

**DISCUSSION**

This investigation demonstrated the time course and mechanisms of both skeletal muscle wasting during cancer cachexia and recovery after chemotherapeutic treatment. Of particular interest was the fact that we were able to study recovery of skeletal muscle using a chemotherapeutic agent that effected a cure so that our observations would not be influenced by ongoing or recurrent cancer cachexia. We showed that wasted skeletal muscle has the ability to generate a strong anabolic response after chemotherapy and that this response was primarily driven initially by a substantial decreased protein degradation and later by increased protein synthesis.

**Wasting of skeletal muscle from cancer cachexia.** Cancer cachexia in C26-bearing mice caused significant wasting of skeletal muscle, and this wasting began shortly after the onset of cachexia, which continued throughout the study. This study measured protein turnover in vivo over a time course to establish the initial and longer-term mechanisms leading to wasting in C26 mice. Protein degradation measured in vivo is determined indirectly as the difference between growth and synthesis and therefore only provides an estimate of protein degradation; these results need to be interpreted with caution. At the very onset of cachexia, protein synthesis was severely reduced and degradation greatly increased in skeletal muscle from tumor-bearing mice. These changes in protein turnover were initiated before any atrophy of skeletal muscle was detected, which just preceded the most catabolic phase of the disease. Protein synthesis in tumor mice remained lower over the experiment but degradation returned to normal. Clearly, initial wasting was mediated by decreased protein synthesis and increased degradation and longer-term wasting solely by decreased synthesis.

Protein wasting in tumor-bearing animals and humans has been shown to be the result of decreased protein synthesis (7), increased protein degradation (27), or both (2, 15, 24). There are differences among tumors in the associated humoral and tumoral response that will influence protein metabolism (29). The present study suggests that some of the differences among tumor models may also reflect the stage at which measurements were made, emphasizing the importance of making measurement over a time course to clearly establish the mechanisms of wasting. These are added complexities that must be considered when establishing mechanisms and developing treatments surrounding the treatment of cancer cachexia. Precise knowledge of the mechanisms of wasting is crucial when devising treatments to minimize wasting and in comprehending the subsequent impact that chemotherapy may have on protein turnover and the processes that lead to recovery.

**Acute effects of chemotherapy on skeletal muscle protein turnover.** Skeletal muscle is of interest when studying recovery from cancer cachexia after chemotherapy. It accounts for >45% of whole body protein mass (19), and the profound muscle weakness and wasting associated with cachexia makes skeletal muscle restoration of considerable importance. To understand the process of recovery from cancer cachexia after chemotherapy, the possible negative effects of
chemotherapy must also be known and considered. Few studies have given attention to the effect of chemotherapeutic agents in well-controlled experimental studies. Most animal studies have tended to focus on the effects of chemotherapy on healthy animals. The hormonal and cytokine milieu, however, is very different during cancer cachexia (29). Le Bricon et al. (14) demonstrated not only that chemotherapeutic agents have a negative impact on protein metabolism but the effect is more severe in tumor-bearing animals. Thus the effect of chemotherapeutic agents on protein metabolism needs to be addressed in the context of cancer cachexia.

In the present study, healthy and tumor-bearing mice injected with cystemustine did not show any major acute alterations to skeletal muscle mass. The chemotherapy, however, reduced protein synthesis somewhat in both groups, but the effect appeared to be greater in the treated tumor-bearing mice. However, the degree of cytotoxicity would depend on dose and type of agent. Decreased protein synthesis induced by cystemustine treatment was not unexpected considering that carmustine, the reference chloroethylnitrosourea, was previously shown to inhibit synthesis of ribosomal RNA and cellular proteins in vitro (12). Chemotherapy did not increase protein degradation as one might expect. The apparent decrease in protein degradation may be a response to limit protein loss due to reduced protein synthesis. This concomitant decrease in degradation likely explains the lack of effect of chemotherapy on protein mass. Interestingly, in parallel studies, we found that cystemustine showed distinct signs of acute cytotoxicity in the small intestine of healthy mice but had little or no effect in tumor-bearing mice (22). In that study, we speculated that the reduced rate of small intestinal protein synthesis in cachectic mice rendered the small intestine less susceptible to the cytotoxic effects of chemotherapy, which normally targets the rapidly proliferating intestinal tissue. The explanation for why there is a differential response between skeletal muscle and small intestine requires further study.

Recovery of skeletal muscle from cancer cachexia after chemotherapy. Complete recovery of skeletal muscle involves restoration of previously existing protein mass. No study to our knowledge has investigated the recovery of skeletal muscle after chemotherapeutic treatment of cancer cachexia. Almost complete recovery of skeletal muscle mass occurred in C26-bearing mice cured with cystemustine. Furthermore, cytotoxicity due to chemotherapy did not appear to seriously impede recovery of skeletal muscle in cured mice. However, this may depend on dose and type of agent used. Le Bricon et al. (14) found that some but not all chemotherapeutic drugs inhibited positive nitrogen balance in tumor-bearing rats.

The present study investigated the mechanisms mediating recovery of skeletal muscle after chemotherapy during cancer cachexia. Skeletal muscle recovery was evident by the second day after chemotherapy, inasmuch as protein synthesis began to increase and degradation decreased to levels well below those of healthy mice. This represents an extraordinarily rapid and dramatic reversal of protein metabolism, especially in degradation. Also, the initial recovery of skeletal muscle occurred under circumstances where protein turnover was severely compromised by cancer cachexia in addition to the effects of chemotherapeutic agent, demonstrating the potential to generate a strong anabolic response.

After the initial phase of recovery, protein synthesis then rose to and subsequently surpassed healthy levels. At the same time, protein degradation increased and subsequently surpassed healthy levels, clearly indicating that the longer-term restoration of skeletal muscle was driven by increased protein synthesis. The fact that recovery was initiated by decreased degradation, rather than increased synthesis, may reflect the negative impact of chemotherapy on protein synthesis. However, because protein synthesis subsequently rose substantially indicates that any cytotoxic effects were not permanent. This also emphasizes the importance of making measurement over a time course, because events are clearly time dependent. In addition, the longer-term mechanisms of recovery may not be universal and need to be studied using other more cytotoxic agents.

**Perspectives**

Having knowledge of the mechanisms involved in restoration of skeletal muscle mass wasted by cancer cachexia is the first step in developing strategies to promote skeletal muscle anabolism. Treatments that decrease degradation and increase skeletal muscle protein synthesis, especially concomitantly, would be of most benefit. However, because chemotherapy can have a negative impact on protein synthesis, which could be a limiting factor in skeletal muscle restoration, treatments promoting synthesis might be of greater benefit. Indeed, our results specifically suggest that treatment strategies should focus on increasing skeletal protein synthesis during the initial stages of recovery. Treatments could be hormonal (e.g., the use of growth hormone or β-adrenergic agents) and/or nutritional. These possibilities await investigation. Finally, we showed that many of the effects of cancer and chemotherapy were time dependent; further studies must select appropriate time points to make measurements to unequivocally ascertain mechanisms of wasting and recovery in the tumor-bearing animal in response to various experimental scenarios.

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REFERENCES


