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Induction of IL-6 by Cytotoxic Chemotherapy Is Associated With Loss of Lean Body and Fat Mass in Tumor-Free Female Mice

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Abstract

Cancer patients treated with cytotoxic chemotherapy experience fatigue and changes in body composition that can impact physical functioning and quality of life during and after treatment. Interleukin-6 (IL-6) is associated with fatigue in cancer survivors and plays an important role in the regulation of body composition. The purpose of the present study was to determine the specific role of IL-6 in cyclophosphamide-doxorubicin-5-fluorouracil (CAF)-induced changes in fatigue, food intake, and body composition using mice lacking IL-6. Female wild-type (WT) and *IL-6*^{-/-} mice were injected with four cycles of CAF or normal saline (NS) administered at 21-day intervals. Daily voluntary wheel-running activity (VWRA), used as a proxy for fatigue, and food intake were monitored daily up to 21 days after the fourth dose. Dual-energy X-ray absorptiometry (DEXA) was used to assess treatment-related changes in lean body mass (LBM), fat mass (FM), and bone mineral content (BMC). Patterns of change in fatigue and food intake did not differ between CAF-treated WT and *IL-6*^{-/-} mice. However, a Genotype × Drug interaction was observed for LBM ($p = 0.047$) and FM ($p = 0.035$) but not BMC ($p = .569$). Whereas WT mice lost LBM and FM during CAF treatment, *IL-6*-deficient mice did not. Treatment-related decreases in levels of the anabolic hormone insulin-like growth factor-1 (IGF-1) may contribute to LBM and FM loss since CAF decreased IGF-1 levels in an IL-6-dependent manner. These findings implicate IL-6 and possibly IGF-1 in the regulation of body composition in breast cancer patients exposed to cytotoxic chemotherapy.

Keywords

fatigue; IL-6; doxorubicin; body composition; cachexia

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Authors' Contribution

LJW conceived of and designed the study. LJW and CRE conducted the experiments and collected data. LJW performed the statistical tests. LJW and JK drafted the manuscript and figures. All authors read and approved the final manuscript.

Declaration of Conflicting Interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Cancer patients undergoing cytotoxic chemotherapy often experience changes in body composition, specifically, decreased lean body mass (LBM), decreased bone mass (BM), and a change (increase or decrease) in fat mass (FM). These changes in body composition may explain the decrease in physical functioning and quality of life and increased risk of bone fracture, cardiovascular disease, and cancer recurrence observed in cancer patients after treatment (Azrad & Demark-Wahnefried, 2014; Vance, Mourtzakis, McCargar, & Hanning, 2011; Weaver et al., 2013; Winters-Stone, Schwartz, Hayes, Fabian, & Campbell, 2012). Researchers have investigated treatment-related changes in dietary intake, reduced physical activity related to fatigue, and reduced resting energy expenditure (REE) as mechanisms of treatment-related changes in body composition (for a recent review see Gadea, Thivat, Planchat, Morio, & Durando, 2012). Although evidence has linked treatment-related declines in physical activity and/or increased dietary intake to gains in FM, the loss of LBM does not appear to be related to these factors (Vance et al., 2011).

The induction of inflammatory cytokines like interleukin-6 (IL-6) by cytotoxic chemotherapy drugs is another potential mechanism underlying these changes in body composition, but it has been the subject of little research till date. Researchers have investigated inflammatory cytokines, including IL-6, as the cause of cachexia, a multifactorial syndrome commonly seen in cancer patients with advanced disease characterized by a progressive loss of skeletal muscle mass with or without loss of FM (Blum et al., 2014; Fearon, Glass, & Guttridge, 2012). Chemotherapy-related increases in IL-6 have been observed in both preclinical models and cancer patients during treatment (Wang et al., 2012; Weymann, Wood, Zhu, & Marks, 2013; Wood et al., 2006). Harden, du Plessis, Poole, and Laburn (2006) demonstrated that lipopolysaccharide (LPS)-induced declines in food intake and voluntary wheel-running activity (VWRA) in rats are partially IL-6 dependent. Increased IL-6 levels are associated with persistent cancer treatment-related fatigue, and investigators have proposed a relationship between fatigue and loss of skeletal muscle mass and function (Al-Majid & McCarthy, 2001; Schubert, Hong, Natarajan, Mills, & Dimsdale, 2007).

We previously developed a clinically relevant adjuvant breast cancer chemotherapy treatment model to determine the specific roles of inflammatory cytokines in the genesis of treatment-related symptoms in mice (Smith et al., 2014). Using this model, we showed that mice injected with four cycles of cytoxan-adriamycin-5-fluorouracil (CAF) administered at 3-week intervals developed acute and persistent fatigue and weight loss. Persistent weight loss, which was evident following four cycles of CAF, was related to losses of FM, BM, and LBM. The purpose of the present study was to use this model to determine the specific role of IL-6 in chemotherapy-related fatigue and changes in body composition using mice engineered to lack IL-6. The anabolic hormone insulin-like growth factor-1 (IGF-1) plays a central role in postnatal growth and is downregulated by inflammatory stimuli such as endotoxin and inflammatory cytokines (O'Connor et al., 2008). Thus, a secondary exploratory aim of the study was to determine whether CAF reduced circulating IGF-1 levels in an IL-6-dependent manner.

Material and Method

Mice

We purchased breeding pairs of IL-6-deficient B6.129S2-*il6^{tm1Kopf}/J* (#002650) and wild-type (WT) C57BL/6J (#000664) mice from Jackson Laboratories (Bar Harbor, ME) and housed them in pathogen-free rooms with a 12-hr light–dark cycle. Mice of the two genotypes were bred under the same conditions. All mice had ad lib access to drinking water supplemented with antibiotics (1.3 mg/ml sulfamethoxazole and 0.3 mg/ml trimethoprim) to prevent infection related to neutropenia, secondary to chemotherapy-induced bone marrow suppression. At the time of sacrifice, mice were terminally anesthetized using isoflurane according to protocols established at the Oregon Health & Science University Department of Comparative Medicine.

Cytotoxic Chemotherapy Administration in Mice

We purchased doxorubicin-HCL as a lyophilized 10-mg tablet from Bedford Labs (Bedford, OH) and dissolved the tablet in sterile, deionized water to attain a stock solution of 1 mg/mL, which we stored at 4°C. We purchased 5-fluorouracil (5-FU) as a 50-mg/mL solution from American Pharmaceutical Partners (Schaumburg, IL) and cyclophosphamide powder from Baxter Healthcare Corporation (Deerfield, IL). We injected drug-treated mice with a combination of cyclophosphamide (Cytoxan), doxorubicin (Adriamycin), and 5-FU at concentrations of 167 mg/kg, 4 mg/kg, and 167 mg/kg, respectively. We first injected mice intraperitoneally (IP) with CA (Cytoxan plus Adriamycin) in a volume of 1-ml normal saline (NS). Approximately 1 hr later, we injected mice IP with 1-ml NS containing 5-FU. We injected NS-treated mice with the same volume of NS without drug. We used this injection volume to reduce localized tissue inflammation at the injection site, provide fluid, and improve absorption of the drug. All injections began at 3 pm. After injection, we returned mice to their home cages.

Assessment of Body Composition

Mice underwent body composition measurement using dual-energy X-ray absorptiometry (DEXA) densitometry using a Lunar PIXImus II (Software Version 1.42.006.010; Lunar Corp, Madison, WI) 3 weeks prior to treatment and then 3 weeks after the last treatment. Prior to scanning, mice were anesthetized by IP injection with 100 mg/kg ketamine and 16 mg/kg xylazine.

Assessment of Fatigue and Food Intake

We used change in VWRA as a proxy for chemotherapy-induced fatigue. Mice were housed singly in pathogen-free rooms (12-hr light–dark cycle) in shoe box cages modified to include an activity wheel with a diameter of 30 cm (Respironics Inc., Sun River, OR). We monitored VWRA automatically using a Mini Mitter magnetic switch and the VitalView data acquisition system (Mini Mitter, Sun River, OR). We placed approximately 10 g (2–3 pellets) of Rodent Diet 5001 (PMI Nutrition International, Brentwood, MO) in the bottom of the cage each day. The following day, we weighed uneaten food and calculated the amount of food eaten during the previous 24 hr.

Experiment 1: Assessment of IL-6 and IGF-1 Expression in Peripheral Blood of CAF- and NS-Treated Mice

For the assessment of IL-6 in peripheral blood, we separated WT female mice into two groups. We injected mice in Group 1 ($n = 50$) with CAF and those in Group 2 ($n = 50$) with NS. We terminally sedated mice with isoflurane and then collected blood from 9 to 10 mice from each group by cardiac puncture at 1, 3, 6, 14, and 24 hr postinjection. We prepared serum from all blood samples and measured the levels of IL-6 and IGF-1 (in 24-hr samples only) as described below.

Experiment 2: Assessment of the Role of IL-6 in Cytotoxic Cancer Chemotherapy–Mediated Changes in Fatigue, Food Intake, and Body Composition

Prior to treatment for Experiment 2, we conducted body composition testing in female WT ($n = 19$) and IL-6-deficient mice ($n = 18$). To determine the contributions of food intake and physical activity level on changes in body composition, we assessed daily food intake and VWRA throughout four cycles of treatment with CAF or NS injected at 21-day intervals. We allowed 2 weeks for mice to become accustomed to the running wheel and established baseline VWRA and food intake levels averaged over 10 days. On Day 11, we separated WT and IL-6-deficient mice into four groups. We injected mice in Groups 1 (WT mice, $n = 9$) and 2 (IL-6-deficient mice, $n = 8$) with CAF, as described above, and mice in Groups 3 (WT mice, $n = 10$) and 4 (IL-6-deficient mice, $n = 10$) with NS without drug. We returned mice to their home cages and monitored daily VWRA and food intake. We repeated these treatments for three more cycles at 21-day intervals and conducted a second round of body composition testing 3 weeks after the last dose of CAF or NS.

Experiment 3: Assessment of the Role of IL-6 in Cytotoxic Cancer Chemotherapy–Mediated Reduction in Circulating IGF-1

WT and IL-6-deficient mice were group housed in pathogen-free rooms (12-hr light–dark cycle) with ad lib access to water. We separated mice into four groups ($n = 5$ in each group) and injected them with either CAF or NS, as described above. To control for the potential effect of nutritional status on serum IGF-1 levels, we withheld mouse chow from all mice for 14 hr postinjection, at which point we terminally anesthetized the mice and collected peripheral blood by cardiac puncture. We collected serum from clotted blood by centrifugation at 8,000 rpm for 5 min at room temperature and stored it at -80°C prior to measurement of IGF-1, as described below.

Measurement of Serum IL-6 and IGF-1

We measured serum IL-6 levels using a bead-based immunofluorescence assay (Luminex Inc., Austin, TX). We obtained cytokine analysis kits from Millipore (Billerica, MA) and performed assays according to the manufacturer-supplied protocol. We collected and analyzed data using the Luminex-100 system version IS (Luminex, Austin, TX). We used a four- or five-parameter regression formula to calculate the sample concentrations from the standard curves. We measured serum levels of IGF-1 by enzyme-linked immunosorbent assay (ELISA) using kits purchased from R&D Systems (Catalog # BD100), and assays were performed in duplicate according to the protocol supplied by the manufacturer.

Analysis

We calculated daily VWRA from the number of wheel turns in a 24-hr period (12-hr light–dark cycle) and used $VWRA_{base10}$ as the average daily VWRA for the 10-day period prior to the start of treatment. We used VWRA during the 12-hr dark phase to calculate the dark-phase average time on wheel, distance run, average speed, and peak speed for the two time periods, that is, (1) the 3 days following the first CAF injection and (2) the last three dark periods of the study. Total distance was the number of wheel turns in 12 hr $\times$ the circumference of the running wheel (.942 m), time on wheel was the number of 1-hr intervals where wheel rotations were >0 , average speed was total distance divided by time on wheel, and peak speed was the maximum speed reached during a 1-hr interval. We used repeated measures analyses of variance (ANOVAs) to examine patterns of change in VWRA and food intake between genotypes and/or treatment groups. We analyzed group differences in body composition measures and serum levels of IL-6 and IGF-1 by ANOVA. Data are presented as the mean $\pm$ standard error of the mean. We used $p < .05$ to indicate statistical significance.

Results

Cytotoxic Cancer Chemotherapy Increases IL-6 Levels

To determine whether CAF could increase the circulating levels of IL-6, in Experiment 1 we injected female WT mice with CAF or NS and measured serum levels of IL-6 at 1, 3, 6, 14, and 24 hr postinjection. Figure 1 shows fold increases in serum levels of IL-6 in CAF-treated WT mice relative to NS-injected WT control mice sacrificed at the same time point. Serum levels of IL-6 increased rapidly following CAF injection and were significantly increased relative to the levels in NS-treated mice 1 hr postinjection, $F(1, 32) = 14.391$, $p = .001$. IL-6 levels remained elevated in CAF-treated mice at 3 hr, $F(1, 32) = 30.140$, $p < .001$, 6 hr, $F(1, 32) = 19.014$, $p < .001$, 14 hr, $F(1, 32) = 42.895$, $p < .001$, and 24 hr postinjection, $F(1, 32) = 31.763$, $p < .001$. Thus, CAF injection led to a rapid and sustained increase in serum levels of IL-6 during the 24-hr postinjection.

Cytotoxic Chemotherapy–Induced IL-6 Production Contributes to the Loss of LBM in Female Mice

In Experiment 2, we sought to determine the specific role of IL-6 in CAF-induced changes in fatigue, food intake, and body composition in WT and IL-6-deficient mice injected with four cycles of CAF or NS at 3-week intervals. Prior to treatment, there was no observed effect of genotype on baseline VWRA, $F(3, 36) = .187$, $p = .905$, or food intake, $F(3, 36) = .299$, $p = .826$. There was, however, an interaction effect for genotype and body composition at baseline. Compared to WT mice, IL-6-deficient mice weighed significantly less (20.3 ± 1.8 g vs. 18.8 ± 0.9 g), $F(1, 36) = 11.301$, $p = .002$. Reduced weight in IL-6-deficient mice was due to a trend to lower FM, $F(1, 36) = 3.62$, $p = .065$, and lower LBM, $F(1, 36) = 9.463$, $p = .004$, and bone mineral content (BMC), $F(1, 36) = 11.67$, $p = .002$. There was, however, no difference in the relative proportions of FM, $F(1, 36) = 1.007$, $p = .323$, LBM, $F(1, 36) = 1.162$, $p = .289$, or BMC, $F(1, 36) = .116$, $p = .736$, between genotypes when normalized to body weight. Figure 2A represents plots of daily VWRA in WT and IL-6-deficient mice administered four cycles of CAF or NS at 21-day intervals. CAF caused a rapid and

significant increase in fatigue (decreased VWRA from baseline) in both genotypes relative to NS-injected mice (Figure 2Ai–ii.). Fatigue was evident for several days post-CAF injection but returned toward baseline prior to the second injection. A similar pattern of fatigue was evident following the final three injections of CAF (Figure 2Ai–ii.). However, after the fourth injection, fatigue did not return to baseline in CAF-treated mice of either genotype (Figure 2Ai–ii.). Although we observed a Time $\times$ Treatment effect, $F(4, 132) = 17.322, p < .0001$, there was no Time $\times$ Drug $\times$ Genotype effect on VWRA, $F(4, 132) = .669, p = .615$, across the four treatment cycles.

Greater than 97% of daily VWRA occurred during the 12-hr dark phase, which is consistent with mice being nocturnal. We calculated dark-phase average time on wheel, distance run, average speed, and peak speed at two time points, that is, (1) the 3 days following the first CAF injection and (2) the last three dark periods of the study prior to the second body composition analysis. These data are presented in Figure 2Bi–iv. At Time 1, WT CAF-treated mice showed reduced time on the wheel, $F(1, 18) = 16.466, p = .001$, distance run, $F(1, 18) = 18.552, p < .001$, average speed, $F(1, 18) = 13.896, p = .002$, and peak speed, $F(1, 18) = 7.530, p = .014$, in the days following injection relative to NS-treated WT mice. We observed a similar effect at Time 1 for CAF-treated IL-6-deficient mice: CAF reduced time on wheel, $F(1, 17) = 38.765, p < .001$, distance run, $F(1, 17) = 27.724, p < .001$, average speed, $F(1, 17) = 16.487, p = .001$, and peak speed, $F(1, 17) = 16.330, p = .001$. Approximately 3 weeks after the fourth treatment, CAF-treated mice of both genotypes still showed a reduction in daily VWRA relative to NS-treated control mice (Figure 2Ai–ii.). At this time, WT CAF-treated mice again spent less time on their wheels, $F(1, 18) = 6.318, p = .022$, and ran a shorter distance, $F(1, 18) = 5.115, p = .037$, than their NS-treated counterparts (Figure 2Bi–iv). Average and peak speed were not significantly different between WT CAF- and NS-treated mice at this time point, although speed and peak speed tended to be lower in CAF-treated mice than in controls, $F(1, 18) = 3.901, p = .065$ and $F(1, 18) = 2.736, p = .116$, respectively. Compared to control *IL-6*^{-/-} mice, CAF-treated *IL-6*^{-/-} mice spent significantly less time on their wheels, $F(1, 17) = 7.757, p = .013$, and ran significantly shorter distances, $F(1, 17) = 9.785, p = .006$, at a significantly lower average speed, $F(1, 17) = 8.674, p = .010$, and peak speed, $F(1, 17) = 6.856, p = .019$, at Time 2. Despite these differences, there was no observed Treatment $\times$ Genotype interaction effect for any of these activity variables at either time point (see Figure 2Bi–iv for *p* values).

Food intake declined immediately following the first CAF injection in WT, $F(1, 18) = 9.9, p = .006$, and *IL-6*^{-/-}, $F(1, 17) = 19.1333, p < .001$, mice but returned to levels similar to those in NS-treated mice by the next day (Figure 3). A similar pattern of food intake was evident following three additional CAF cycles. A compensatory increase in food intake occurred frequently after each CAF dose in mice of both genotypes. We observed a Time $\times$ Drug effect, $F(4, 132) = 4.208, p = .003$, but no Time $\times$ Drug $\times$ Genotype effect on food intake across each of the four treatment cycles, $F(4, 132) = .933, p = .447$.

Figure 4 summarizes changes in body composition during treatment. We observed a Genotype $\times$ Drug interaction on LBM, $F(3, 37) = 4.261, p = .047$. Although CAF caused a significant decrease in LBM in WT mice, $F(1, 17) = 12.020, p = .003$, we observed no such decrease in CAF-treated IL-6-deficient mice, $F(1, 17) = .796, p = .386$. We also observed a

Genotype $\times$ Treatment interaction for FM, $F(3, 37) = 4.843$, $p = .035$, with FM lower, albeit not significantly, in WT CAF-treated mice than in NS-treated control mice, $F(1, 17) = 3.013$, $p = .101$. In contrast, FM in CAF-treated IL-6-deficient mice tended to be higher than in IL-6-deficient NS-treated control mice, $F(1, 17) = 2.104$, $p = .166$. BM was significantly reduced in both *IL-6*^{-/-}, $F(1, 17) = 6.849$, $p = .019$, and WT CAF-treated mice, $F(1, 17) = 11.061$, $p = .004$, but there was no Genotype $\times$ Drug interaction effect, $F(3, 37) = .330$, $p = .569$.

The observed Genotype $\times$ Treatment interactions in FM and LBM did not appear to be due to genotype differences in total food intake or total VWRA during the study because there were no significant Genotype $\times$ Treatment interactions on average daily food intake, $F(3, 37) = .938$, $p = .340$, or average daily VWRA, $F(3, 37) = .358$, $p = .554$. Taken together, our data suggest that CAF-induced IL-6 plays a role in the treatment-induced changes in body composition independent of food intake and total VWRA.

CAF Decreases Circulating IGF-1 Levels in an IL-6-Dependent Manner

Inflammatory cytokines decrease the production of IGF-1, an anabolic hormone that plays a central role in the maintenance of body composition. We reasoned that the CAF-induced changes in body composition observed in WT mice might be related to IL-6-mediated reduction in circulating IGF-1 levels. In Experiment 3, to test this hypothesis, we first determined whether CAF could reduce circulating IGF-1 levels in WT mice. We measured serum levels of IGF-1 in peripheral blood collected from CAF- and NS-treated WT mice 24 hr postinjection. We found that CAF treatment caused a statistically significant reduction in levels of IGF-1 in serum, $F(1, 17) = 6.647$, $p = .020$; Figure 5A. To determine whether this CAF-mediated reduction in circulating IGF-1 levels was dependent upon IL-6, we injected WT and IL-6-deficient mice with CAF or NS and collected peripheral blood at 14 hr postinjection. Because the IGF-1 levels are influenced by nutritional status (Livingstone, 2013) and CAF treatment causes a reduction in food intake, we removed food from all mice following injection. Figure 5B shows fold change in serum IGF-1 in CAF-treated mice relative to control mice. We again observed a significant decrease in blood levels of IGF-1 in WT CAF-treated mice, $F(1, 9) = 5.520$, $p = .047$. In contrast, there was no significant change in serum IGF-1 levels in CAF-treated *IL-6*^{-/-} mice, $F(1, 9) = .004$, $p = .950$. Taken together, these data suggest that IL-6 is required for CAF-induced declines in circulating IGF-1 levels.

Discussion

Prior clinical and preclinical studies have implicated IL-6 in the weight loss associated with advanced cancer (Fearon et al., 2012). In this context, IL-6 is secreted by tumor cells directly and/or by the host immune system in response to the tumor. The present study is the first to demonstrate a specific role for IL-6 in the weight loss associated with cytotoxic chemotherapy in the absence of tumor burden. We showed that CAF, a clinically relevant cytotoxic chemotherapy regimen used in the treatment of early-stage breast cancer, increases circulating levels of IL-6, a finding consistent with those of other preclinical and clinical studies (Wang et al., 2012; Wood et al., 2006). The induction of IL-6 by CAF was rapid and

yielded a sustained increase in serum IL-6 levels during the 24 hr postinjection. Smith et al. (2014) found that, in contrast to IL-6, serum levels of IL-1 β and tumor necrosis factor- α were highly variable in the hours following CAF injection, which likely reflects their short half-life.

In the present study, CAF also caused a rapid increase in fatigue and anorexia that, in the case of fatigue, persisted for several days before returning to baseline. Although the CAF-induced increase in serum IL-6 levels coincided with the onset of fatigue and anorexia, IL-6 deficiency had no impact on either variable since patterns of fatigue and anorexia in IL-6-deficient mice were no different than those in WT mice. Our findings are in contrast to those from prior rodent studies that demonstrated a role for IL-6 in lipopolysaccharide (LPS)-induced sickness behavior (Bluthe, Michaud, Poli, & Dantzer, 2000; Harden, du Plessis, Poole, & Laburn, 2006). In the study by Harden and colleagues (2006), IL-6 antisera dampened the LPS-mediated decline in VWRA and food intake in rats, while Bluthe, Michaud, Poli, and Dantzer (2000) found that the decline in social exploration and body weight following LPS injection was significantly dampened in mice lacking IL-6. Gender differences may explain these incongruities, since investigators performed the prior studies with male rodents, and we used female mice in the present study. Differences in the nature of the inflammatory stimuli provided by cytotoxic chemotherapy and LPS also likely contributed to these disparate results. The inflammatory-cytokine and sickness-behavior responses to LPS are rapid, yet transient, and accompanied by fever (Grossberg et al., 2011). Unlike LPS, cytotoxic chemotherapy causes widespread tissue damage and prolonged sickness-behavior-like symptoms and does not cause fever (Weymann et al., 2013). While IL-6 did not appear to play a role in CAF-mediated fatigue in the present study, additional CAF-induced cytokines and chemokines may have played a role. Monocyte chemoattractant protein-1 (MCP-1) is an attractive candidate. We showed previously that CAF increases MCP-1 levels in WT mice (Smith et al., 2014). In addition, Mahoney et al. (2013) used MCP-1-deficient mice to show that a 5-FU-mediated decline in VWRA was MCP-1 dependent (Mahoney et al., 2013).

While fatigue and food intake were no different in CAF-treated IL-6-deficient mice relative to their WT counterparts, we observed a striking difference in CAF-induced changes in body composition between the two genotypes. Although CAF-treated WT mice lost LBM and FM relative to their sham-treated counterparts, CAF-treated IL-6-deficient mice did not. These changes in body composition were independent of food intake and physical activity level since there were no significant differences between WT and IL-6-deficient CAF-treated mice in the amount of food eaten or VWRA during treatment. Our findings are consistent with the hypotheses that implicate inflammatory processes rather than simply reduced caloric intake or reduced physical activity in cancer cachexia. Thus, while nutritional support and increased physical activity are key components of managing cancer cachexia, it is also important to target the inflammatory processes that drive anorexia, hypercatabolism, and hypoanabolism (Fearon, Arends, & Baracos, 2013).

The sparing of LBM and FM in CAF-treated IL-6-deficient mice in the present study suggests that losses of LBM and FM in CAF-treated WT mice were due to increased energy expenditure and/or enhanced catabolism and/or decreased anabolism. Several studies in

cancer patients with advanced disease have shown that weight loss is associated with increased REE and levels of inflammatory cytokines, including IL-6 (Cao et al., 2010; Deans et al., 2009). Of note, studies of early-stage cancer patients undergoing cytotoxic chemotherapy have found an inconsistent relationship between changes in body composition and REE. While some studies showed that REE increased during treatment, others found no change or a decrease (Campbell, Lane, Martin, Gelmon, & McKenzie, 2007; Demark-Wahnefried et al., 2001; Harvie, Campbell, Baildam, & Howell, 2004). In addition, research has shown that loss of FM in cachectic cancer patients is related to enhanced lipolysis (Ryden & Arner, 2007; Zuidgeest-van Leeuwen et al., 2002). IL-6 has been implicated in lipolysis, and our finding that, relative to sham-treated mice, CAF-treated WT mice lost FM while IL-6-deficient CAF-treated mice did not supports this idea (Ji et al., 2011; Wolsk, Mygind, Grondahl, Pedersen, & van Hall, 2010).

To our knowledge, this is the first study to demonstrate a specific role for IL-6 in the regulation of circulating IGF-1 following exposure to cytotoxic chemotherapy. Hepatic IGF-1, the primary source of circulating IGF-1, is regulated by growth hormone (GH) secreted from the anterior pituitary via activation of the signal transducer and activator of transcription 5 (STAT5; Rotwein, 2012). During systemic inflammation, STAT5 signaling is reduced, leading to reduced IGF-1 expression despite normal levels of circulating GH. Ahmed et al. (2007) showed that IL-6 reduces the transcription of hepatic IGF-1 expression by inhibiting STAT5 signaling. Further work is needed to determine whether the CAF-induced decrease in circulating IGF-1 is mediated by impaired pituitary GH secretion or hepatic GH signaling or decreased production by other tissues. Although the gain-and-loss-of-function studies have shown a central role for hepatic IGF-1 in the maintenance of musculoskeletal mass (List et al., 2014), additional work is also needed to elucidate the specific role that IGF-1 plays in CAF-related loss of LBM.

In summary, the data from the present study implicate IL-6 in cytotoxic chemotherapy—related loss of FM and LBM. This work supports the need for follow-up clinical studies that examine the relationships among treatment-related changes in IL-6, IGF-1, and body composition. Understanding the mechanisms underlying cancer treatment-related symptoms will allow the development of targeted strategies aimed at their prevention or management in cancer patients.

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Figure 1.

Cytotoxic cancer chemotherapy increases circulating inter-leukin (IL)-6 levels in wild-type (WT) mice relative to normal saline (NS)-treated mice. Fold increase in serum levels of IL-6 in cyclophosphamide-doxorubicin-5-fluorouracil (CAF)-treated ($n = 50$) relative to normal NS-treated mice ($n = 50$) sacrificed at 1, 3, 6, 14, and 24 hr postinjection ($n = 4-5$ mice per group per time point). Each bar represents the mean $\pm$ standard error of mean (SEM) of each value. The asterisks indicate a statistically significant difference in serum protein level (pg/mL) between groups. *** $p < .0001$.

Figure 2.

Cytotoxic chemotherapy-induced changes in voluntary wheel-running activity (VWRA) in wild-type (WT) and *IL-6*^{-/-} mice. A, Daily VWRA and food intake in (i) WT and (ii) *IL-6*^{-/-} mice injected with cyclophosphamide-doxorubicin-5-fluorouracil (CAF; *n* = 17, filled circle) or normal saline (NS; *n* = 20 open circle). The black arrows represent the time points at which mice were administered CAF or NS. One-way analysis of variance (ANOVA) was used to detect differences between the CAF and NS groups. Each data point represents the mean of each value. Error bars are not included to allow discrimination of data trends. (B) Dark-phase change from baseline in average (i) time on wheel, (ii) distance run, (iii) and speed, and in (iv) peak speed calculated at Time 1 (the three dark phases following the first CAF injection) and Time 2 (the last three dark phases of the study prior to body composition analysis). Two-way ANOVA was used to detect significant Treatment × Genotype interactions (see underlined *p* values above each bar cluster). Each bar represents the mean ± standard error of mean (SEM) of each value. **p* < .05, ***p* < .001, ****p* < .0001, †*p* = .065, and ††*p* = .116 for comparison between treatment groups within genotypes.

Figure 3.

Cytotoxic chemotherapy–induced changes in food intake in wild-type (WT) and *IL-6*^{-/-} mice. Daily food intake in (i) WT and (ii) *IL-6*^{-/-} mice injected with cyclophosphamide-doxorubicin-5-fluorouracil (CAF; $n = 17$, filled circle) or normal saline (NS; $n = 20$, open circle). The black arrows represent the time points at which mice were administered CAF or NS. One-way analysis of variance (ANOVA) was used to detect differences between the CAF and NS groups. Each data point represents the mean of each value. Error bars are not included to allow discrimination of data trends. * $p < .05$ for comparison between treatment groups within genotypes.

Figure 4.

Cytotoxic chemotherapy-induced changes in body composition in wild-type (WT) and *IL-6*^{-/-} mice. Body composition analysis was performed 3 weeks before and 3 weeks after treatment. Bars show mean treatment-related changes in fat mass (FM), lean body mass (LBM), and bone mineral content (BMC) derived from pre- and posttreatment dual-energy absorptiometry (DEXA) scans on cyclo-phosphamide-doxorubicin-5-fluorouracil (CAF)- or normal saline (NS)-treated wild-type (WT) and IL-6-deficient mice. Two-way analysis of variance (ANOVA) was used to detect significant Treatment × Genotype interactions (see underlined *p* values above each bar cluster). **p* < .05, ***p* < .001, and ****p* < .0001 for comparison between treatment groups within genotypes.

Figure 5.

Effects of cytotoxic chemotherapy treatment on circulating insulin-like growth factor (IGF)-1 levels in wild-type (WT) and *IL-6*^{-/-} mice. A, Serum levels of IGF-1 in nonfasted WT mice 24 hr after injection with cyclophosphamide-doxorubicin-5-fluorouracil (CAF) or normal saline (NS). Each bar represents the mean $\pm$ standard error of mean (SEM) of each value. B, Fold increase in fasting serum IGF-1 levels in CAF-treated WT and IL-6-deficient mice relative to NS-treated controls at 14 hr postinjection. Each bar represents the mean $\pm$ SEM of each value. * $p < .05$ for comparison of IGF-1 level (A) or change (B) between groups.