Interleukin-6 and cachexia in Apc\(^{Min/+}\) mice

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\(^1\)Integrative Muscle Biology Laboratory, Exercise Science Department, \(^2\)Department of Biological Sciences, and the \(^3\)Center for Colon Cancer Research, University of South Carolina, Charleston, South Carolina; and \(^4\)Department of Cellular and Molecular Pharmacology, Medical University of South Carolina, Charleston, South Carolina

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Baltgalvis KA, Berger FG, Pena MM, Davis JM, Muga SJ, Carson JA. Interleukin-6 and cachexia in Apc\(^{Min/+}\) mice. Am J Physiol Regul Integr Comp Physiol 294: R393–R401, 2008. First published December 5, 2007; doi:10.1152/ajpregu.00716.2007.—The Apc\(^{Min/+}\) mouse has a mutation in the Apc tumor suppressor gene and develops intestinal polyps, beginning at 4 wk of age. This mouse develops cachexia by 6 mo, characterized by significant loss of muscle and fat tissue. The purpose of the present study was to determine the role of circulating interleukin-6 (IL-6) and the polyp burden for the development of cachexia in Apc\(^{Min/+}\) mice. At 26 wk of age, mice exhibiting severe cachectic symptoms had a 61% decrease in gastrocnemius muscle weight, complete loss of epididymal fat, a 10-fold increase in circulating IL-6 levels, and an 89% increase in intestinal polyps compared with mildly cachectic animals. Apc\(^{Min/+}\)/IL-6\(^{-/-}\) mice did not lose gastrocnemius muscle mass or epididymal fat pad mass while overall polyp number decreased by 32% compared with Apc\(^{Min/+}\) mice. Plasmid-based IL-6 overexpression in Apc\(^{Min/+}\)/IL-6\(^{-/-}\) mice led to a decrease in gastrocnemius muscle mass and epididymal fat pad mass and increased intestinal polyp burden. IL-6 overexpression did not induce cachexia in non-tumor-bearing mice. These data demonstrate that IL-6 is necessary for the onset of adipose and skeletal muscle wasting in the Apc\(^{Min/+}\) mouse and that circulating IL-6 can regulate Apc\(^{Min/+}\) mouse tumor burden.

CACHEXIA IS CHARACTERIZED as an overall state of ill health, accompanied by a loss of lean body mass and fat mass, weakness, fatigue, anemia, metabolic abnormalities, inflammation, and impaired immune function, with or without anorexia (2–4, 24). This condition is associated with other life-threatening diseases, such as cancer and AIDS, and negatively affects life quality and survival (2, 26, 36). Almost 50% of all cancer patients experience progressive wasting of adipose tissue and/or skeletal muscle mass (37), predominantly during the terminal stages of disease. Cancer patients can lose up to 30% of their original body weight, and cachexia accounts for ~20–33% of cancer deaths. In particular, gastrointestinal and lung cancer patients are extremely susceptible to cachexia (16, 31). The Apc\(^{Min/+}\) mouse is an established model of colorectal cancer and cachexia (28, 30). This mouse develops intestinal polyps as early as 4 wk of age and becomes cachectic between 3 and 6 mo of age. Use of Apc\(^{Min/+}\) mice to study cachexia has many advantages in that these mice have a polypl burden that mimics human colorectal cancer, chronic low levels of circulating interleukin-6 (IL-6), and lack of anorexia. However, the mechanisms inducing wasting in these mice are poorly understood.

One potential candidate for inducing cachexia is IL-6, a pleiotropic cytokine that modulates a variety of physiological responses and activates genes associated with cellular proliferation, differentiation, and apoptosis (20). Elevated circulating IL-6 levels have been associated with cachexia (21). Intraperitoneal IL-6 treatment (40), intracerebroventricular IL-6 injections in the lateral ventricle, IL-6 transgenic mice (38), and colon-26 tumor-bearing mice with elevated plasma IL-6 levels (45) all demonstrate an ~25% decrease in gastrocnemius muscle weight. Blocking IL-6 through neutralization antibodies results in attenuation of IL-6-induced muscle wasting in rodents (14, 35, 39, 44). In contrast, some studies administering exogenous IL-6 to rodents fail to find an association between high circulating IL-6 levels and cachexia (12, 15, 33, 42, 43). The disparities between studies on IL-6 and cachexia point to indirect effects of IL-6 on skeletal muscle wasting during cancer. One such indirect mechanism may be IL-6-induced tumor growth. IL-6 is also a potent tumor growth factor (8, 13, 34), and the contribution of IL-6-induced tumor growth to cachexia has not been elucidated.

IL-6 elicits its effect by binding to a plasma membrane receptor complex that contains the common signal-transducing receptor chain glycoprotein 130 (9, 20, 23). Ligation of this receptor activates signal transducers and activators of transcription (STAT-3) (9, 11, 20), which is generally associated with cell growth, differentiation, development, and survival (6). Skeletal muscle and myocytes are sensitive to IL-6, and treatment with IL-6 results in phosphorylation of STAT-3 (1, 17, 19, 32, 41). Skeletal muscle STAT-3 is activated during atrophy (18, 22), but it is unknown whether muscle STAT-3 activation leads to cachexia.

The purpose of the present study was to determine the relationship between circulating IL-6 and polyp burden for the development of cachexia in Apc\(^{Min/+}\) mice. We hypothesized that increased circulating IL-6 would both increase the polyp burden and overall wasting in Apc\(^{Min/+}\) mice. In addition to wild-type and Apc\(^{Min/+}\) mice, Apc\(^{Min/+}\)/IL-6\(^{-/-}\) mice were developed to ablate IL-6. A plasmid-based in vivo electroporation system delivered into skeletal muscle was used to overexpress IL-6 in Apc\(^{Min/+}\) and Apc\(^{Min/+}\)/IL-6\(^{-/-}\) mice, as well as wild-type mice.

MATERIALS AND METHODS

Animals. C57BL/6, Apc\(^{Min/+}\), and Apc\(^{Min/+}\)/IL-6\(^{-/-}\) mice were originally purchased from Jackson Laboratories (Bar Harbor, ME), and breeding was maintained at the University of South Carolina’s
animal resource facility as previously described (28). Apc\(^{Min+}\) mice (n = 12) were housed individually in cages with voluntary activity wheels and killed at 26 wk of age (see activity protocol below). Apc\(^{Min+}\)/IL-6\(^{-/-}\) mice were genotyped for the mutant Apc allele, IL-6, and neomycin resistance. The primer sequences for IL-6 were as follows: forward 5'-TTC CAT CCA GTT GCC TTC TTG G-3' and reverse 5'-TTC TCA TTT CCA CGA TTT CCC AG-3'. The reverse primer sequence for neomycin resistance was as follows: 5'-CCG GAG AAC CTG CGT GCA ATC C-3'.

Electroporation of plasmid DNA in the quadriceps muscle was used after 10 wk of IL-6 overexpression.

under brief isoflurane anesthesia from the retroorbital eye sinus 7 days to examine the effect of circulating IL-6 on muscle mass. To take advantage of this variability, mice were categorized as >2 mm, 1–2 mm, and <1 mm in the small and large intestine.

Total RNA isolation and cDNA synthesis. Total RNA was isolated from the gastrocnemius muscles using TRIzol reagent (Invitrogen, Carlsbad, CA) as previously described (27). Extracted RNA was treated with 10 units of DNase I to degrade any residual contaminating genomic DNA. cDNA was reverse transcribed from 3 \(\mu\)g of total RNA using 1 \(\mu\)l of random hexamers and 50 units of Superscript III reverse transcriptase (Invitrogen) in a final volume of 20 \(\mu\)l at 25°C for 10 min, followed by 42°C for 60 min, and 70°C for 15 min. Real-time PCR. Real-time PCR was performed using reagents from Applied Biosystems (Foster City, CA). Gene expression for IL-6 (FAM dye) was carried out in 25-\(\mu\)l reactions consisting of 2 \(\times\) Taqman Universal PCR master mix (AmpliTaq Gold DNA Polymerase, Buffer, dNTP mix, AmpErase UNG, MgCl\(_2\)), 1.0 \(\mu\)l cDNA, RNase-free water, and 60 nM of each primer. The sequences for the primers were as follows: cytoplasmic forward 5'-TGT GCC AGG GTG GTG ACT T-3', cytoplasmic reverse 5'-TCA AAT TTC TCT CCT CCG TAG ATG GAC TT-3'. Samples were analyzed on an ABI 7300 Sequence Detection System. Reactions were incubated for 2 min at 50°C and 10 min at 95°C, followed by 50 cycles consisting of a 15-s denaturing step at 95°C and a 1-min annealing/extension step at 60°C. Data were analyzed by ABI software using the cycle threshold (CT), which is the cycle number at which the SYBR or FAM fluorescence emission is midway between detection and saturation of the reaction. The 2\(^{-\Delta\Delta CT}\) method (25) was used to determine changes in gene expression between treatment groups with the cyclophilin C\(_\alpha\) as the correction factor.

Western blotting. Western blotting was performed as previously described (28). Briefly, frozen gastrocnemius muscle was homogenized in Mueller buffer, and protein concentration was determined by the Bradford (7) method. Crude muscle homogenate (30–60 \(\mu\)g) was fractionated on 8–12% SDS-polyacrylamide gels. Gels were transferred to polyvinylidene difluoride membranes overnight. Membranes were Ponceau stained to verify equal loading of each gel. Membranes were blocked overnight in 5% milk in Tris-buffered saline with 0.1% Tween 20 (TBS-T). Primary antibodies for total and phosphorylated STAT-3 (Tyr705) were purchased from Cell Signaling (Danvers, MA). Antibodies were diluted 1:1,000 to 1:2,000 in 5% BSA in TBS-T followed by overnight incubation with membranes at 4°C. Anti-rabbit

following each electrophoration and at death to ensure validity of the procedure. The IL-6 level for each animal was averaged for all time points assessed over the experimental period. In previous studies using this kit, we have detected 3–30 pg IL-6/ml Apc\(^{Min+}\) mouse plasma (28, 29).

Tissue collection. Mice were injected with a ketamine-xylazine-acepromazine cocktail (1.4 ml/kg body wt), and gastrocnemius muscles, epidymal fat pads, tibias, and intestines were excised. Tibia length was measured as an indicator of animal body size. The gastrocnemius muscle was rinsed in PBS, snap-frozen in liquid nitrogen, weighed, and stored at -80°C until further analysis. The small intestines were removed as described previously (29). Briefly, the intestines were dissected, cleared of mesentery adipose tissue, and fixed in 10% buffered formalin for 24 h. Plasma was collected via the inferior vena cava with heparinized needles, stored on ice, and centrifuged at 1,000 \(g\) for 10 min at 4°C, and the plasma was stored at -80°C until further analysis.

Polyp counts. Polyp counts were performed as described previously (29). Briefly, formalin-fixed intestinal sections from all animals were stained with hematoxylin and eosin, mounted on slides, and counted by a single investigator who was blinded to the treatments. Polyps were counted under a dissecting microscope, using tweezers to pick through the intestinal villi and identify polyps. Polyps were categorized as >2 mm, 1–2 mm, and <1 mm in the small and large intestine.

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Statistical analysis. Data were analyzed with a two-tailed Student's t-test (GraphPad Prizm 4.00 for Windows) and are presented as the mean ± SEM. A value of P < 0.05 was considered statistically significant.
IgG horseradish peroxidase-conjugated secondary antibodies (GE Healthcare Life Sciences, Piscataway, NJ) were incubated with the membranes at 1:2,000 to 1:5,000 dilutions for 2 h in 5% milk in TBS-T. Enhanced chemiluminescence (GE Healthcare Life Sciences) was used to visualize the antibody-antigen interactions. Film was digitally scanned, and blots were quantified by densitometry using scientific imaging software (Scion Image, Frederick, MD). The Ponceau-stained membranes were also digitally scanned and the 45 kDa actin bands were quantified by densitometry and used as a protein-loading correction factor for each lane.

Statistical analysis. Repeated-measures ANOVA was used to examine changes in body weight, food intake, and wheel running activity over time in mice categorized by stage of cachexia. One-way ANOVAs or independent t-tests were used to determine significance for all other variables. Post hoc analyses were performed with Student-Newman-Keuls’s methods. When the assumption of normality failed, a Kruskal-Wallis one-way ANOVA on ranks was performed. Post hoc analyses were performed with Dunn’s method. Significance was set at P < 0.05.

RESULTS

Voluntary activity and body weight. Body weight was traced over the entire study in ApcMin/+ mice that had access to voluntary activity wheels. There were no differences in body weight between mildly and severely cachectic ApcMin/+ mice at the beginning of the study (P = 0.904; Fig. 1A). The severely cachectic mice started to decrease in body weight by 18 wk of age (P = 0.015) and differed in body weight by the completion of the study at 26 wk of age (P < 0.001). Body weights in severely cachectic ApcMin/+ mice were reduced 20% compared with mildly cachectic mice at the study’s end (26.2 ± 0.3 vs. 20.9 ± 0.6 g; P < 0.001). Food intake was also measured weekly; there were no differences between the mildly cachectic and severely cachectic mice at any time point (P = 0.115; Fig. 1B). All of these mice had access to voluntary activity wheels, which were used as an indicator of each animal’s health. Running distance per day was not different between the two groups at the beginning of the study (P = 0.975; Fig. 1C). However, by 15–18 wk of age, the severely cachectic mice started to decrease running wheel distance (P < 0.001) and were running significantly less than the mildly cachectic group through the completion of the study.

Muscle and fat pad mass. Skeletal muscle weights were measured to determine the amount of skeletal muscle wasting in ApcMin/+ mice stratified by their degree of cachexia. Severely cachectic ApcMin/+ mice had a 61% decrease in gastrocnemius muscle weight and complete elimination of the epididymal fat pad compared with mildly cachectic mice (Fig. 2A). Other hindlimb muscles were examined. In severely cachectic mice, soleus muscle mass was reduced 30% (P = 0.003), plantaris muscle mass was reduced 57% (P < 0.001), and tibialis anterior muscle mass was reduced 53% (P = 0.002) compared with mildly cachectic mice. However, tibia length, an indicator of body frame size, did not differ by degree of cachexia (16.4 ± 0.0 vs. 16.5 ± 0.1 mm; P = 0.297).

Polyp number. To determine whether the intestinal tumor burden varied with cachexia severity, polyp counts were also performed in these mice. Total polyp number was 89% higher.
in severely cachectic mice compared with mildly cachectic mice ($P < 0.001$; Fig. 2B). However, when stratified by polyp size, polyps 1–2 mm in diameter and >2 mm in diameter were greater in severely cachectic mice compared with mildly cachectic mice.

**Plasma IL-6 and muscle STAT-3 activation.** Plasma IL-6 levels were increased ~10-fold in mice with severe cachexia ($P = 0.020$; Fig. 2C). Overall, there was a strong correlation (Fig. 2D) between tumor number and plasma IL-6 ($r = 0.789$, $P = 0.002$). To determine whether downstream targets of IL-6 signaling were activated in cachectic muscle, Western blotting was performed to assess STAT-3 activation in mildly and severely cachectic $Apc^{Min}$ mouse gastrocnemius muscle (Fig. 2E). The ratio of phosphorylated STAT-3 to total STAT-3...
increased 4.5-fold in severely cachectic mice compared with mildy cachectic mice (P = 0.004). There was a significant inverse correlation between STAT-3 activation (P/total STAT-3) and gastrocnemius muscle weight (r = −0.831; P = 0.002). Gastrocnemius muscle IL-6 mRNA did not change with severity of cachexia (38.40 ± 0.35 vs. 38.22 ± 0.25 CT; P = 0.91), suggesting that circulating IL-6 rather than muscle-produced IL-6 was contributing to STAT-3 activation.

**IL-6 ablation.** The effect of IL-6 ablation on cachexia development and progression was examined with Apc<sup>Min</sup>/IL-6<sup>−/−</sup> mice. At 13 wk of age, Apc<sup>Min</sup> mice had already begun epididymal fat pad loss (Fig. 3A), but there were no differences in gastrocnemius muscle mass (Fig. 3B) between the three strains. At 26 wk of age, Apc<sup>Min</sup>/IL-6<sup>−/−</sup> mice did not demonstrate wasting and had gastrocnemius muscle and epididymal fat pad masses similar to C57BL/6 mice. Maintenance of fat and muscle mass was not due to increased caloric intake, since daily food intake was actually reduced 11% in Apc<sup>Min</sup>/IL-6<sup>−/−</sup> mice compared with Apc<sup>Min</sup> mice (0.147 ± 0.003 vs. 0.165 ± 0.002 g food/g body wt; P < 0.001).

To determine whether skeletal muscle was sensitive to circulating IL-6 levels, Western blotting was performed to determine STAT-3 activation (Fig. 3C). The ratio of phosphorylated to total STAT-3 was lowered 83% in Apc<sup>Min</sup>/IL-6<sup>−/−</sup> mice compared with Apc<sup>Min</sup> mice.

**IL-6 overexpression.** Mice were electroporated in the quadriceps muscle with either an IL-6 expression plasmid or control plasmid. C57BL/6 mice and Apc<sup>Min</sup>/IL-6<sup>−/−</sup> were electroporated every 2 wk for a total of 10 wk, starting at 16-wk of age. Apc<sup>Min</sup> mice were only electroporated for a total of 4 wk since they undergo cachexia spontaneously by 6 mo of age (Fig. 4A). Average plasma IL-6 levels were elevated significantly in mice receiving the IL-6 plasmid compared with mice receiving the control plasmid in C57BL/6 mice, Apc<sup>Min</sup> mice, and Apc<sup>Min</sup>/IL-6<sup>−/−</sup> mice (Table 1). The plasma IL-6 levels obtained with overexpression were similar to humans with cachexia before death (21).

There were no differences in body weight at the beginning of the study between the different treatment groups and within any strain (Table 1). Apc<sup>Min</sup> mouse body weight was decreased 11% by IL-6 overexpression (P = 0.010), and Apc<sup>Min</sup>/IL-6<sup>−/−</sup> mouse body weight decreased 21% (P = 0.012) compared with mice receiving the control plasmid. Apc<sup>Min</sup> mice receiving IL-6 had a 70% reduction in epididymal fat pad mass (P = 0.028), and Apc<sup>Min</sup>/IL-6<sup>−/−</sup> mice had a 63% decrease in epididymal fat pad mass (P = 0.030) compared with control mice (Fig. 4B). IL-6 overexpression in the circulation also reduced gastrocnemius muscle mass 23% in Apc<sup>Min</sup> mice (P = 0.004) and 32% in Apc<sup>Min</sup>/IL-6<sup>−/−</sup> mice (P = 0.007; Fig. 4C). Plasmid-based IL-6 overexpression induced cachexia in mice with a polyb burden but not in wild-type mice. The body weights of C57BL/6 mice were not affected by IL-6 overexpression. C57BL/6 epididymal fat pad mass and gastrocnemius muscle weight were not affected by circulating IL-6 overexpression. Food intake was not affected by IL-6 overexpression within any of the strains (data not shown).

During IL-6 overexpression, phosphorylated STAT-3 was induced in all strains of mice (Figs. 4D). It appears that skeletal muscle is sensitive to circulating IL-6 levels and STAT-3 is activated even in the absence of muscle mass loss. Because...
IL-6 can act in a paracrine manner, gastrocnemius IL-6 mRNA was measured to determine if IL-6 produced by the muscle was responsible for STAT-3 activation. Gastrocnemius muscle IL-6 mRNA did not change with IL-6 overexpression in wild-type mice ($P=0.943$) or in $Apc^{	ext{Min}+}$ /$IL-6^{-/-}$ mice ($P=0.34$). Thus changes in skeletal muscle STAT-3 activation appear to be due to circulating IL-6 rather than muscle-produced IL-6.

Polyp burden and IL-6. We examined if $Apc^{	ext{Min}+}$ mouse intestinal polyp burden was sensitive to circulating IL-6 levels. Total polyp number was 32% lower in the 26-wk-old $Apc^{	ext{Min}+}$ /$IL-6^{-/-}$ compared with $Apc^{	ext{Min}+}$ mice ($P=0.014$; Fig. 5A). Polyp size was also affected in the $Apc^{	ext{Min}+}$ /$IL-6^{-/-}$ mouse with polyps $>1$ mm in diameter being reduced by 32% compared with $Apc^{	ext{Min}+}$ mice ($P=0.023$; Fig. 5B).

IL-6 overexpression increased both intestinal and colon polyp number and size. Total polyp number was 64% greater with IL-6 electroporation in $Apc^{	ext{Min}+}$ /$IL-6^{-/-}$ mice (Fig. 5C). Polyps $>1$ mm in diameter also increased 61% with IL-6.
overexpression (P = 0.030; Fig. 5D). Similar results were found with IL-6 overexpression in ApcMin+/− mice. ApcMin+/− mouse total polyp number increased 82% with IL-6 overexpression (55 ± 13 vs. 100 ± 5 polyps; P = 0.005). Polyp size also increased with IL-6 overexpression in polyps >1 mm in diameter by 98% (47 ± 14 vs. 92 ± 5; P = 0.005). Overall, these results indicate that changes in circulating IL-6 are associated with a greater tumor burden and a greater loss of adipose and muscle tissue.

**DISCUSSION**

A strength of the ApcMin+/− mouse as a cachectic model is the variable wasting seen between mice. This variable wasting rate may be related to significant differences in tumor burden between mice. To examine different stages of muscle wasting, we classified ApcMin+/− mice by the degree of wasting they exhibited. ApcMin+/− mice with the most severe cachectic symptoms also had the highest circulating IL-6 levels and the largest intestinal polyp burden. The importance of IL-6 was also demonstrated by the lack of wasting and a lower overall tumor burden in ApcMin+/−/IL-6−/− mice compared with age-matched ApcMin+/− mice. Systemic IL-6 overexpression in ApcMin+/− and ApcMin+/−/IL-6−/− mice induced wasting and polyp formation. However, IL-6 overexpression was not sufficient to induce skeletal muscle wasting in nontumor-bearing mice. These data suggest that systemic IL-6 is essential for the development of cachexia in ApcMin+/− mice and is associated with a greater tumor burden.

The current study reports that severity of cachexia in ApcMin+/− mice is associated with both a greater number and larger intestinal polyps. Although others have suggested that cachexia-inducing tumors are not necessarily large tumors (37), our data suggest that a critical tumor burden is necessary to induce cachexia in the ApcMin+/− mouse. The ApcMin+/− mouse primarily develops adenomas, but there is not metastasis (30). It is possible that the increase in adenoma number or growth creates an environment that promotes muscle wasting. Additionally, we had previously shown that plasma IL-6 levels were elevated in cachectic ApcMin+/− mice compared with age-matched, wild-type mice (28). The current study extended this finding by showing that, in 26-wk-old ApcMin+/− mice, increased plasma IL-6 levels were also associated with increased polyp number and size. Because IL-6 is a potent tumor growth factor (8, 13, 34), IL-6 may be leading to an increase in polyp

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**Table 1. IL-6 overexpression reduces body weight in mice with a polyp burden but not in wild-type mice**

<table>
<thead>
<tr>
<th>Control (n = 10)</th>
<th>+ IL-6 (n = 10)</th>
<th>Control (n = 8)</th>
<th>+ IL-6 (n = 10)</th>
<th>Control (n = 8)</th>
<th>+ IL-6 (n = 6)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plasma IL-6, pg/ml</td>
<td>1.7±0.8</td>
<td>152.5±17.9*</td>
<td>9.7±2.8</td>
<td>181.1±29.6*</td>
<td>0±0</td>
</tr>
<tr>
<td>Pre body wt, g</td>
<td>27.7±1.0</td>
<td>27.3±0.4</td>
<td>24.0±0.5</td>
<td>24.6±0.4</td>
<td>26.0±0.3</td>
</tr>
<tr>
<td>Post body wt, g</td>
<td>27.1±1.0</td>
<td>28.9±0.4</td>
<td>24.0±0.5</td>
<td>21.8±0.5*†</td>
<td>27.1±0.7</td>
</tr>
</tbody>
</table>

Values are means ± SE; n, no. of mice. IL-6, interleukin-6. Plasma IL-6 was measured after each electroporation and averaged for each mouse. Values are means ± SE. Changes in body wt were analyzed with a repeated-measures one-way ANOVA. Changes in plasma IL-6 were analyzed with independent t-tests. Significance was set at P < 0.05. *Different from control. †Different from Pre.

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**Fig. 5. ApcMin+/− mouse polyp burden varies with changes in circulating IL-6.** A: total polyp burden in 26-wk-old ApcMin+/− and ApcMin+/−/IL-6−/− mice. B: polyp burden stratified by size in 26-wk-old ApcMin+/− and ApcMin+/−/IL-6−/− mice. C: total polyp burden in ApcMin+/−/IL-6−/− mice following IL-6 overexpression. D: polyp burden stratified by size in ApcMin+/−/IL-6−/− mice following IL-6 overexpression. Values are means ± SE. Data were analyzed with independent t-tests. Significance was set at P < 0.05. *Different from ApcMin+/− mice or control.
burden, promoting the cachectic environment driven by the tumor. Although the association between high circulating IL-6 levels and muscle wasting has been demonstrated previously (21, 38, 40, 45), the current study shows that even low, chronic levels of IL-6 are associated with muscle mass loss. This is important because the levels of circulating IL-6 seen in ApcMin/+ mice are similar to humans with cachexia (21).

To examine the importance of IL-6 for development of cachexia and intestinal/colon polyps, we generated ApcMin/+/-/H11001 mice. Unlike 26-wk-old ApcMin/+ mice, age-matched ApcMin/+/-/IL-6/-/- mice did not exhibit wasting of muscle and fat pad mass. This demonstrates the dependence of IL-6 on the induction of cachexia. These data coincide with other models of cachexia showing that blocking the effects of IL-6 have positive effects on skeletal muscle mass (14, 35, 39, 44). In the current study, ApcMin/+/-/IL-6/-/- mice also had a lower intestinal tumor burden than ApcMin/+ mice. Although tumor growth is associated with cachexia, many studies that delay or reverse cachexia through the inhibition of IL-6 or other inflammatory cytokines fail to detect a change in tumor growth (5, 10, 21).

We find that the induction of cachexia showing that blocking the effects of IL-6 have positive effects on skeletal muscle mass (14, 35, 39, 44). In the current study, ApcMin/+/-/IL-6/-/- mice also had a lower intestinal tumor burden than ApcMin/+ mice. Although tumor growth is associated with cachexia, many studies that delay or reverse cachexia through the inhibition of IL-6 or other inflammatory cytokines fail to detect a change in tumor growth (5, 10, 35). This may be related to the tumor model used to induce cachexia. An advantage of the ApcMin/+ mouse is the ability to count and classify by polyp size throughout the intestine and colon. Therefore, it is possible that the ablation of IL-6, which leads to preservation of muscle and fat mass in ApcMin/+ mice, is mediated through the inhibition of tumor growth. One limitation of the ApcMin/+/-/IL-6/-/- mouse model is that IL-6 is ablated in both skeletal muscle and tumors, making it impossible to determine the source of IL-6 that is important for the induction of cachexia. Nevertheless, this has important implications for clinicians treating cachectic patients because methods for controlling the increase in plasma IL-6 seen during cancer may be beneficial for both suppressing tumor growth and maintaining skeletal muscle and adipose tissue mass.

To determine if IL-6 was sufficient to induce cachexia, circulating IL-6 was increased in wild-type, ApcMin/+ and ApcMin/+/-/IL-6/-/- mice. Although the circulating levels obtained through overexpression were physiological, it should be noted that they were at least 10-fold higher than those seen in cachetic ApcMin/+ mice. Overexpression of circulating IL-6 induced wasting in both ApcMin/+ and ApcMin/+/-/IL-6/-/- mice but not in wild-type mice. Others have also shown that IL-6 induces cachexia in mice with tumors (33, 35, 43), similar to our finding in ApcMin/+ mice. Additionally, ApcMin/+ and ApcMin/+/-/IL-6/-/- mice receiving IL-6 also had a greater tumor burden than mice receiving the vector control. An additional finding of the current study was that elevated circulating IL-6 induced wasting and polyp growth in the absence of tissue-level expression. ApcMin/+/-/IL-6/-/- mice lacked IL-6 expression in intestinal polyps, adipose tissue, and skeletal muscle and underwent cachexia in the presence of high circulating IL-6. It has been suggested that tumor-produced IL-6 is essential for cachexia (44), but the current study does not support tumor production of IL-6 as necessary for the induction of cachexia. In addition, IL-6 did not induce wasting in wild-type, non-tumor-bearing mice, suggesting other factors related to the overall tumor environment may also be involved in the regulation of cachexia. Further work is needed to discover the specific interaction between the tumor and IL-6 that mediates wasting.

IL-6 signaling at the cellular level involves the phosphorylation and dimerization of STAT-3. The current study examined STAT-3 phosphorylation in wasting skeletal muscle. Skeletal muscle STAT-3 activation has been detected in other forms of atrophy, such as sarcopenia (18) and following crush injury (22), but STAT-3 activation has not been studied during cancer cachexia. Greater muscle STAT-3 activation was associated with more muscle mass loss in ApcMin/+ mice. Overexpression of circulating IL-6 in ApcMin/+ mice did induce STAT-3 activation. However, STAT-3 activation also occurred in wild-type mice that did not have muscle wasting. Although skeletal muscle STAT-3 phosphorylation is sensitive to circulating IL-6, this signaling pathway does not appear to be sufficient to induce skeletal muscle mass loss. This adds further support that indirect mechanisms of IL-6, possibly related to other factors related to the overall tumor burden of the animal, are important for inducing muscle mass loss during cancer.

Perspectives and Significance

This study supports a role of systemic IL-6 in the development of cachexia and intestinal polyp growth in the ApcMin/+ mouse. ApcMin/+ mice exhibiting a greater severity of cachexia and a larger intestinal tumor burden also had the highest circulating IL-6 levels. Circulating IL-6 overexpression also accelerated ApcMin/+ mouse cachexia and increased intestinal polyp number. Wasting was not induced by circulating IL-6 overexpression in wild-type mice, which have no intestinal tumors. Together, these data suggest an interaction between intestinal tumors and high circulating IL-6 levels, which create an environment favoring catabolism of skeletal muscle and adipose tissue. This was further demonstrated by the absence of cachexia in tumor-bearing mice that lack IL-6 and adding back systemic IL-6-induced cachexia in these mice. These findings have important implications for treatment of cancer and the associated cachexia. The ability to inhibit specific targets of IL-6 regulation may serve beneficial to both tumor progression and the maintenance of total body mass. Future work will attempt to identify muscle-specific and other systemic-related changes induced by IL-6 that promote adipose tissue and muscle catabolism.

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GRANTS

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Apc\(^{Min+}\) MICE AND CACHEXIA


