ORIGINAL ARTICLE

The effect of exercise on IL-6-induced cachexia in the $Apc^{Min/+}$ mouse

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Abstract

Background Cachexia involves unintentional body weight loss including diminished muscle and adipose tissue mass and is associated with an underlying disease. Systemic overexpression of IL-6 accelerates cachexia in the $Apc^{Min/+}$ mouse, but does not induce wasting in control C57BL/6 mice. With many chronic diseases, chronic inflammation and metabolic dysfunction can be improved with moderate exercise. A direct effect of regular moderate exercise on the prevention of IL-6-induced cachexia in the $Apc^{Min/+}$ mouse has not been investigated. The purpose of this study was to assess the effects of exercise on the development of cachexia in the $Apc^{Min/+}$ mouse.

Methods Mice were randomly assigned to moderate treadmill exercise (18 m/min, 1 h, 6 days/week, 5% grade) or cage control (CC) groups from 6 to 14 weeks of age. At 12 weeks of age, mice were electroporated with either IL-6-containing or control plasmid into the quadriceps muscle. Mice were killed after 2 weeks of systemic IL-6 overexpression or control treatment.

Results IL-6 overexpression induced an 8% loss in body weight in CC mice, which was significantly attenuated by exercise. IL-6 overexpression in CC mice increased fasting insulin and triglyceride levels, which were normalized by exercise, and associated with increased oxidative capacity, an induction of AKT signaling, and a repression of AMPK signaling in muscle. These exercise-induced changes occurred despite elevated inflammatory signaling in skeletal muscle.

Conclusion We conclude that moderate-intensity exercise can attenuate IL-6-dependent cachexia in $Apc^{Min/+}$ mice, independent of changes in IL-6 concentration and muscle inflammatory signaling. The exercise effect was associated with improved insulin sensitivity and improved energy status in the muscle.

Keywords Inflammation · Colorectal cancer · Insulin resistance · Oxidative capacity

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1 Introduction

Cachexia, characterized by severe loss of body weight, including both muscle and adipose mass, develops in numerous diseases, including cancer, HIV-AIDS, and chronic renal failure [1]. This condition is also associated with chronic inflammation, hypermetabolism, fatigue, impaired immune function, and overall weakness, leading ultimately to increased morbidity and mortality. Nearly 50% of all cancer patients are diagnosed with cachexia, and cachexia plays a significant role in 20% of all cancer-related deaths [2]. Although modeling cachexia in rodents has been proven extremely beneficial for understanding the regulation of this wasting process, the underlying disease may ultimately impact the specific mechanisms responsible for the initiation and progression of the wasting process [3]. The $Apc^{Min/+}$ mouse is an established model of colon cancer that develops

a slowly progressing cachexia when compared with many other cancer cachexia models and provides physiologic relevance to the human condition. At codon 850 in the *Adenomatous polyposis coli* (*Apc*), gene there is a nonsense mutation that predisposes the mice to intestinal adenomas [4]. Cachexia is initiated around 14 weeks of age, and the average life span of these mice is approximately 20–26 weeks. The initiation and progression of cachexia in this mouse is directly related to the intestinal tumor burden and circulating IL-6 levels [5].

Regular moderate-intensity exercise reduces the risk for colon cancer and increases survival after diagnosis with colon cancer [6]. A well-established mechanism that exercise has, which may play a role in cancer prevention, is associated with lowering the level of inflammation [7–9]. Several studies have shown that exercise also decreases the incidence of tumor growth in the *Apc*^*Min/+* mouse and other rodent cancer [10–16]. Although exercise has been widely mentioned as having a great potential for preventing and slowing the development of cancer cachexia, few studies address the mechanisms related to this potential benefit. A major limitation to mechanistic studies is that the therapy should attenuate cachexia, independent of effects on the underlying disease [17]. Treating the underlying disease is mechanistically and physiologically different from treating the cachexia associated with the disease. We recently demonstrated that *Apc*^*Min/+* mice decrease voluntary wheel running distance with the progression of cachexia [12] and that this change is associated with a decrease in muscle oxidative capacity in both primarily fast- and slow-type skeletal muscle [18]. Chronic fatigue, which also leads to a decrease in activity and potentially a decrease in oxidative capacity, is common among cancer patients [19, 20]. The decrease in muscle oxidative capacity is one potential mechanism for stimulating the progression of cachexia [18] that may also play a part in altered metabolism during cachexia. It remains to be determined whether maintenance of muscle oxidative capacity through increased muscle contraction, i.e., exercise, can slow or prevent the progression of cachexia in the absence of effects on the underlying disease.

Insulin resistance develops in many cancer patients [21, 22], and the association between insulin resistance and cancer cachexia is widely acknowledged [17]; however, a direct role for insulin resistance in the development and progression of cancer cachexia is uncertain [22]. Pharmacological agents that improve insulin resistance have been effective, decreasing polyp formation and attenuating wasting in tumor-bearing mice [22, 23]. Exercise is also commonly used to increase insulin sensitivity in various disease states, including diabetes and obesity [24, 25]. In healthy rodents, exercise improves both glucose tolerance [26] and insulin sensitivity [27]. Further examination for the potential for exercise to inhibit progression of cachexia

through improved insulin action [28] and/or a reduction in chronic systemic inflammation [29–32] is well justified.

Plasma concentrations of interleukin-6 (IL-6), an inflammatory cytokine, are correlated with the progression of cachexia in late-stage cancer patients [33]. Although not all patients or animal models demonstrate this IL-6 dependency, the *Apc*^*Min/+* mouse and the C26 ectopic tumor cell models of colon cancer are well-characterized models in which elevated IL-6 levels have an established role in the progression of cachexia [5, 34]. The regulatory function of IL-6 is not well defined. It has both pro-inflammatory and anti-inflammatory properties as well as the ability to activate transcription of genes involved in cell proliferation, differentiation, and apoptosis [35]. Contracting skeletal muscle synthesizes and releases IL-6, and circulating IL-6 increases transiently with exercise [9]. The function of transiently increased, exercise-induced IL-6 has been linked to metabolic signaling with exercise [9], distinct from chronically elevated IL-6 levels associated with inflammation [3]. Chronically elevated IL-6 levels have been associated with insulin resistance and can be used to predict the development of type 2 diabetes in humans [36]. It has also been shown to increase fasting glucose levels in a dose-dependent manner in healthy human subjects [37]. IL-6 has been shown to suppress insulin action through the activation of STAT3, which then acts on the insulin receptor suppressing the activation of IRS-1 and downstream targets [38, 39].

Increased circulating IL-6 levels are associated with the progression of cachexia in *Apc*^*Min/+* mice [5]. We have also demonstrated that IL-6 overexpression in *Apc*^*Min/+* mice accelerates the progression of cachexia and increases the intestinal/colon tumor burden [5]. Although there is an inverse relationship between voluntary wheel running distance and the development of cachexia in *Apc*^*Min/+* mice [12] and regular treadmill exercise can reduce tumor growth in these mice [40], the effect of regular moderate exercise on the prevention and progression of IL-6-induced cachexia in the *Apc*^*Min/+* mouse has not been investigated. The purpose of the current study was to determine the effect of regular moderate treadmill exercise on the development and progression of IL-6-induced cachexia in the *Apc*^*Min/+* mouse. We hypothesized that exercise training during systemic IL-6 overexpression would prevent muscle mass loss and fat mass loss and also attenuate changes in systemic glucose metabolism. Additionally, we hypothesized that regular moderate exercise training would benefit skeletal muscle by increasing oxidative capacity, improving metabolic signaling, and reducing muscle inflammatory signaling despite high circulating IL-6 levels and intestinal tumors. To test these hypotheses, treadmill-trained or cage control non-cachectic *Apc*^*Min/+* mice were subjected to 2 weeks of systemic IL-6 overexpression between 12 and 14 weeks of age. Age-matched wild-type mice served as additional controls.

Changes in body weight, physical activity, grip strength, and glucose tolerance were monitored over the 2-week study. Muscle and fat mass were examined at the end of the study and the quadriceps muscle examined for changes in protein expression related to oxidative capacity, inflammatory signaling, and metabolic signaling. We have previously demonstrated that skeletal muscle IL-6 mRNA expression is not induced during the progression of cachexia [18], but elevated systemic IL-6 is the source of increased STAT3 signaling in muscle. Although not a primary outcome of the study, muscle protein expression data are also presented for the quadriceps muscle directly overexpressing IL-6 due to electroporation as an opportunity to compare systemic effects with the combination of systemic and local IL-6 over-expression. Muscle expression data are also presented for wild-type mice overexpressing IL-6 but not having a tumor burden.

2 Methods

2.1 Animals

$Apc^{Min/+}$ male mice, purchased from Jackson Laboratories, were crossed with C57BL/6 female mice at the Animal Resource Facilities at the University of South Carolina. Using a tail snip taken at the time of weaning, mice were genotyped for heterozygous expression of the Apc gene using RT-PCR. Male mice were housed four to five per cage, with $Apc^{Min/+}$ mice kept in separate cages from control C57BL/6 mice. All mice were kept on a 12:12-h light/dark cycle starting at 7:00 AM. Mice had unrestricted access to rodent chow (HarlanTeklad Rodent Diet, no. 8604) and water. Mice were weighed weekly. A subset of $Apc^{Min/+}$ mice ($n=10$) was used for an additional experiment to examine IL-6 and triglyceride levels with varying degrees of cachexia. While body weights, muscle weights, and fat pad mass from this subset of mice have been previously published [18], the IL-6 and triglyceride measurements from these mice have not been previously published. Blood was collected at 10, 14, and 20 weeks and analyzed for triglycerides and IL-6; mice were killed at 20 week and stratified by degree of cachexia into mild or severe groups based on extent of weight loss [18]. The University of South Carolina's Institutional Animal Care and Use Committee approved all animal experimentation.

2.2 Treadmill protocol

At 5 weeks of age, mice were grouped into either exercise or cage control (Fig. 1); after 3 days of acclimation, they started their training as previously described [40]. Briefly, acclimation consisted of running at a 5% grade for a total of 20 min

with a gradual increase in speed starting at 10 m/min and increasing to 18 m/min. After the acclimation period, mice started on a training regimen that consisted of a 5-min warm-up at 10 m/min at 5% grade, followed by 55 min of running at 18 m/min at 5% grade. All training was conducted at the beginning of the dark cycle (1900 hours) in the dark. Mice were encouraged to run by gentle prods, as needed. Mice ran 6 days a week. After electroporation at 12 weeks, the mice received a 2-day break from exercise and then mice ran until 14 weeks of age, when they were killed.

2.3 Grip strength and RotoRod performance

In a subset of mice ($n=5$–6 per treatment group), combined hindlimb and forelimb rodent grip strength was measured with the Grip Tester (Columbus Instruments, Columbus, OH). Mice were placed with all four limbs on a metal grid mounted at a 45° angle connected to a force transducer. Mice were pulled by the tail until they let go of the grid. Each mouse went through a series of two sets of five repetitions of force measurements, with a 2- to 3-min rest period between each set. The highest and the lowest scores from each set of five repetitions were removed and the remainders averaged to provide a mean force measurement for each mouse.

Neuromuscular performance was assessed ($n=5$–6 per treatment group) with the RotoRod (Columbus Instruments). The protocol consisted of a ramping protocol from 0 to 25 rpm over a period of 90 s. The protocol continued at 25 rpm from 90 to 120 s. Each mouse performed the protocol three times, with each trial separated by a 1- to 2-min rest period. The longest time of the three tests was recorded for each mouse. Mice were tested for grip strength and RotoRod performance at 11 and 14 weeks of age. The mice were acclimated to the testing procedures for 3 days prior to the start of testing.

2.4 Cage activity monitoring

At 4 and 12 weeks of age, a subset of mice ($n=5$–6) were single-housed and placed in activity monitor cages (Opto-M3 Activity Meter, Columbus Instruments). Activity was measured for 10 h during the dark cycle (9 PM–7 AM); the number of beams crossed in an X–Y plane was recorded for two consecutive nights. Food consumption was also recorded during this time while the mice were single-housed.

2.5 IL-6 overexpression

At 12 weeks of age, mice were electroporated with either an empty vector or an IL-6 plasmid as previously described [5]. The IL-6 plasmid, driven by the CMV promoter, was used to increase endogenous IL-6 production in the mice.

The mice were anesthetized with a mixture of isoflurane and oxygen during the procedure. While unconscious, the right leg was shaved and cleaned with alcohol. A small incision was made over the right quadriceps muscle and 50 μg of either control vector or IL-6 plasmid injected into the muscle. To promote uptake of the plasmid into the myofibers, a series of eight 100-V pulses lasting 50 ms each were used on the quadriceps muscle. The skin was then closed with a wound clip. Mice were killed 2 weeks later.

2.6 Tissue collection

At the time of killing (14 weeks), mice were given a subcutaneous injection of ketamine–xylazine–acepromazine cocktail (1.4 mL/kg body weight) and tissues were collected as previously described [40]. Briefly, tissues were dissected out, weighed, and then snap-frozen in liquid nitrogen and stored at −80°C.

2.7 Intestinal tissue collection

Intestinal tissue collection was performed as described previously, with a slight modification [41]. Briefly, the small intestines were dissected at the distal end of the stomach and at the proximal end of the cecum. The large intestine was removed from the distal end of the cecum to the anus. Mesentery adipose tissue was removed with tweezers and the small intestine cut into four equal sections. All intestinal sections were flushed with phosphate-buffered saline (PBS), opened longitudinally with a pair of scissors, and flattened with a cotton swab between two pieces of blotting paper. Intestinal sections were fixed in 4% paraformaldehyde (PFA) in PBS overnight and transferred to PBS for storage at 4°C for further analysis.

2.8 Polyp counts

Polyp counts were performed as previously described [40, 41]. Intestinal sections from all animals were fixed with 4% PFA, stained and briefly in 0.1% methylene blue, then placed under a dissecting microscope. The polyp number was counted by the same investigator in a blind manner using tweezers to pick through the intestinal villi and identify polyps. Polyp sizes were categorized as large (>1 mm in diameter) or small (≤1 mm).

2.9 Glucose tolerance test

To determine whether IL-6 overexpression or exercise had an effect on glucose clearance, a glucose tolerance test was conducted at 12 and 14 weeks of age in a subset of animals ($n=6$–7) after an overnight fast. A 20% glucose solution (0.1 mL/g body weight) was administered via intraperitoneal injection. Blood glucose was measured with a handheld glucometer (Bayer CONTOUR®) prior to the glucose injection and at 15, 30, 60, 90, and 120 min following the glucose injection. Blood was collected from the tail vein for insulin and glucose analysis at each time point. HOMA index was calculated fasting insulin (μU/m)×fasting glucose (mmol/l)/22.5 [42].

2.10 Enzyme-linked immunosorbent assays

Blood was collected at 12 and 14 weeks of age via a retro-orbital sinus puncture and centrifuged at 10,000×g for 10 min. Plasma was collected and stored at −80°C until analysis. Using commercial ELISA kits for insulin (Millipore, Billerica, MA), IL-6 (Invitrogen, Fredrick, MD), and adiponectin and leptin (R&D Systems, Minneapolis, MN) according to the manufacturer's instructions, circulating levels of fasting insulin, IL-6, adiponectin, and leptin were measured.

2.11 Plasma triglycerides and glycerol

After an overnight fast, blood was collected from the tail vein in capillary tubes. Plasma triglycerides were determined in 3 μL aliquots enzymatically using a colorimetric assay from ThermoDMA (Thermo Electron Corp., Melbourne, Australia). Glycerol was measured enzymatically using a glycerol assay kit (Cayman, Ann Arbor, MI).

2.12 Western blotting

Briefly, frozen quadriceps were homogenized in Mueller buffer and the protein concentration determined by the Bradford method. Crude homogenates (30–60 g) were fractionated on 8–15% polyacrylamide gels. Gels were transferred to PVDF membranes overnight, and Ponceau staining was used to visually confirm the gel transfer and equal loading. Membranes were blocked in 5% Tris-buffered saline with 0.1% Tween 20 (TBST) milk for 1 h at room temperature. Primary antibodies for phosphorylated-STAT3 (Y705), phosphorylated NFkB p65 (S468), p-AKT (T308), p-AMPK (T172), cytochrome C, COX IV, total AKT, total AMPK, total STAT3, and total NFkB (p65; Cell Signaling) were incubated at dilutions of 1:100 to 1:2,000 overnight at 4°C in 1% TBST milk. Secondary anti-rabbit or anti-mouse IgG-conjugated secondary antibodies were incubated with the membranes at 1:2,000 to 1:5,000 dilutions for 1 h in 1% TBST milk. Enhanced chemiluminescence (GE Healthcare) was used to visualize the antibody–antigen interactions and developed by autoradiography (Kodak, Biomax, MR). Digitally scanned blots were analyzed by measuring the integrated optical density of each band using digital imaging software (Scion Image, Frederick, MD).

2.13 Statistical analysis

Results are reported as the means ± SE. Variables were analyzed with two-way ANOVA (IL-6 overexpression × exercise) to assess significant effects and interactions within each genotype. Post hoc analyses were performed with Student–Newman–Keuls methods. A pre-planned t test was run (Min × Min + IL-6) in the cage control animals. The accepted level of statistical significance was $p<0.05$.

3 Results

3.1 Body weight, growth, food consumption, and tumor burden

It has previously been shown that overexpressing IL-6 can accelerate cachexia in $Apc^{Min/+}$ mice, but does not induce cachexia in wild-type C57BL/6 mice [5]. The current study extends our previous work by overexpressing IL-6 at a young age, just prior to the onset of cachexia, then evaluating the effects of exercise despite increasing plasma IL-6 concentrations. IL-6 was overexpressed for 2 weeks, starting at 12 weeks of age. Exercise with or without IL-6 overexpression had no effect on the overall rate of growth, as measured by the change in tibia length. Systemic IL-6 overexpression is known to cause growth retardation in rodents [43]. Two weeks of IL-6 overexpression tended to suppress the growth rate in wild-type mice ($p=0.06$, Fig. 2a), but did not cause them to lose weight. Exercise alone had no effect on body weight in either wild-type or $Apc^{Min/+}$ mice. IL-6 overexpression significantly reduced the body weight of $Apc^{Min/+}$ mice at 14 weeks of age (7.8%; Fig. 1b and Table 1), while exercise effectively prevented the IL-6-induced decrease in body weight in $Apc^{Min/+}$ mice (Fig. 2b). IL-6 levels in non-exercising cage control $Apc^{Min/+}$ mice were significantly correlated with body weight loss from 12 to 14 weeks of age (Fig. 2c). Two weeks of IL-6 overexpression in $Apc^{Min/+}$ mice induced a significant body weight loss that was suppressed by exercise. Food consumption was monitored both before and after the 2 weeks of IL-6 overexpression in sedentary and exercised mice (Table 1). At 12 weeks of age, food consumption was not statistically different between sedentary wild-type (0.12±0.01 g/g body weight per day) and sedentary $Apc^{Min/+}$ mice (0.13±0.01 g/g body weight per day). Two weeks of IL-6 overexpression significantly altered food intake. In wild-type mice, there was a main effect of IL-6 regardless of exercise to decrease food consumption. Non-exercising $Apc^{Min/+}$ mice overexpressing IL-6 also tended to decrease food consumption ($p=0.06$). Exercise alone in the $Apc^{Min/+}$ mice significantly decreased food consumption, and exercise with IL-6 overexpression restored food consumption to control levels (Table 1). Food consumption did not correlate with changes in body weight in the cage control $Apc^{Min/+}$ ($r^2=0.14$, $p=0.86$) or wild-type mice ($r^2=0.38$, $p=0.53$) overexpressing IL-6. Plasma IL-6 levels from electroporated animals ranged from 21 to 165 pg/mL in $Apc^{Min/+}$ mice and from 0 to 8 pg/mL in vector-treated $Apc^{Min/+}$ mice. Exercise had no effect on circulating IL-6 levels or the overall rate of growth in $Apc^{Min/+}$ mice. However, exercise
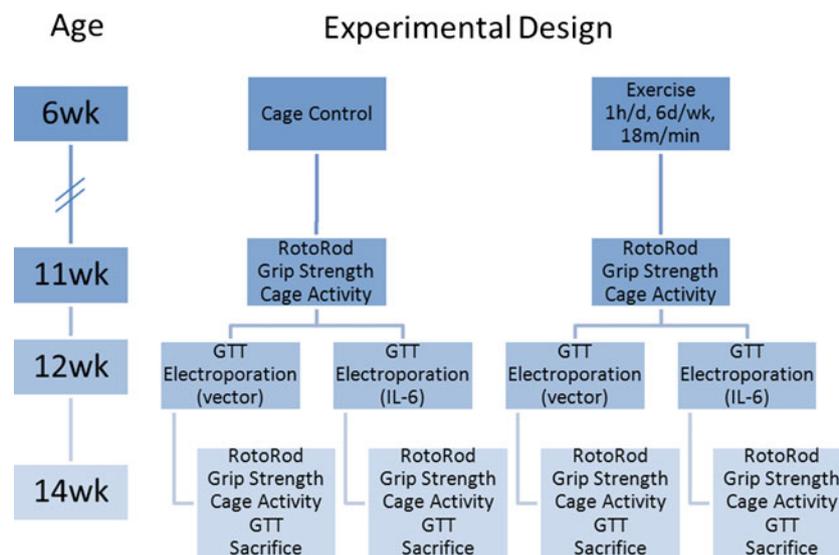

Fig. 1 Schematic of experimental design. Mice started the exercise regimen at 6 weeks of age, after being acclimated to the treadmill at 5 weeks of age. Mice ran 6 days/week for 1 h at 18 m/min at the beginning of the dark cycle. At 12 weeks of age, functional measurements were taken including grip strength and RotoRod performance. A glucose tolerance test was conducted and then the mice were electroporated. After 2 weeks of IL-6 overexpression (14 weeks), functional measures and a glucose tolerance test were conducted and mice were killed

Table 1 Effect of exercise and IL-6 overexpression on body weight and growth in wild-type and $Apc^{Min/+}$ mice

N	C57BL/6				$Apc^{Min/+}$			
	CC vector 12	CC IL-6 11	Ex vector 13	Ex IL-6 12	CC vector 12	CC IL-6 15	Ex vector 14	Ex IL-6 12
BW 12 weeks	24.9±0.4	24.3±0.4	24.2±0.4	24.7±0.6	23.2±0.4	23.5±0.3	22.7±0.4	22.7±0.4
BW 14 weeks	25.9±0.3	24.6±0.4	25.2±0.4	25.5±0.7	23.2±0.3	21.8±0.4[a]	22.8±0.4	22.5±0.5[a]
Tibia length	17.2±0.1	17.2±0.1	17.1±0.1	17.3±0.1	17.0±0.1	16.9±0.1	17.1±0.1	17.1±0.1
Plasma IL-6 (pg/mL)	0.0±0.0	103.2±12.1[a]	0.0±0.0	118.7±12.1[a]	5.5±2.4	102.5±23.8[a]	5.8±2.2	87.9±6.0[a]
% Polyps >1 mm	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	59.3±5.9	54.5±3.6	30.8±3.6[c]	40.8±2.9[c]
Spleen (mg)	147±18	120±18	146±31	151±27	284±30	358±22[a]	287±32	359±48[a]
14-week food consumption	0.16±0.01	0.10±0.01[a]	0.14±0.01	0.11±0.01[a]	0.16±0.01	0.11±0.01[b]	0.11±0.01[b]	0.16±0.03

Body weights were measured at 12 weeks (pre) and again at 14 weeks (post) after 2 weeks of IL-6 overexpression (cage control). Tibia length was measured at 14 weeks as an index of body size. Plasma IL-6 levels were measured after 2 weeks of overexpression. Incidence of large polyps (≥1 mm) was measured in the distal half of the small intestine at 14 weeks of age as an index of tumor burden. Values are means±SE. Data were analyzed with two-way ANOVA within each genotype. Significance was set at $p≤0.05$

BW body weights, *CC* cage control

[a] Effect of IL-6

[b] Different from the CC vector

[c] Effect of exercise

did reduce the incidence of large tumors and increase daily food consumption in $Apc^{Min/+}$ mice overexpressing IL-6. Two weeks of IL-6 overexpression did not significantly alter total tumor number (data not shown); however, exercise significantly decreased the proportion of large tumors in $Apc^{Min/+}$ mice by ~20% (Table 1). This is consistent with previous data demonstrating that moderate treadmill exercise decreased the intestinal tumor burden in $Apc^{Min/+}$ mice [13, 40]. Decreased tumor burden is thought to be a result of a decrease in inflammation, but with the current model, IL-6 remained high even with exercise (Table 1).

3.2 Muscle, fat, spleen, and testes mass

Quadriceps muscle mass was recorded at the time of killing. The study performed electroporation on the right quadriceps muscle. The experimental design allowed the examination of the right quadriceps muscle overexpressing IL-6 and the left quadriceps muscle only exposed to high systemic IL-6. Systemic IL-6 overexpression caused a 13% decrease in mass of the non-electroporated left quadriceps muscle from $Apc^{Min/+}$ mice, and this loss was blocked by exercise. In contrast, systemic IL-6 overexpression in wild-type mice did not affect quadriceps muscle mass (Fig. 2d). However, local muscle IL-6 overexpression caused muscle mass loss in both the wild-type (−13%) and the $Apc^{Min/+}$ mice (−30%) in the electroporated right quadriceps, which could not be rescued by exercise (Fig. 2e).

Wild-type mouse epididymal fat pad mass was not affected by IL-6 overexpression, but there was a significant main effect

of exercise to reduce epididymal fat mass 25% regardless of IL-6 concentration. $Apc^{Min/+}$ mouse fat pad mass was reduced 30% by IL-6. Despite the positive effects of exercise on body weight and muscle mass loss in $Apc^{Min/+}$ mice, epididymal fat pad mass loss was not attenuated by exercise (Fig. 2f). As expected, spleen weight was twofold greater in control $Apc^{Min/+}$ mice compared with control wild-type mice. IL-6 overexpression further increased $Apc^{Min/+}$ spleen weight by 25%; exercise had no effect on this IL-6 effect, which is consistent with the lack of effect of exercise on inflammation. There was no effect of IL-6 overexpression or exercise on wild-type spleen weight (Table 1).

IL-6 overexpression in sedentary $Apc^{Min/+}$ mice that lost body weight also had a reduction in quadriceps muscle and epididymal fat pad mass (Fig. 2f) and an increased spleen weight (Table 1). When comparing the sedentary IL-6-overexpressing $Apc^{Min/+}$ mice that lost body weight to the exercising IL-6-overexpressing $Apc^{Min/+}$ mice that were weight-stable, the exercising mice maintained quadriceps mass in the systemically exposed muscle. Exercise had no effect on the IL-6-induced loss of epididymal fat or increase in spleen size and was unable to rescue muscle mass in the right quadriceps overexpressing IL-6.

3.3 Physical activity and functional performance

It has previously been shown that voluntary wheel running, as a measure of cage activity, decreases with severity of cachexia [10–16]. In the present study, ambulatory cage activity was measured as the number of times the mouse
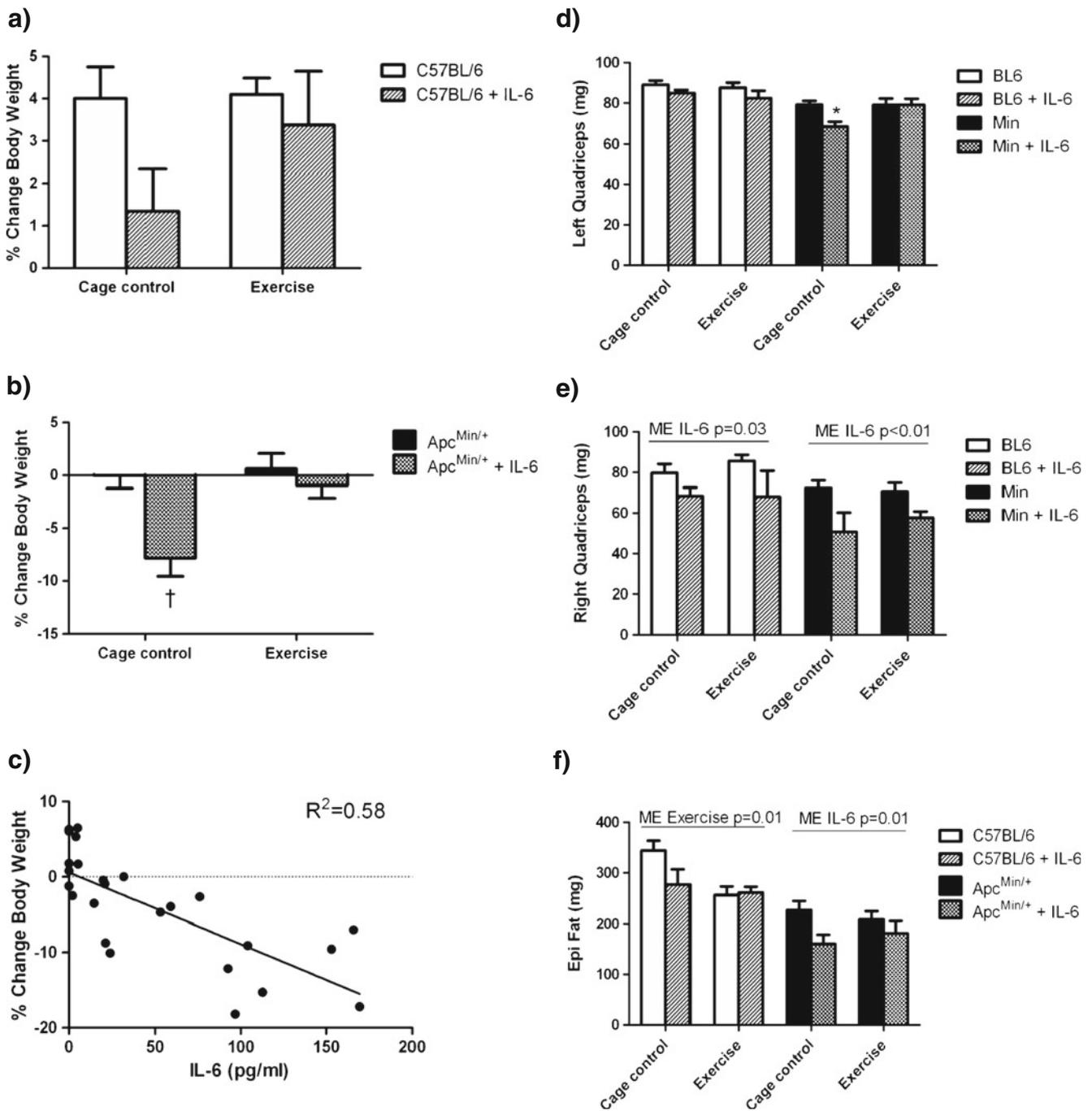
Fig. 2 Effect of exercise and IL-6 overexpression on body weight and muscle and fat mass in wild-type and $Apc^{Min/+}$ mice. IL-6 was overexpressed for 2 weeks in mice from 12 to 14 weeks of age undergoing treadmill exercise or serving as cage control. The percent change in body weight was calculated from values at 12 and 14 weeks of age in wild-type (**a**) and $Apc^{Min/+}$ mice (**b**). **c** The percent body weight loss correlates to the levels of IL-6 in cage control $Apc^{Min/+}$ mice. **d** Changes in quadriceps weight from systemic IL-6 overexpression. **e** Changes in quadriceps weight from local IL-6 overexpression. **f** Changes in epidydimal fat pad weight from 2 weeks of IL-6 overexpression. Values are the means ± SE. Data were analyzed with two-way ANOVA. Significance was set at $p \le 0.05$. †Different from all others

crosses a new beam along the x–y axis in the cage during a 10-h period of the dark cycle. Sedentary control $Apc^{Min/+}$ mice cage activity was not different from sedentary wild-type mice at 14 weeks of age (Table 2). IL-6 overexpression significantly increased cage activity in sedentary wild-type mice, but had no effect on cage activity in sedentary $Apc^{Min/+}$ mice. Exercise alone did not change cage activity in $Apc^{Min/+}$ or wild-type mice. When exercise was combined with IL-6 overexpression, cage activity was significantly reduced in both wild-type and $Apc^{Min/+}$ mice (Table 2).

Neuromuscular performance was measured by the time spent on the RotoRod. There was no difference in RotoRod performance between sedentary wild-type and sedentary $Apc^{Min/+}$ mice (Table 2). Neither IL-6 overexpression nor exercise treatments had any effect on RotoRod performance in either wild-type or $Apc^{Min/+}$ mice (Table 2). Volitional strength was measured using grip strength testing of all four limbs. Interestingly, we found that 14-week sedentary $Apc^{Min/+}$ mice had significantly greater strength compared with wild-type mice (Table 2). While IL-6 overexpression had no significant effects on strength in wild-type mice, both IL-6 overexpression and exercise significantly reduced strength in $Apc^{Min/+}$ mice. There was no interaction between IL-6 and exercise on strength loss in $Apc^{Min/+}$ mice. Non-exercising wild-type mice overexpressing IL-6 had significantly greater strength than control mice, and exercise had no effect on strength, regardless of IL-6 concentration (Table 2). In the $Apc^{Min/+}$ mice, volitional strength was decreased by both IL-6 overexpression and by exercise (Table 2). IL-6 overexpression in the sedentary $Apc^{Min/+}$ mice induced body weight loss and decreased volitional strength while not affecting voluntary cage activity. IL-6 overexpression that induced $Apc^{Min/+}$ weight loss also decreased strength without effecting neuromuscular performance (or cage activity). Comparing the sedentary IL-6-overexpressing $Apc^{Min/+}$ mice that lost body weight to the exercising IL-6-overexpressing $Apc^{Min/+}$ mice that were weight-stable, exercising mice actually had decreased cage activity and did not improve strength despite maintaining muscle mass.

3.4 Glucose and fatty acid metabolism

Insulin resistance often accompanies cachexia. The effect of IL-6 overexpression on glucose and lipid metabolism was measured in all mice at 12 and 14 weeks. Measurements included fasting triglycerides, fasting insulin, fasting glucose, and a glucose tolerance test (Fig. 3). Additionally, the HOMA-IR index was calculated as an index of insulin resistance [42]. At 12 weeks of age, fasting triglycerides were increased in the cage control $Apc^{Min/+}$ mice compared with wild-type mice, and $Apc^{Min/+}$ mice had a significantly higher fasting glucose/insulin ratio (Table 3). Six weeks of treadmill exercise lowered the elevated triglycerides in $Apc^{Min/+}$ mice at 12 weeks, but did not affect the elevated glucose/insulin ratio. At 14 weeks, neither IL-6 overexpression nor exercise had any effect on the measured parameters in wild-type mice. In $Apc^{Min/+}$ mice, IL-6 overexpression increased fasting triglycerides by ~2.5-fold; exercise reversed this increase (Fig. 3a). IL-6 overexpression induced fasting insulin levels by ~60% in $Apc^{Min/+}$ mice; exercise also reversed this increase (Fig. 3b). Although $Apc^{Min/+}$ mice had elevated fasting plasma glucose

Table 2 Effect of exercise and IL-6 overexpression on physical activity and performance in wild-type and $Apc^{Min/+}$ mice

	C57BL/6				$Apc^{Min/+}$			
	CC vector	CC IL-6	Ex vector	Ex IL-6	CC vector	CC IL-6	Ex vector	Ex IL-6
N	6	5	6	6	5	5	5	6
Activity monitor counts	10,799±1,108	18,259±2,083[a]	11,765±746[b]	7,202±1,742[c]	8,037±1,143	10,193±2,301	12,180±2,120[d]	4,001±1990[e]
Average time on rod (s)	98.9±13.7	88.9±23.6	104.9±14.5	71.7±9.5	112.9±8.9	84.1±9.8	99.6±9.1	94.8±17.0
Grip strength/BW (N/g)	0.079±0.002	0.095±0.002[a]	0.083±0.002	0.079±0.003	0.099±0.002	0.075±0.003[a]	0.082±0.002	0.087±0.006

At 14 weeks of age, mice were monitored for changes in physical activity and performance measures. The amount of cage activity measured during the dark cycle. The amount of time spent on the RotoRod with a progressive increase in speed up to 25 rpm. Volitional strength was measured in the forelimb and hindlimb. Values are the means ± SE. Data were analyzed with repeated measures two-way ANOVA within each genotype. Significance was set at $p \leq 0.05$

[a] Effect of IL-6 within cage control, $p = 0.03$

[b] Effect of IL-6 within exercise, $p = 0.02$

[c] Effect of exercise within IL-6, $p = 0.006$

[d] Effect of IL-6 within exercise, $p = 0.01$

[e] Effect of exercise within IL-6, $p = 0.03$

compared with wild-type mice, there was no further increase on IL-6 overexpression. Exercise did not affect fasting glucose in $Apc^{Min/+}$ mice at 14 weeks regardless of IL-6 overexpression (Fig. 3c). Insulin resistance was calculated using the HOMA-IR index. At 12 weeks of age, $Apc^{Min/+}$ mice had high HOMA-IR compared with the wild-type mice, which was improved by exercise training

(Table 3). After 2 weeks of IL-6 overexpression, the HOMA-IR increased by ~50% in $Apc^{Min/+}$ mice, and this change was attenuated by exercise (Fig. 3d). IL-6 over-expression, which induced body weight loss in the sedentary $Apc^{Min/+}$ mice, also increased fasting triglyceride levels, fasting insulin levels, and the HOMA-IR index while not affecting the already elevated fasting glucose
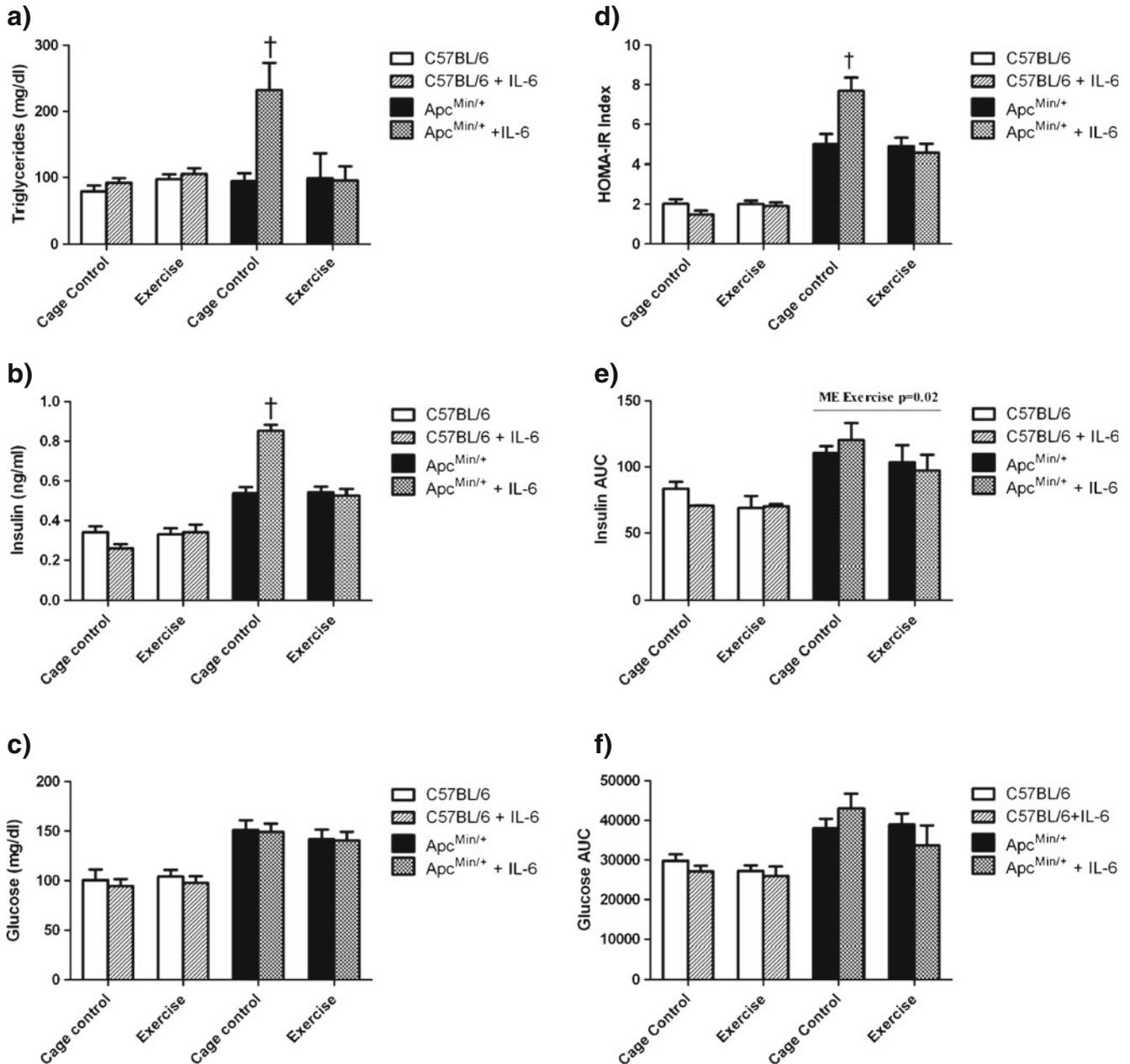
Fig. 3 Glucose metabolism after 2 weeks of IL-6 overexpression with and without exercise intervention. At 14 weeks of age, fasting triglycerides (a), fasting insulin (b), and fasting glucose (c) were measured after a 5-h fast. Insulin resistance was monitored by calculating the HOMA-IR index (d) and a glucose tolerance test. In a subset of mice ($n=5$–7/group), an intraperitoneal glucose tolerance test was conducted after an overnight fast. Blood glucose was measured at 0, 15, 13, 60, 90, and 120 min. Insulin area under the curve (AUC) (e) and glucose AUC (f) were calculated from values obtained during the glucose tolerance test. Values are the means ± SE. Data were analyzed with two-way ANOVA within each genotype. Significance was set at $p \leq 0.05$. *Interaction between IL-6 and cage control

Table 3 Effect of exercise on glucose metabolism in 12-week-old wild-type and $Apc^{Min/+}$ mice

N	C57BL/6		$Apc^{Min/+}$	
	Cage control 23	Exercise 25	Cage control 27	Exercise 26
Fasting glucose (mg/dL)	108.5±6.6	117.8±6.1	140.4±5.2[a]	137.8±5.4[a]
Fasting triglycerides (mg/dL)	72.7±4.3	90.2±8.2	104.9±10.2[b]	93.5±10.7
Fasting insulin (ng/mL)	0.46±0.08	0.52±0.05	0.48±0.02	0.44±0.02
Glucose/insulin	194.8±45.7	191.9±34.7	305.4±15.7[a]	321.6±26.1[a]
HOMA	3.1±0.1	3.2±0.2	4.2±0.3[a]	3.5±0.2[a]

Measurements were taken prior to 2 weeks of IL-6 overexpression. Glucose was taken after an overnight fast using a handheld glucometer and blood taken from the tail vein. Triglycerides and insulin were measured after a 5-h fast with blood taken from the tail vein. Values are the means±SE. Data were analyzed with two-way ANOVA. Significance was set at $p \leq 0.05$

[a] Main effect of genotype

[b] Interaction between genotype and exercise

levels. When comparing the sedentary IL-6-overexpressing $Apc^{Min/+}$ mice that lost body weight to the exercising IL-6-overexpressing $Apc^{Min/+}$ mice that were weight-stable, exercise reduced fasting triglycerides, fasting insulin, and the HOMA-IR index.

To further investigate insulin resistance, a glucose tolerance test was administered to a subset of mice. After 6 weeks of exercise, there was no difference in insulin area under the curve (AUC) between wild-type and $Apc^{Min/+}$ mice (BL-6 CC=87±7 AUC; BL-6 Ex=88± 4 AUC; Min CC=98±5 AUC; Min Ex=99±7 AUC). There was also no difference in glucose AUC at 12 weeks of age regardless of genotype or exercise (BL-6 CC= 15,359±1,969 AUC; BL-6 Ex=16,097±2,978 AUC; Min CC =20,672±3,026 AUC; Min Ex=18,126±2,062 AUC). Insulin and glucose AUC were also calculated after 2 weeks of IL-6 overexpression (Fig. 3e, f). There was a main effect for exercise to decrease the insulin AUC in the $Apc^{Min/+}$ mice regardless of IL-6 treatment (Fig. 3e). Glucose AUC was unchanged by IL-6 or exercise treatments in both wild-type and $Apc^{Min/+}$ mice (Fig. 3f). Thus, while altered glucose clearance is associated with cancer in $Apc^{Min/+}$ mice, it appears to at least initially be independent of IL-6-induced muscle wasting.

IL-6 overexpression increased fasting triglyceride levels ~2.5-fold in $Apc^{Min/+}$ mice, which was reversed by exercise (Fig. 3a). We next examined a subset of mice out to 20 weeks of age to determine the correlation between fasting triglyceride levels and the development of cachexia. At 20 weeks of age, mice were separated by the degree of body weight loss into mild or severe categories of cachexia. Triglycerides were only increased in the $Apc^{Min/+}$ mice that developed severe cachexia (Fig. 4a). Additionally, this increase in triglyceride levels was apparent at 14 weeks of age, which was prior to body weight loss in these mice. Triglycerides continued to increase as body weight loss

progressed. The level of circulating triglycerides was also significantly correlated with the percentage of body weight loss (Fig. 4b) and with circulating IL-6 levels (Fig. 4c). These data demonstrate that elevated fasting triglycerides are a useful predictor of risk for cachexia in $Apc^{Min/+}$ mice, and future work is needed to determine whether they are also useful for predicting cachexia in cancer patients.

Plasma glycerol levels, which are indicative of the rate of lipolysis, were significantly higher in sedentary control $Apc^{Min/+}$ mice compared with sedentary control wild-type mice (Fig. 5a). There was a main effect of exercise to decrease fasting glycerol levels in wild-type mice regardless of IL-6 overexpression. In $Apc^{Min/+}$ mice, there was no effect of exercise or IL-6 on glycerol levels (Fig. 6a). In cage control $Apc^{Min/+}$ mice, plasma glycerol was significantly correlated with the percent change in body weight, but this correlation was lost with exercise training (Fig. 5b). Although there appears to be considerable variability in response, weight loss in sedentary $Apc^{Min/+}$ mice is related to an increased rate of lipolysis. However, the preventative effect of exercise on IL-6-induced body weight loss is apparently not related to changes in the rate of lipolysis. This corresponds to the inability of exercise to prevent loss of epididymal fat pad mass (Fig. 2f).

3.5 Circulating adiponectin and leptin

To assess their role in the progression of cachexia, we measured the circulating levels of adiponectin and leptin. Adiponectin, a hormone secreted from fat, modulates several metabolic processes, including glucose homeostasis and fatty acid catabolism. Adiponectin levels were decreased in sedentary control $Apc^{Min/+}$ mice compared with sedentary control wild-type mice. In both $Apc^{Min/+}$ and wild-type mice, there was a main effect for IL-6 over-expression to decrease adiponectin levels, regardless of
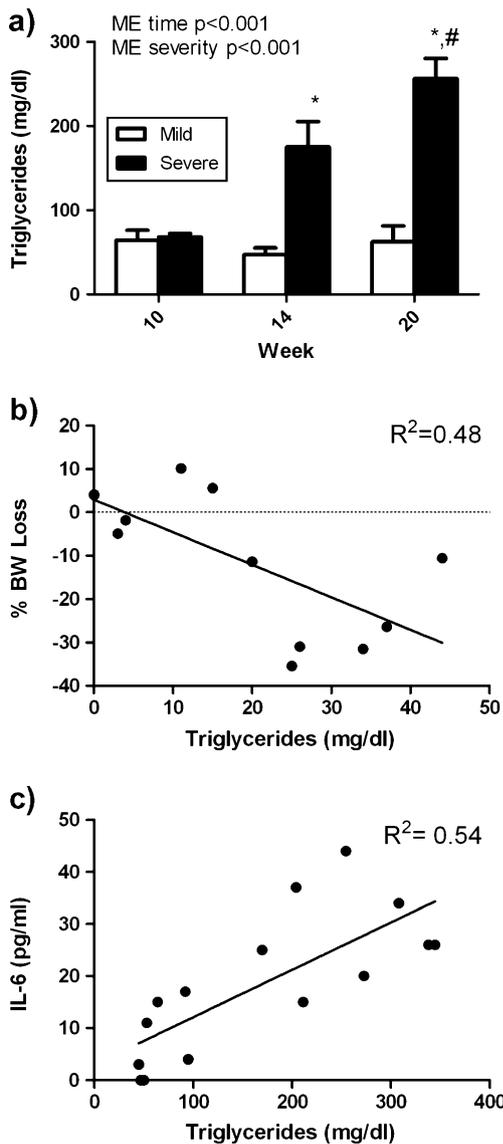
Fig. 4 Circulating triglycerides are increased with severity of cancer cachexia. Fasting triglycerides were measured at 10, 14, and 20 weeks of age in $Apc^{Min/+}$ mice and data stratified by cachexia severity. **a** Fasting triglycerides measured at 10, 14, and 20 weeks from mild and severely cachectic $Apc^{Min/+}$ mice. Samples were taken from the same mice at three different ages. **b** Circulating triglycerides correlate with percent body weight loss from peak body weight to body weight at 20 weeks ($p=0.01$). **c** Circulating triglycerides correlate with plasma IL-6 levels at 20 weeks of age ($p=0.001$). Values are the means ± SE. Data were analyzed with repeated measures two-way ANOVA and Pearson's correlation. Significance was set at $p \leq 0.05$

overexpression to increase leptin levels regardless of exercise (Fig. 5d). While fat mass loss proceeds with the development of cachexia, the relationship of circulating adiponectin and leptin to body weight loss remains to be established.

3.6 Skeletal muscle oxidative capacity

To determine whether IL-6 overexpression altered the quadriceps muscle adaptive response to the exercise training, we measured cytochrome C and COXIV protein expression as an indicator of muscle oxidative capacity. Systemic IL-6 overexpression had no effect on the protein expression of cytochrome C and COX IV in $Apc^{Min/+}$ mouse (Fig. 6b); however, systemic IL-6 caused a decrease in protein expression of cytochrome C and COX IV in wild-type mice (Fig. 6a). There was a main effect of exercise to increase cytochrome C and COX IV protein expression regardless of IL-6 overexpression in both the wild-type (Fig. 6a) and $Apc^{Min/+}$ mice (Fig. 6b).

We also examined the effects of both local and systemic IL-6 overexpression on quadriceps muscle oxidative capacity with and without exercise training. The quadriceps muscle from $Apc^{Min/+}$ mice undergoing both systemic and local IL-6 overexpression (Fig. 6b, d) also demonstrated a significant main effect of exercise to increase cytochrome C and COXIV protein expression (Fig. 6d); however, the overall exercise induction appeared attenuated when compared with muscle exposed to increased systemic IL-6 only. In the IL-6-overexpressing quadriceps muscle from wild-type mice, exercise significantly induced COX IV, but has little effect on cytochrome C protein expression (Fig. 6c).

3.7 Skeletal muscle inflammation

To determine whether exercise could alter muscle inflammatory signaling induced by IL-6 overexpression, we examined STAT3 and NFkB signaling. Systemic IL-6 overexpression had no effect on total STAT3 protein expression in either wild-type ($p=0.22$) or $Apc^{Min/+}$ mice ($p=0.75$); similarly, total NFkB protein expression was not altered in either wild-type ($p=0.68$) or $Apc^{Min/+}$ mice ($p=0.48$, data not shown). Systemic Il-6 overexpression induced the ratio of phosphorylated to total STAT twofold in wild-type mice (Fig. 7a) and fourfold in $Apc^{Min/+}$ mice (Fig. 7b). The activation of NFkB signaling, the ratio of phosphorylated to total, was increased fivefold in wild-type muscle and 38-fold in $Apc^{Min/+}$ mouse muscle by systemic IL-6 overexpression. The systemic IL-6 induction of muscle inflammatory signaling was not attenuated by exercise training in both the wild-type and $Apc^{Min/+}$ mice (Fig. 7a, b), demonstrating that exercise can benefit muscle mass independent of decreased STAT and NFkB signaling.

exercise. Exercise had no effect on the level of circulating adiponectin (Fig. 5c). Leptin levels were not different between sedentary control $Apc^{Min/+}$ mice compared with sedentary control wild-type mice. Wild-type and $Apc^{Min/+}$ mice leptin levels responded differentially to exercise and IL-6 overexpression. There was a main effect of exercise to decrease circulating leptin levels regardless of IL-6 treatment. In $Apc^{Min/+}$ mice, there was a main effect of IL-6
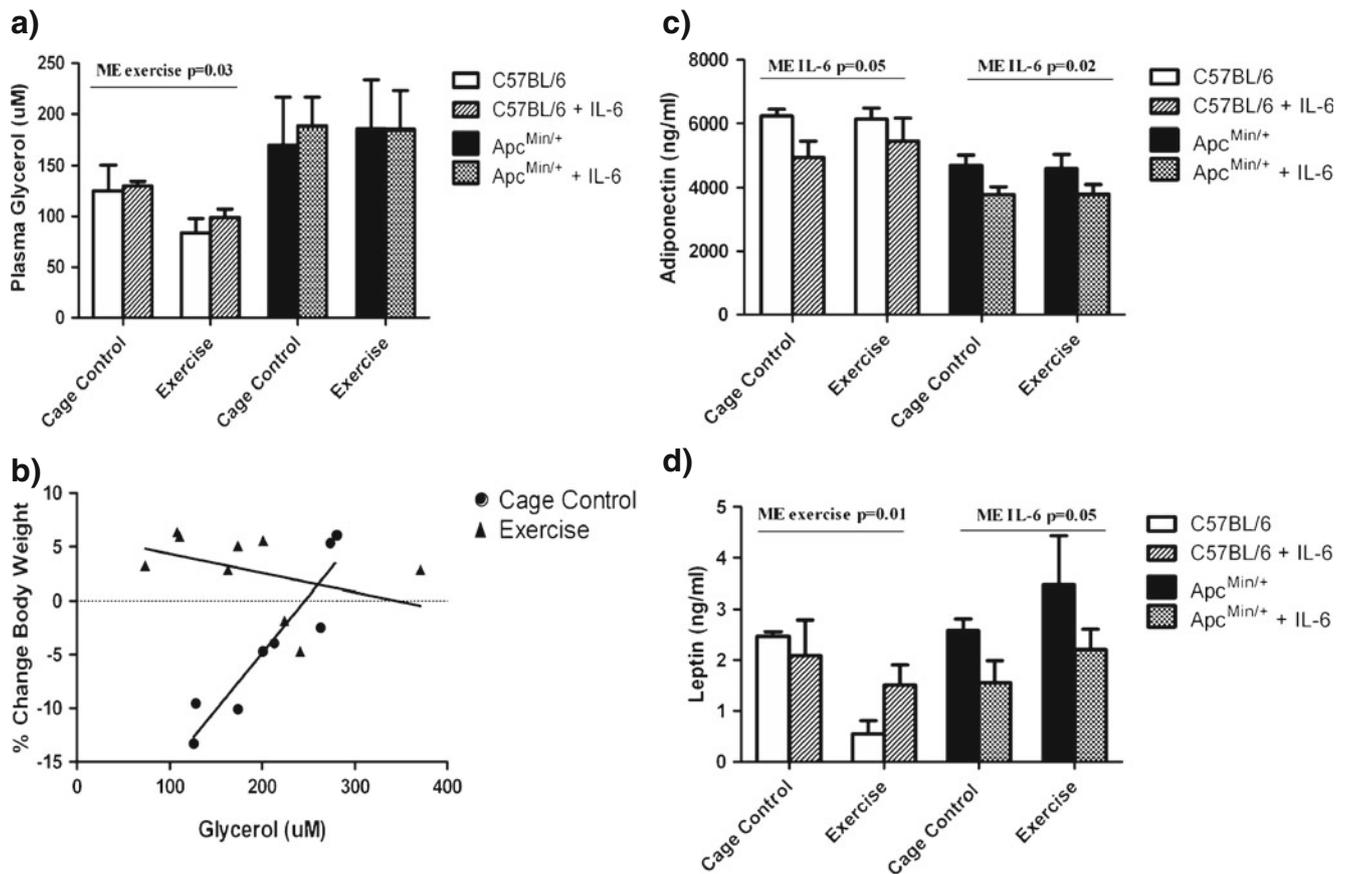
Fig. 5 Lipid metabolism after 2 weeks of IL-6 overexpression with and without exercise training. **a** At 14 weeks of age, plasma glycerol was measured in a subset of mice ($n=5$) after a 5-h fast. **b** Fasting glycerol levels in cage control $Apc^{Min/+}$ mice (*circles*) correlated with percent change in body weight ($R^2=0.87$, $p<0.001$). Fasting glycerol levels in exercised $Apc^{Min/+}$ mice (*triangles*) were not correlated with percent change in body weight ($R^2=0.18$, $p=0.26$). Plasma concentrations of adiponectin (**c**) and leptin (**d**) were measured after 2 weeks of IL-6 overexpression. Mice were fasted for 5 h prior to the collection of plasma samples at the time of killing. Values are the means±SE. Data were analyzed with two-way ANOVA. Pearson's correlation analysis was performed to compare percent change in body weight with glycerol levels in the $Apc^{Min/+}$ mice. Significance was set at $p \leq 0.05$

The quadriceps muscles subjected to both local and systemic IL-6 overexpression with or without exercise training were also examined. Total STAT3 (wild type: $p=0.52$; $Apc^{Min/+}$: $p=0.08$) or total NFkB (wild type: $p=0.75$; $Apc^{Min/+}$: $p=0.11$) protein expression was not significantly changed by local IL-6 overexpression with or without exercise (data not shown). There was a significant main effect of local IL-6 overexpression to induce STAT3 and NFkB activation in wild-type muscle (Fig. 7c) and $Apc^{Min/+}$ mice (Fig. 7d) regardless of exercise. Exercise training did not significantly attenuate or accentuate the induction of inflammatory signaling in $Apc^{Min/+}$ mice.

3.8 Skeletal muscle metabolism

To determine whether exercise training would alter IL-6-induced regulation of muscle metabolism, we examined adenosine monophosphate-activated protein kinase (AMPK; T172) and protein kinase B (Akt); (T308) activation. Systemic

IL-6 overexpression in $Apc^{Min/+}$ mice increased muscle AMPK activation threefold; exercise training significantly attenuated this increase (Fig. 8b). There was no effect of systemic IL-6 overexpression on AKT activation in $Apc^{Min/+}$ mice, but exercise training has a significant main effect on AKT activation regardless of IL-6 overexpression (Fig. 8b). In the wild-type mouse, neither IL-6 overexpression nor exercise training significantly altered AMPK activation (Fig. 8a). There was no effect of IL-6 or exercise on total AMPK protein expression levels in wild-type or $Apc^{Min/+}$ mice (data not shown). Similarly, total AKT protein expression was not affected by IL-6 or exercise in wild-type or $Apc^{Min/+}$ mice (data not shown).

In muscle subjected to both local and systemic IL-6 overexpression, there was a trend ($p=0.06$) for IL-6 to increase AMPK activation in wild-type mice (Fig. 8c). In $Apc^{Min/+}$ mice, there was a main effect of exercise to reduce AMPK activation regardless of IL-6 expression (Fig. 8d), which was due to a significant increase in total AMPK
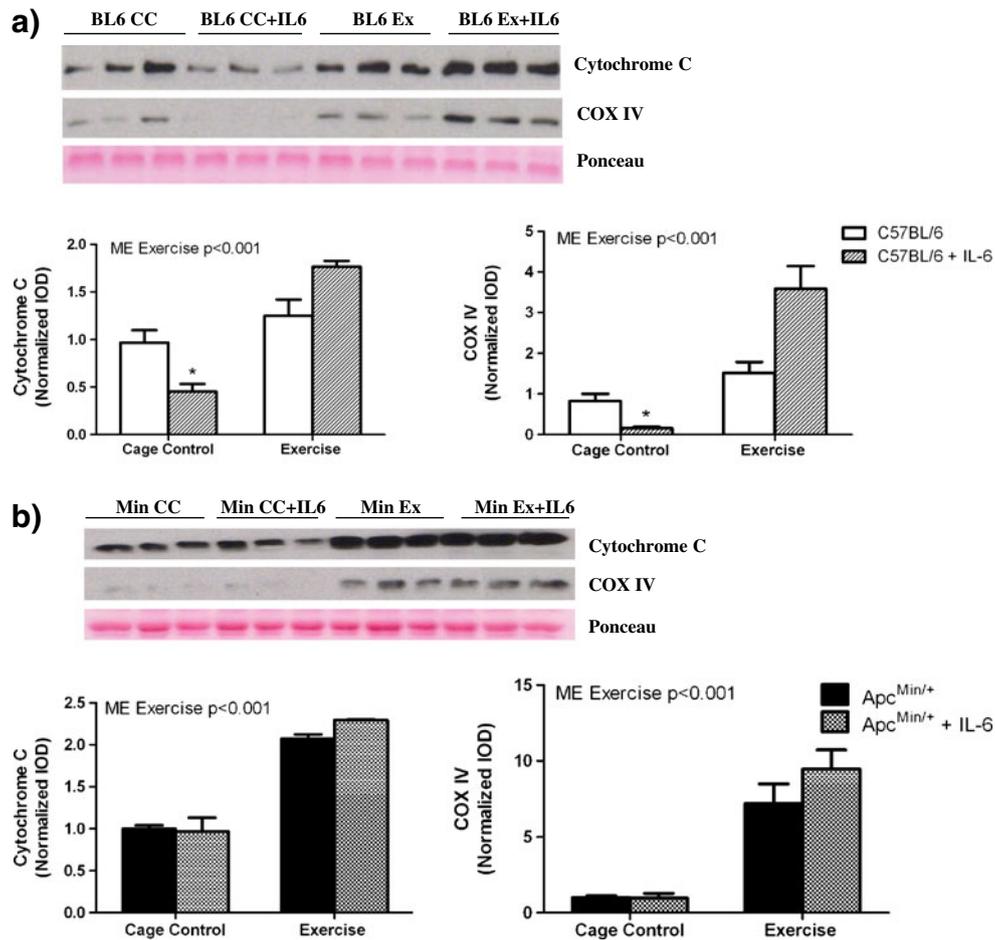
Fig. 6 Effect of local and systemic IL-6 overexpression and exercise on muscle oxidative capacity. To measure the effect of systemic IL-6 overexpression, the relative protein expression of cytochrome C and COX IV was measured in the wild-type left quadriceps muscle (**a**) and the $Apc^{Min/+}$ left quadriceps muscle (**b**). The effects of local IL-6 overexpression on protein expression of cytochrome c and COX IV were measured in the right quadriceps muscle of wild-type (**c**) and $Apc^{Min/+}$ (**d**) mice. All data were normalized to cage control (*CC*). Values are the means±SE. Data were analyzed with two-way ANOVA. Significance was set at $p \le 0.05$. *Effect of IL-6 within cage control

protein expression with exercise (p=0.006). While local IL-6 overexpression induced muscle AKT activity in wild-type mice (Fig. 8c), and this induction was attenuated by exercise training, this response was not significant in the $Apc^{Min/+}$ mice due to variability in the activation of AKT with exercise (Fig. 8d).

4 Discussion

Exercise has been shown to aid in the prevention of cancer and is thought to be of therapeutic value in slowing the progression or even preventing the development of cachexia. Although many studies have examined the effects of aerobic exercise training on the development and progression of cancer [11, 41, 45], the direct examination of exercise on the prevention of muscle loss and progression of cachexia was often not the primary outcome analyzed by these studies. Several studies suggest the use of exercise training to ameliorate muscle wasting in conditions of cachexia, as reviewed by Zinna and Yarasheski [44], but the underlying mechanisms of the protective effects of exercise remains poorly defined. To our knowledge, our study presented here is the first mechanistic examination of controlled moderate-intensity aerobic exercise on systemic and muscle metabolic changes that normally occur with the progression of cachexia. Additionally, this study was conducted under conditions where systemic inflammation was maintained. While our study confirms that regular treadmill exercise can reduce the intestinal polyp burden in $Apc^{Min/+}$ mice, we demonstrate the novel finding that moderate treadmill exercise is effective in preventing IL-6-induced body weight and muscle loss in $Apc^{Min/+}$ mice. The exercise-induced attenuation of cachexia was associated with improved insulin
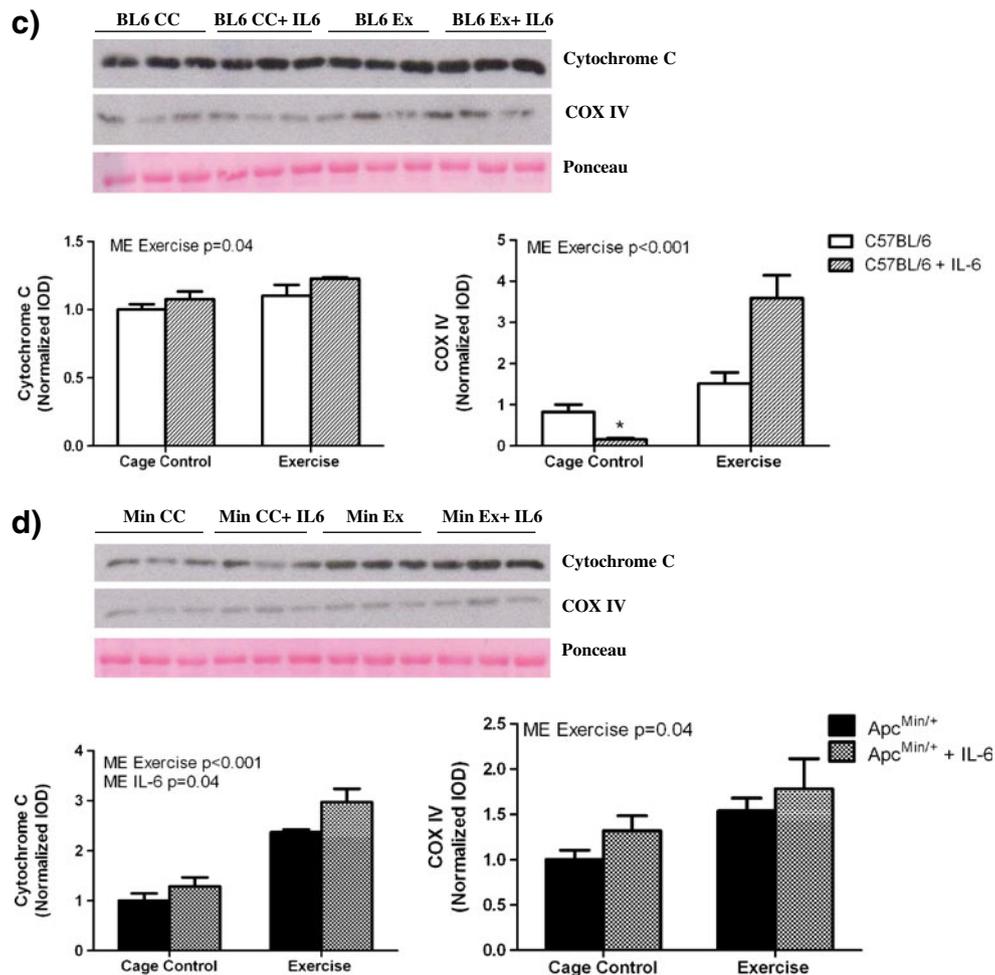
Fig. 6 (continued)

sensitivity and glucose metabolism despite high circulating IL-6 levels. The exercise attenuation of muscle mass loss was associated with an increased muscle oxidative metabolic capacity, an attenuation of IL-6-induced muscle AMPK activation, and increased muscle AKT activation. All of these exercise-induced changes that benefited muscle mass retention occurred without a reduction in muscle inflammatory signaling. Overall, exercise-induced improvements in insulin resistance, lipid metabolism, and food consumption appear important for muscle and body weight maintenance in the $Apc^{Min/+}$ mouse.

The current study confirms that systemic IL-6 overexpression is sufficient to decrease body weight and muscle mass in sedentary $Apc^{Min/+}$ mice, as previously reported [5, 41]. However, this current study extends prior observations by demonstrating that only 2 weeks of IL-6 overexpression in a younger, weight-stable, non-cachectic $Apc^{Min/+}$ mouse can induce significant muscle and body weight loss. Prior studies with $Apc^{Min/+}$ mice had examined 4 weeks of IL-6 over-expression, starting at a more advanced 16 weeks of age [5,

41]. Although muscle mass and body weight were maintained in exercising $Apc^{Min/+}$ mice despite high circulating IL-6 levels, exercise was not able to prevent epididymal fat loss. This finding suggests that initial preservation of fat may not be required to preserve body weight and muscle mass during the onset of cachexia. Decreased physical activity and increased fatigue occur with cancer cachexia [19, 20]; however, we did not find decreased cage activity to be associated with IL-6-induced body weight loss in $Apc^{Min/+}$ mice. This is interesting because we have reported that $Apc^{Min/+}$ mouse voluntary wheel running distance is inversely correlated with the degree of cachexia [12], and hindlimb muscle oxidative capacity is severely repressed in late-stage cachexia [18]. Thus, our current data suggest that this loss of oxidative capacity may not be due to a decreased cage activity. We did find that the combination of IL-6 over-expression and exercise, which prevented cachexia, also repressed cage activity. However, it is a possibility that the decreased cage activity was associated with fatigue, related to the daily exercise protocol. Cancer patients' daily activity
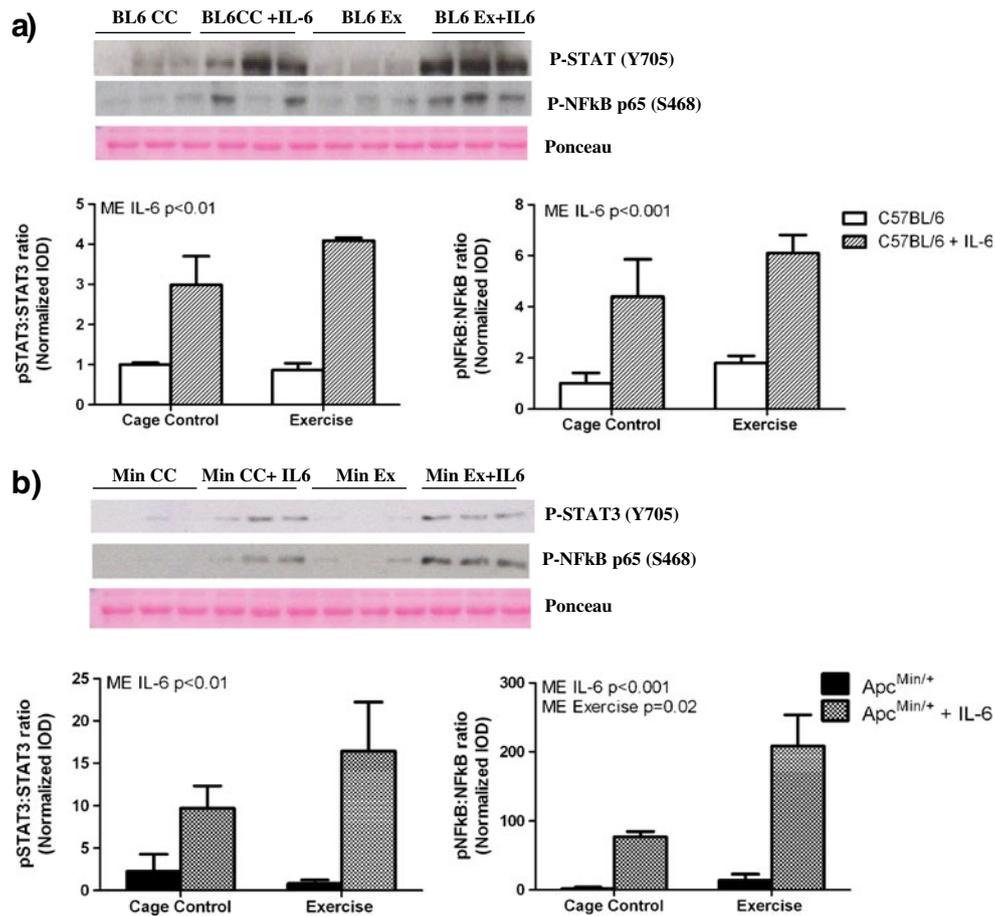
Fig. 7 Effect of local and systemic IL-6 overexpression and exercise on STAT3 and NFkB markers of muscle inflammation. To measure the effect of systemic IL-6 overexpression, the relative protein expression of p-STAT3(Y705) and p-NFkB(S468) was measured and normalized to levels of total STAT3 and NFkB in the wild-type left quadriceps muscle (**a**) and the $Apc^{Min/+}$ left quadriceps muscle (**b**). The effects of local IL-6 overexpression on the protein expression of p-STAT3(Y705) and p-NFkB(S468) were measured and normalized to the levels of total STAT3 and NFkB in the wild-type right quadriceps muscle (**c**) and the $Apc^{Min/+}$ right quadriceps muscle (**d**). All data were normalized to cage control (*CC*). Values are the means ± SE. Data were analyzed with two-way ANOVA. Significance was set at $p \leq 0.05$

levels have been reported comparable to healthy activity levels, but cancer patients also had significantly reduced spontaneous activity [45]. In support of repressed volitional effort, we found that exercise, despite positive effects on body weight and muscle mass, did not improve IL-6-suppressed voluntary grip strength in $Apc^{Min/+}$ mouse. However, we found no change in performance on the RotoRod, similar to our previous results with cachectic mice [41], which we interpret to mean muscle neuromuscular control was maintained during cachexia. Additionally, exercising $Apc^{Min/+}$ mice were able to complete all moderate-intensity treadmill exercise sessions. Since the decreased cage activity was found in mice that did not develop cachexia, it appears that the decreased cage activity and volitional strength could be associated with centrally mediated mechanisms that induce fatigue and possibly related to IL-6 overexpression, However, these fatigue-related changes were independent of the processes that preserve body weight and muscle mass, but would certainly impact life quality.

Insulin resistance is a hallmark of cachexia [21, 22, 46, 47], so improved insulin sensitivity may be one mechanism by which exercise could protect against cachexia. Besides inducing weight loss, IL-6 overexpression in $Apc^{Min/+}$ mouse increased fasting triglycerides, fasting insulin, and the HOMA index, which are all indicative of increased insulin resistance. Regular moderate-intensity treadmill exercise was able to normalize all of these parameters. Exercise has well-documented effects on insulin sensitivity and glucose clearance, even in obese and type 2 diabetic patients [48–50]. Interestingly, the exercise-induced changes in glucose metabolism occurred without a reduction in circulating IL-6 concentration. Although chronically elevated IL-6 levels have been associated with increased glucose uptake [51] and insulin resistance, the relationship of IL-6 with insulin resistance is still equivocal [52–54]. The administration of metformin and rosiglitazone, insulin sensitizers, to cachectic rodents with high IL-6 levels can decrease tumor growth and markers of muscle proteolysis [22, 23]. Triglyceride levels
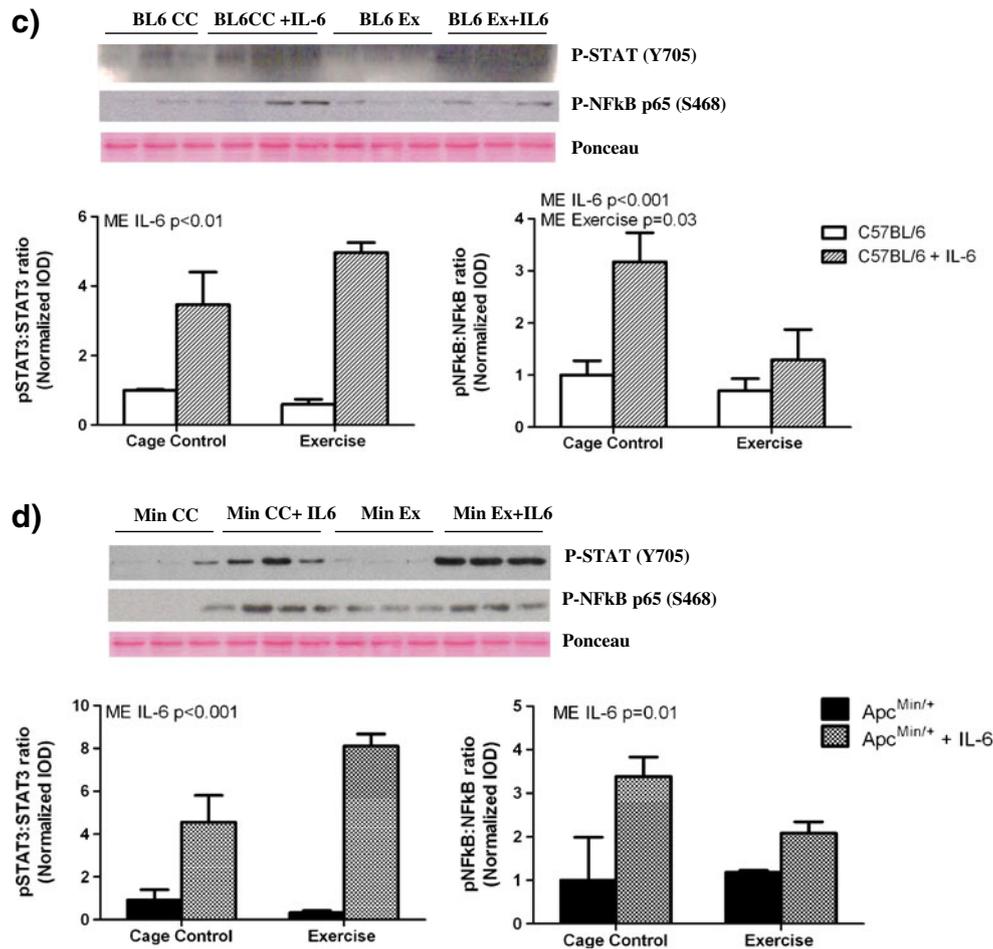
Fig. 7 (continued)

are commonly elevated in cachexia [55, 56], but little is understood regarding their role in the development and progression of the disease. As in our study, exercise has been shown to reduce triglyceride levels in cachectic tumor-bearing rats after 8 weeks of exercise training [57]. However, we extend these findings to show in the $Apc^{Min/+}$ mouse that elevated fasting triglyceride levels are predictive of weight loss, being elevated earlier and to a greater extent in mice that eventually develop severe cachexia. Early increases in triglycerides have been noted in other models of cancer cachexia as well, but the implication to use them as a predictive measure of cachexia has not been addressed [58]. We also found that fasting triglyceride levels were strongly correlated with the degree of body weight loss and the circulating IL-6 concentration. Adiponectin is an insulin-sensitizing adipokine and contributes to the regulation of muscle insulin resistance and oxidative capacity [59]. While IL-6 overexpression reduced the circulating levels of adiponectin, the preventative effect of exercise on weight loss was not correlated with the altered levels of these hormones. The disconnect between the IL-6 reduction in adiponectin and the improved insulin sensitivity in exercising mice requires

further investigation, but could be attributed to exercise-induced adiponectin-independent effects on metabolism.

Skeletal muscle oxidative capacity and mitochondrial function can influence muscle glucose uptake and has been associated with insulin resistance and type 2 diabetes [60, 61]. Additionally, altered mitochondria content is also related to a diminished ability of the muscle to efficiently oxidize fatty acids and has been suggest to decrease muscle metabolic flexibility [62]. Decreased skeletal muscle oxidative capacity has been reported with many wasting conditions [63–66], and we have found that both red and white hindlimb muscles from the severely cachectic $Apc^{Min/+}$ mice have reduced mitochondrial content and oxidative protein expression. The loss of muscle oxidative capacity in the later stages of cachexia [18] also corresponds with severe insulin resistance, as seen by the inability to clear glucose during a glucose tolerance test late in cachexia [67]. In the present study, IL-6 overexpression for 2 weeks in $Apc^{Min/+}$ mice that were weight-stable at the beginning of the IL-6 treatment did not suppress quadriceps muscle oxidative protein expression. This finding confirms our earlier observations that oxidative protein loss occurs later in the
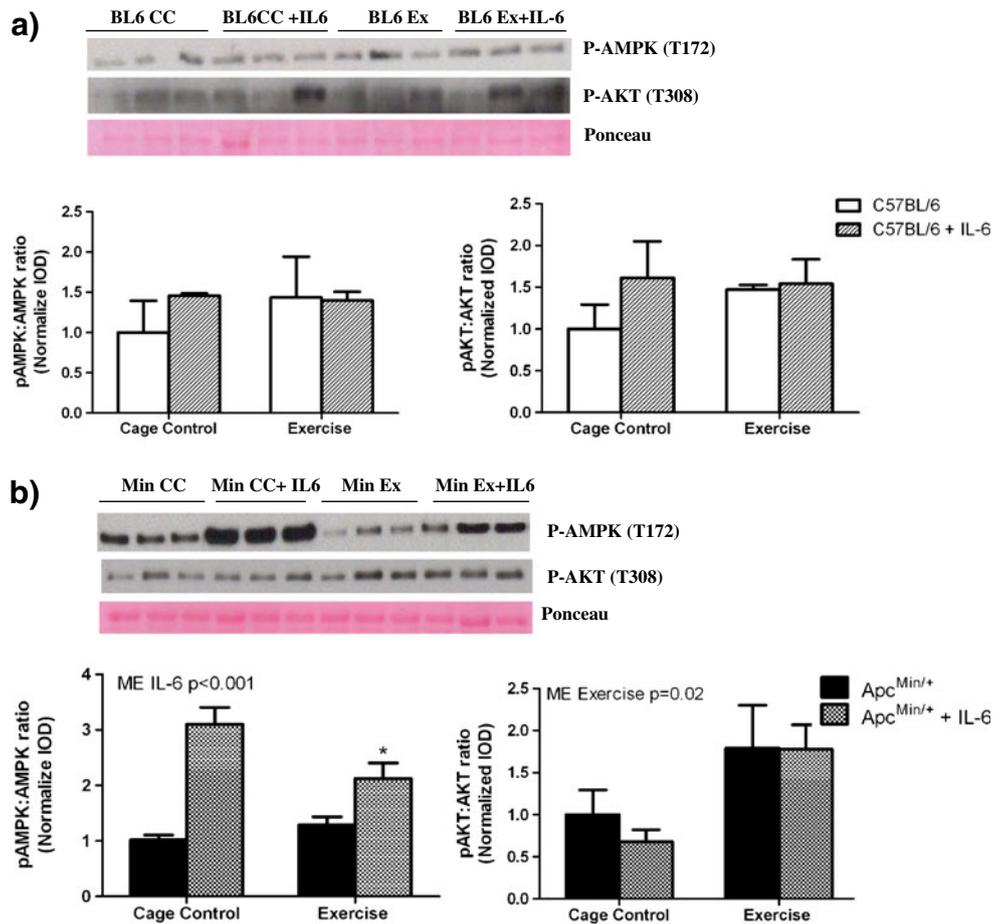
Fig. 8 Effect of local and systemic IL-6 overexpression and exercise on AKT and AMPK markers of energy status. To measure the effect of systemic IL-6 overexpression, the relative protein expression of phosphorylated (T172) AMPK and phosphorylated (T308) AKT was measured and normalized to the levels of total AMPK and AKT in the wild-type left quadriceps muscle (**a**) and the $Apc^{Min/+}$ left quadriceps muscle (**b**). The effects of local IL-6 overexpression on the protein expression of phosphorylated (T172) AMPK and phosphorylated (T308) AKT were measured and normalized to the levels of total AMPK and AKT in the wild-type right quadriceps muscle (**c**) and the $Apc^{Min/+}$ right quadriceps muscle (**d**). Values are the means±SE. Data were analyzed with two-way ANOVA. Significance was set at $p \leq$ 0.05. *Effect of exercise within IL-6

progression of cachexia in $Apc^{Min/+}$ mice since mice had only lost approximately 8% of their body weight at the end of the 2-week treatment. These observations further demonstrate that the initial loss of muscle mass and the corresponding increase in insulin resistance at the onset of cachexia are independent of muscle oxidative protein loss. Related to muscle plasticity with exercise, our study confirms that tumor-bearing mice can improve skeletal muscle oxidative capacity and further extends these findings to demonstrate that oxidative changes can occur while circulating IL-6 levels are maintained at chronically high levels. Improvements in oxidative capacity are closely linked to improvements in insulin resistance [68, 69] and could be a mechanism through which exercise is working to improve insulin sensitivity during the onset of cachexia.

Improvements in insulin resistance and attenuation of the cachectic condition may also be explained by alterations in muscle metabolic signaling related to insulin and energy status. Muscle anabolic signaling induced by insulin and insulin-like growth factor 1 occurs through the activation of the PI3K/AKT/mTOR pathway. Activated AKT can also decrease the transcription of genes involved in ATP-dependent ubiquitin proteasome degradation of muscle protein [70]. AMPK is activated in muscle by a decrease in available energy and also contraction [71] AMPK activation promotes several cellular processes including the inhibition of protein synthesis through mTOR inhibition, increased glucose uptake, increased protein degradation, and induction of mitochondrial biogenesis [71]. Additionally, the chronic elevation of IL-6 has been shown to stimulate AMPK [72, 73]. In the $Apc^{Min/+}$ mouse, AMPK and AKT activation are both increased with the progression of cachexia, and their activation is associated with decreased muscle mass [74]. However, at the onset of cachexia or during pre-cachexia,
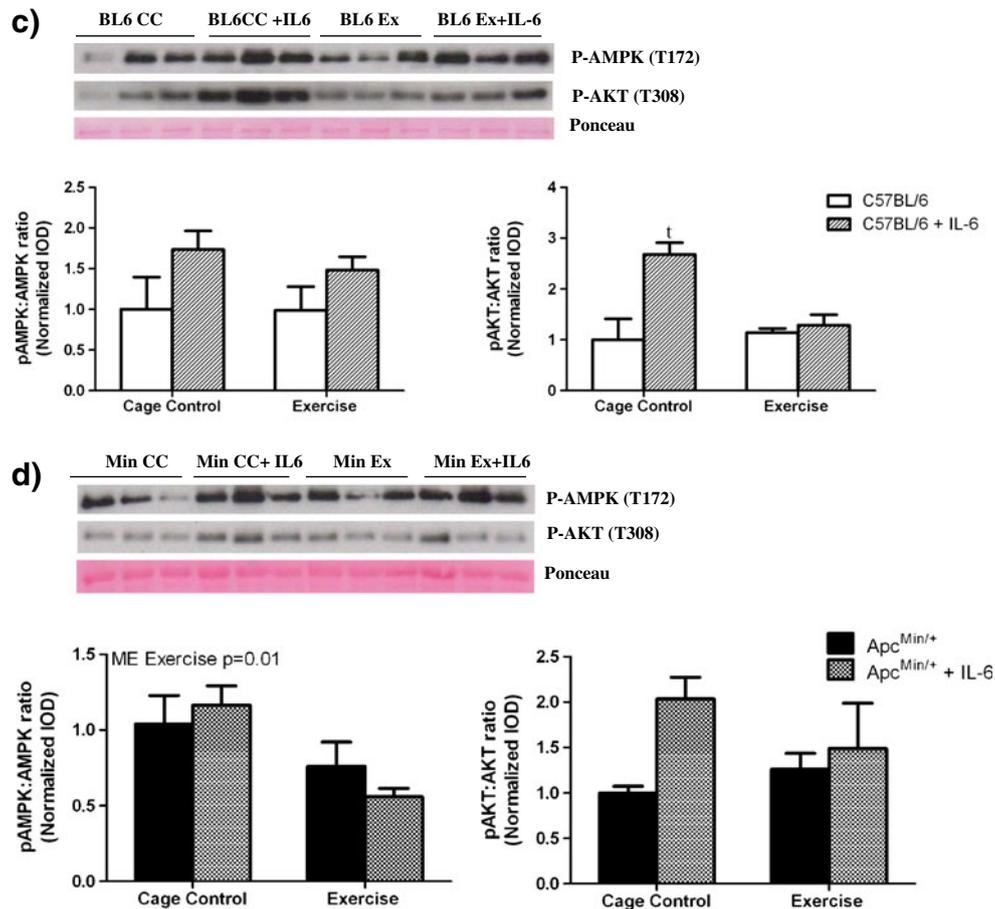
c)

d)

Fig. 8 (continued)

neither AKT nor AMPK is activated [74]. Furthermore, attenuation of further body weight and muscle mass loss during the progression of cachexia by systemic administration of an IL-6r antibody, a potential therapeutic target for treatment of cancers [75], to cachectic $Apc^{Min/+}$ mice also significantly decreases AKT and AMPK activation [74]. This IL-6r antibody decrease in AMPK and AKT activation was also associated with a reduction of STAT3 phosphorylation. Interestingly, regular moderate exercise was able to attenuate IL-6-increased phosphorylation of AMPK independent of any reduction in the increased level of muscle STAT signaling. IL-6 overexpression induced quadriceps muscle inflammatory signaling related to STAT and NFkB activation that was not attenuated by exercise training. Thus, exercise may be acting through alternative pathways that can circumvent inflammation-induced muscle mass loss. Exercise may be decreasing the activation of AMPK through improved oxidative capacity, which may be relieving energy stress of the cells; however, this still requires further investigation.

While preventing or slowing loss of skeletal muscle mass is a widely acknowledged goal for the treatment of cachexia, loss of adipose tissue mass and its impact on cachexia progression is less well understood. Adipose tissue has the potential to alter

cachexia progression through several mechanisms, including its role in fuel supply, thermal regulation, organ protection, insulin resistance, as a source of inflammation, and as a source of adipokines. A study by Das et al. [76] reported that when white adipose tissue was maintained through an ablation of adipose triglyceride lipase, muscle mass was maintained. In the present study, exercise prevented skeletal muscle mass loss without affecting epididymal fat mass loss. IL-6 has been reported to activate lipolysis in adipose tissue [77]. We examined lipolysis by measuring plasma glycerol, which is a reliable indicator of lipolysis and is increased in cachectic patients [78]. Consistent with previous reports, wild-type mice had decreased fasting plasma glycerol levels in response to exercise training [79, 80], which correlates with the exercise-induced decrease in fat mass in these mice. However, in non-exercising $Apc^{Min/+}$ mice, plasma glycerol levels were not associated with body weight loss. While glycerol was increased in the pre-cachectic $Apc^{Min/+}$ mouse compared with wild-type mice, the lipolysis rate was related to cancer progression rather than cachexia.

IL-6 overexpression decreased the circulating levels of the adipokine leptin in $Apc^{Min/+}$ mice regardless of exercise, which corresponded with fat mass loss. Leptin levels have

been extensively examined in cachexia [81–83]. Smiechowska et al. [84] showed that patients with cachexia had decreased leptin levels, cancer patients without weight loss had increased leptin compared with controls, and that plasma leptin correlated with insulin resistance. Leptin is well acknowledged as a factor in appetite control. In the current study, IL-6 decreased food consumption in both the wild-type mice and the $Apc^{Min/+}$ mice, but only the $Apc^{Min/+}$ mice lost body weight and leptin was not suppressed by IL-6 expression in the wild-type mice, suggesting that IL-6 alone does not regulate leptin levels. Furthermore, food consumption was not significantly correlated with body weight loss in cage control mice overexpressing IL-6. Since food consumption varied greatly by animal, it was not possible to strongly link altered food consumption and body weight loss in the $Apc^{Min/+}$ mouse; future research with a pair feeding design might be better able to demonstrate the effect of food consumption on energy balance and the progression of cachexia in $Apc^{Min/+}$ mouse.

The current study's method of IL-6 overexpression using in vivo quadriceps muscle electroporation allowed the novel comparison of systemic IL-6 overexpression, the non-electroporated quadriceps, with the electroporated quadriceps subjected to both local and systemic IL-6 overexpression. Our prior studies have only examined non-electroporated muscle subjected to systemic IL-6 overexpression since we have never found an induction of IL-6 mRNA in $Apc^{Min/+}$ mouse hindlimb muscle [18]. Hence, local overexpression does not appear physiological to our condition of cachexia, but serves as an interesting secondary observation. Although exercising $Apc^{Min/+}$ mice maintained quadriceps muscle mass when subjected to systemic IL-6 overexpression, exercise was not able to preserve quadriceps muscle mass directly over-expressing IL-6. There was a significant effect of local IL-6 overexpression regardless of exercise to increase cytochrome C protein expression, increase STAT activation, and increase NFkB activation. There was no effect of local IL-6 expression on COXIV protein expression, AMPK activation, or AKT activation. This differed from systemic IL-6 over-expression only in that cytochrome C protein was not induced and AMPK activation was induced. These were all main effects of local IL-6 overexpression regardless of the exercise treatment. The exercise-induced increase in COXIV protein expression was attenuated in the local IL-6-overexpressing quadriceps muscle when compared with systemic IL-6 overexpression only (approximately 1.5-fold versus 9-fold, respectively).

In conclusion, we have shown that exercise effectively prevents loss of body weight and muscle mass even in the presence of high circulating IL-6 levels. Although the protective mechanisms of exercise need further study, our data support the effect of exercise on insulin resistance as a potential mechanism that could be mediated through im-

proved oxidative capacity, AKT signaling, and energy sensing (decreased AMPK) without altering muscle inflammatory signaling. We have shown that $Apc^{Min/+}$ mice with moderate cachexia have altered glucose and lipid metabolism. Our study also points to the need for further understanding on the role of adipose tissue loss and changes in plasma adipokines during the initiation and progression of cachexia. Further research is necessary to determine whether measurement of plasma triglyceride levels may be useful for predicting risk and assessing response to therapy for cancer cachexia.

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