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IGF-1 is downregulated in experimental cancer cachexia

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insulin-like growth factor-1; muscle wasting; atrogin-1; pentoxifylline; formoterol

WASTING OF LEAN BODY MASS, mainly due to loss of skeletal muscle protein, is frequently observed in several clinical conditions such as sepsis, cancer, acquired immunodeficiency syndrome (AIDS), or chronic inflammatory diseases. Muscle depletion may eventually result in functional alterations that increase mortality and impair patients' management and recovery. Studies performed in experimental models of cancer cachexia as well as in neoplastic patients have shown that the loss of muscle mass mainly derives from protein hypercatabolism, whereas changes in protein synthesis seem to occur less frequently (8, 9, 29, 45, 59). The ATP-ubiquitin-dependent degradative pathway plays a pivotal role in this catabolic response, although recent reports suggest that the lysosomal compartment, Ca^{2+}-dependent proteolysis, and caspases may be involved as well (reviewed in Refs. 22, 45). Classic hormones, proinflammatory cytokines, and other humoral factors have been involved in the pathogenesis of muscle wasting. TNF-α, in particular, is considered to play a major role in activating muscle protein breakdown, although little is known about the underlying mechanisms (reviewed in Ref. 3).

Recent data point to the importance of the signal transduction pathway activated by insulin-like growth factor-1 (IGF-1) in the regulation of skeletal muscle mass. IGF-1 is an anabolic growth factor that stimulates muscle protein synthesis as well as proliferation and differentiation of satellite cells (32). It has been reported to exert antiapoptotic effects on muscle cells (38), to suppress proteolysis, and to inhibit the ubiquitin-proteasome system (15, 37). The observation that transgenic mice overexpressing IGF-1 specifically in the skeletal muscle show a hypertrophic phenotype is consistent with the role played by this growth factor in the regulation of muscle mass (44). Finally, catabolic states such as sepsis and cancer have been associated with reduced levels of circulating IGF-1 (4, 29).

Aims of the present study have been to investigate *1*) whether muscle wasting in cancer cachexia may be associated with perturbations of the IGF-1 system and *2*) whether pharmacological agents known to exert protective effects against muscle loss may interfere with the IGF-1 pathway. For this purpose, IGF-1 mRNA levels have been determined in the skeletal muscle of rats bearing the Yoshida AH-130 ascites hepatoma (Refs. 20, 22, and references therein). In separate experiments, rats were treated with an inhibitor of TNF-α synthesis, pentoxifylline (PTX), alone or combined with formoterol (FRT), a β_2-adrenergic agent previously shown to be effective in preventing cachexia (12), or with IGF-1 alone.

The results show that skeletal muscle wasting in the AH-130 tumor-bearing rats is associated with marked reduction of IGF-1 mRNA levels. Although the mechanisms by which this alteration may concur with muscle atrophy remain to be elucidated, the present study suggests that perturbations of IGF-1 may be relevant to the onset of cancer-related muscle depletion.

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MATERIALS AND METHODS

The study was performed on male Wistar rats weighing ~150 g. They were housed on a regular dark-light cycle (light from 8:00 AM to 8:00 PM), with free access to food (Piccioni, Brescia, Italy) and water throughout the experimental period, and cared for in compliance with the Italian Ministry of Health Guidelines (no. 86609 EEC) and the NIH *Guide for the Care and Use of Laboratory Animals* (NIH 1996). The experimental protocol was approved by the Bioethical Committee of the University of Torino. The rats were randomized in two groups (n = 8 each), namely, controls and tumor bearers. The latter received an intraperitoneal inoculation of Yoshida AH-130 ascites hepatoma cells (~10^8 cells/rat). In a different set of experiments, the animals were divided into four groups (controls and tumor hosts, either treated or untreated). Treated animals received subcutaneous injections of IGF-1 (Genentech, South San Francisco, CA; 5 mg/kg, twice a day, n = 6), PTX (Sigma, St. Louis, MO; 10 mg/kg, once a day, n = 6), or PTX (10 mg/kg) plus FRT (Industriale Chimica, Saronno, Italy; 3 mg/kg, once a day, n = 8). PTX, FRT, and IGF-1 doses were decided on the basis of available literature, with little modifications for the latter (12, 15, 21). Others have used PTX doses higher than those employed in the present investigation (17). Although IGF-1 and FRT doses used in the present study are higher than those reported in the literature, no side effects were recorded in the treated animals. Both controls and tumor hosts were then killed at 8:00 AM under light ether anesthesia 2, 4, or 7 days after transplantation. Immediately before death, blood was collected from the abdominal aorta. The tumors were harvested from the peritoneal cavity, and volume and cellularity were evaluated. Several tissues were rapidly excised, weighed, frozen in liquid nitrogen, and stored at −80°C.

RT-PCR. Total RNA was extracted from the gastrocnemius muscle or from the liver with the TriPure reagent (Roche, Indianapolis, IN) and quantified spectrophotometrically. RNA integrity was checked by electrophoresis on 1.2% agarose gel containing 10% MOPS (0.2 M) and 18% formaldehyde.

IGF-1 [Ea isoform; primers used recognize a region between exon 3 and exon 6, bypassing exon 5, expression of which gives the Eb isoform (cf. Ref. 44)] and IGF-1 receptor (IGF-1R) mRNA levels were analyzed using semiquantitative RT-PCR using the kit Ready-to-Go RT-PCR beads (Hybond C; Amersham Italia, Milan, Italy). Aliquots of total RNA (0.5 μg) were added to a RT-PCR reaction mixture containing PCR buffer (10 mM Tris·HCl, pH 9.0, 60 mM KCl, and 1.5 mM $MgCl_2$), 2.0 units of *Taq* DNA polymerase, 10 μM of each primer, and 200 μM dNTP. Primers for IGF-1, IGF-1R, atrogin-1, MuRF1, ubiquitin, and 18S ribosomal subunit, described in Table 1, were obtained according to published sequences (Invitrogen, Milan, Italy).

Amplification conditions consisted of 1 min of denaturation at 95°C, 1 min of annealing at 60°C, and 2 min of polymerization at 72°C for each step for 25 cycles, followed by extension at 72°C for 7 min. Positive and negative controls were performed. PCR products were electrophoresed on 2% agarose gels and visualized with ethidium bromide. A standard DNA ladder was used to estimate the length of each PCR product. Quantification was performed using densitometric analysis: individual product bands and representative background were excised from each gel lane and analyzed using specific software (Phoretix). The results were normalized according to 18S ribosomal subunit expression, amplified with the following conditions: 30 s of denaturation at 95°C, 1 min of annealing at 55°C, and 2 min of polymerization at 72°C for 10 cycles, followed by extension at 72°C for 7 min. Comparisons among groups were made in the linear phase of amplification.

Table 1. *Oligonucleotide sequences*

18S	5′-CGCAGAATTCCCACTCCCGACCC-3′
	3′-CCCAAGCTCCAACTACGAGC-5′
IGF-1Ea	5′-CACATCTCTTCTACCTGGC-3′
	3′-GTAGGTCTTGTTTCCTGCAC-5′
IGF-1R	5′-TCTTGGATGCGGTGTCCAATAAC-3′
	3′-GCAGCACTCATTGTTCTCGTTGC-5′
Atrogin-1	5′-CCATCAGGAGAAGTGGATCTATGTT-3′
	3′-GCTTCCCCCAAAGTGCAGTA-5′
MuRF-1	5′-GGACGGAAATGCTATGGAGA-3′
	3′-AACGACCTCCAGACATGGAC-5′
Ubiquitin	5′-GGTAAGACCATCACCCTGGA-3′
	3′-AGGGTGGACTCCTTCTGGAT-5′

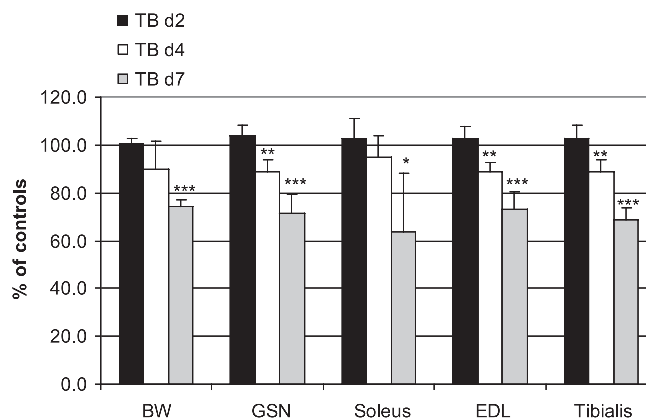

Fig. 1. Body and muscle weight in rats bearing the AH-130 hepatoma (tumor bearing rats, TB). Data are means ± SD (n = 8) expressed as percentages of controls. BW, body weight; GSN, gastrocnemius; EDL, extensor digitorum longus; d2, d4, and d7, *days 2*, *4*, and *7*. *P < 0.05; **P < 0.01; ***P < 0.001 vs. controls.

Insulin and IGF-1 circulating levels. Insulin and IGF-1 were measured using commercially available kits, as directed by the manufacturer (IDS, Boldon, Tyne and Wear, UK).

Data presentation. Results are expressed as means ± SD. Where not differently specified, n = 8 for both controls and tumor hosts. Significance of the differences has been evaluated using ANOVA.

RESULTS

The Yoshida AH-130 ascites hepatoma elicited in the rat hosts a progressive loss of body weight and a concomitant severe reduction in the weight of several muscles such as gastrocnemius, extensor digitorum longus (EDL), tibialis anterior, and soleus (Fig. 1). As previously reported, muscle weight loss in the present model system of cachexia strictly reflects protein depletion (cf. Refs. 56, 57). Food intake is significantly reduced in tumor hosts (see Fig. 6). However, studies were not performed in pair-fed groups, because previous results have shown that muscle protein waste in the AH-130 hosts is mainly associated with acceleration of protein breakdown rates, whereas in pair-fed animals, the decrease of skeletal muscle protein content is due to impaired protein synthesis (57). The conclusion was that reduced food intake and metabolic competition by the tumor did not justify the hypercatabolic state in the AH-130 bearers and that pair feeding does not provide reliable controls. Indeed, the effects of malnutrition in the AH-130 bearers are likely overwhelmed and subverted in the frame of the tumor-host interplay that leads to a distinctively peculiar syndrome. This view also is shared by more recent investigators, in particular Lecker et al. (41).

IGF-1 and IGF-1R. IGF-1 mRNA expression in the gastrocnemius of AH-130 hosts showed a marked decrease with

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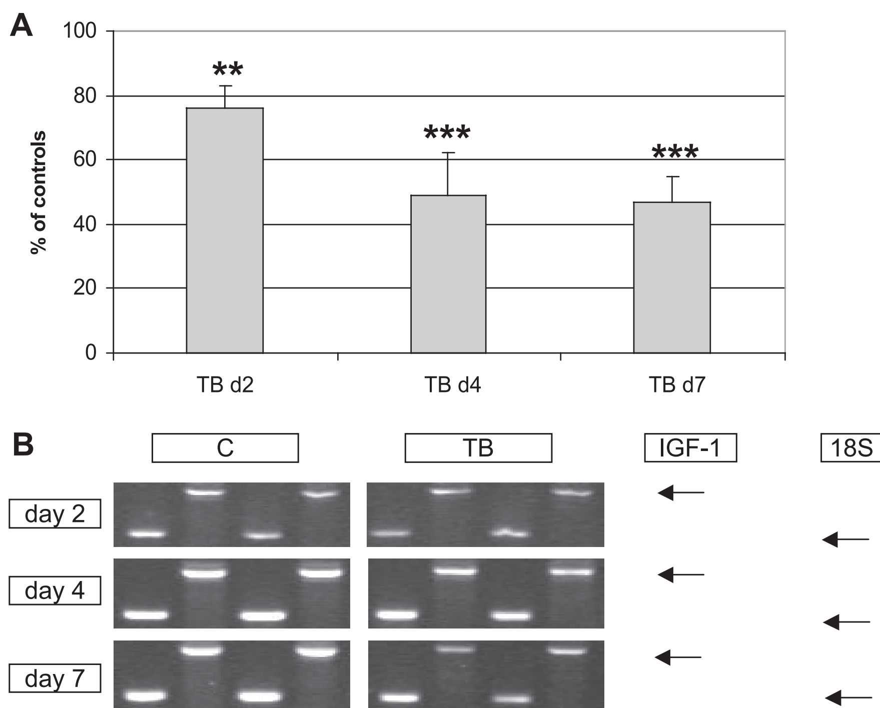

Fig. 2. Insulin-like growth factor-1 (IGF-1) mRNA levels in the gastrocnemius of tumor-bearing rats. *A*: densitometric analysis. Data are means ± SD (n = 8) expressed as percentages of controls [controls (C) = 122 ± 6 arbitrary units (a.u.)]. $**P < 0.01$; $***P < 0.001$ vs. controls. *B*: representative RT-PCR pattern of expression.

respect to controls (Figs. 2 and 7). The reduction (to 80% of controls) could be observed already 2 days after tumor transplantation, although it did not reach significance in all the experiments. Subsequently, on *days 4* and *7*, the mRNA level ranged between 70 and 50% of controls.

The expression of IGF-1R in the gastrocnemius, although significantly reduced at *day 4* after tumor transplantation, was markedly increased at *day 7* (Fig. 3*A*). Also at the latter time point, the levels of insulin receptor mRNA were about twice those of controls (209%, P = 0.00014).

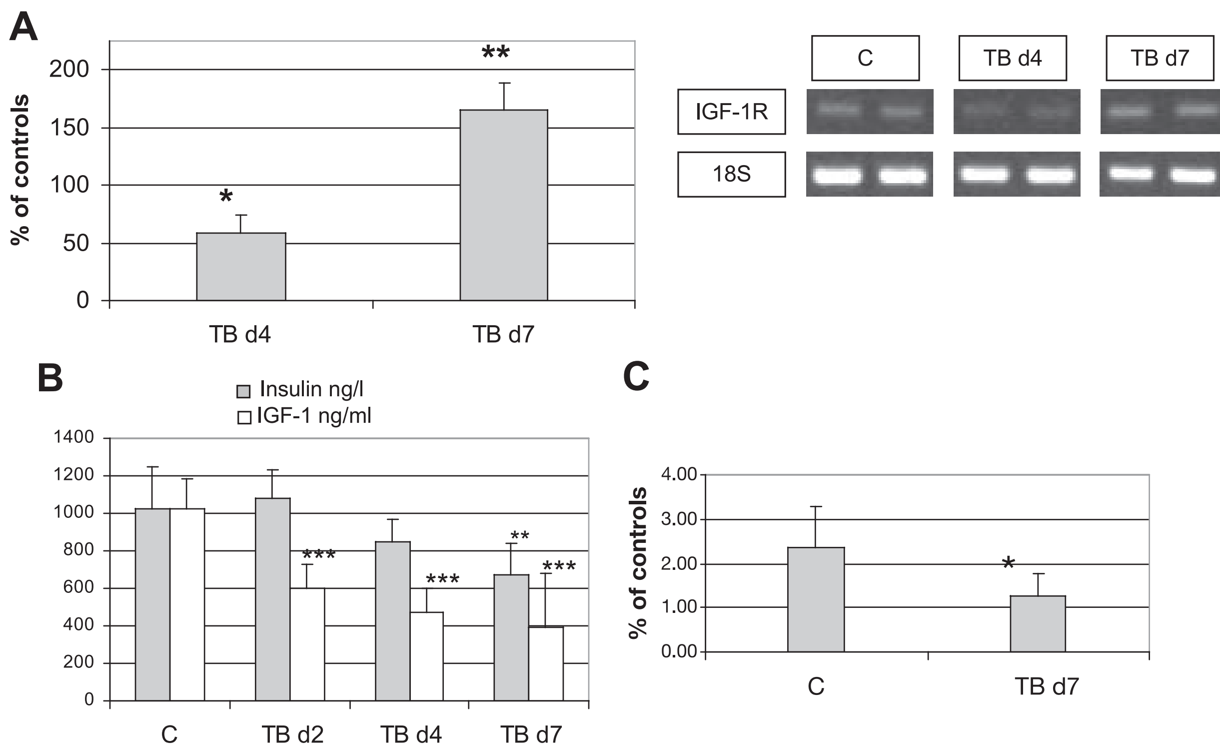

Fig. 3. *A*: IGF-1 receptor (IGF-1R) mRNA levels in the gastrocnemius of tumor-bearing rats. *Left*, densitometric analysis; *right*, representative RT-PCR pattern. Data (means ± SD; n = 8) are expressed as percentages of controls (C = 17 ± 3 a.u.). $*P < 0.05$; $**P < 0.005$ vs. controls. *B*: IGF-1 and insulin circulating levels in controls and tumor-bearing rats. Data (fed values) are means ± SD (n = 8). $**P < 0.005$; $***P < 0.001$ vs. controls. *C*: IGF-1 mRNA levels in the liver of controls and tumor-bearing rats. Data are means ± SD (n = 6) expressed as arbitrary units. $*P < 0.05$ vs. controls.

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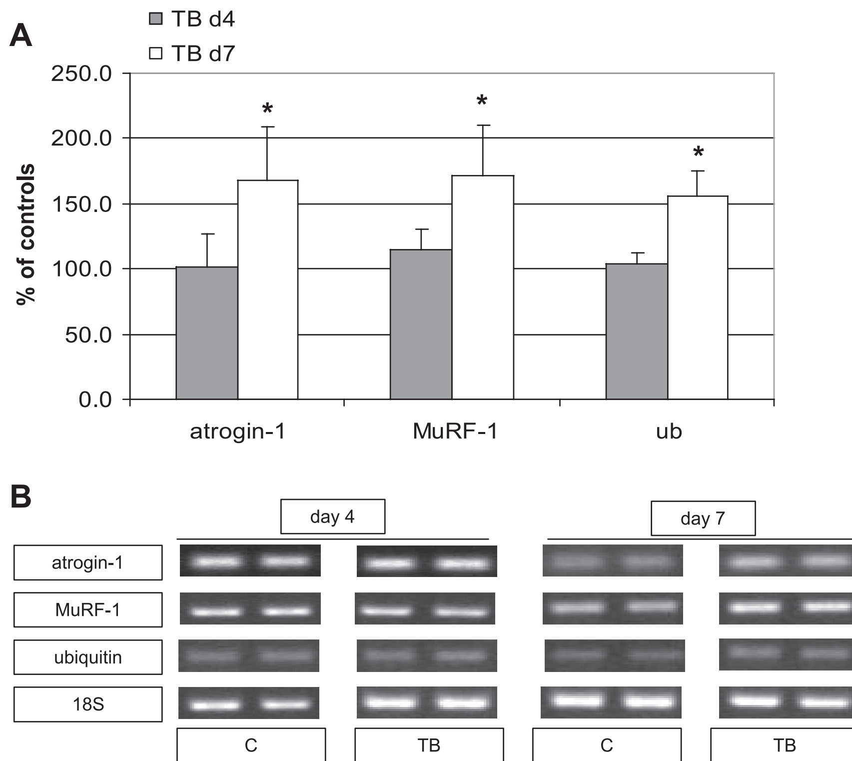

Fig. 4. Expression of atrogin-1, MuRF1, and ubiquitin in the muscle of tumor-bearing rats. *A*: densitometric analysis. Data are means ± SD (n = 8) expressed as percentages of controls (atrogin-1 = 91 ± 8 a.u., MuRF1 = 63 ± 12 a.u., ubiquitin = 35 ± 12 a.u., averages of *day 4* and *day 7* values). $*P < 0.05$ vs. controls. *B*: representative RT-PCR pattern.

Plasma IGF-1 was markedly reduced in the AH-130 hosts as early as 2 days after tumor transplantation, whereas the decrease of insulin was comparatively delayed and less pronounced (Fig. 3*B*). Low circulating IGF-1 levels also were recently reported in cachectic cancer patients (35). Consistently with the notion that circulating IGF-1 is mostly of hepatic origin, IGF-1 mRNA levels in the liver were significantly reduced (Fig. 3*C*).

Atrogin-1, MuRF1, and ubiquitin mRNA. It was previously reported that impairment of IGF-1 signaling may also result in muscle atrophy by enhancing the expression of molecules pertaining to the ATP-ubiquitin-dependent proteolytic system, particularly the muscle-specific ubiquitin ligases atrogin-1 and MuRF1 (cf. Refs. 50, 55). Consistent with these reports, and with our previous observations showing that muscle wasting in the AH-130 hosts is associated with hyperactivation of the ATP-ubiquitin-dependent proteolytic system (reviewed in Ref. 22), the levels of atrogin-1, MuRF1, and ubiquitin mRNA were higher in *day 7* AH-130 hosts than in controls, whereas no changes could be detected in *day 4* tumor bearers (Fig. 4).

Effects of systemic IGF-1 administration. None of the phenotypic changes induced by the tumor were corrected by systemic administration of IGF-1. As shown in Table 2, the body weight loss and the marked depletion observed in both the skeletal muscle (gastrocnemius and soleus) and the adipose tissue of *day 4* AH-130 bearers were not affected by IGF-1 administration. Similarly, hypertrophy of the spleen, frequently reported in the AH-130 model system, was not modified by the treatment. Both liver and heart mass did not differ between controls and tumor hosts, and treatment with IGF-1 did not affect this pattern. Cumulative food intake was not significantly different between treated and untreated controls (controls = 93 ± 4 g, IGF = 99 ± 5 g) or tumor hosts (AH-130 = 66 ± 3 g, AH-130 + IGF = 76 ± 6 g, $P < 0.001$ vs. controls and IGF for both groups). Finally, tumor growth, evaluated as total cell number, was comparable in treated and untreated rats.

Effects of PTX and FTR administration. The last point of the present study was to investigate whether the protection against muscle wasting exerted by pharmacological treatments (cf. Refs. 20, 22) may be associated with restoration of normal IGF-1 mRNA levels. In agreement with previous observations (21), systemic administration of PTX to the AH-130 hosts resulted in a significant attenuation of both soleus and heart wasting at *day 4* after tumor transplantation. As for the gas-

Table 2. *Effect of recombinant IGF-1 administration to tumor-bearing rats*

	Final Body Weight, g	Tumor (Total Cells)	Tissue Weight, mg/100 g initial body weight					
			Liver	Heart	Gastrocnemius	Soleus	WAT	Spleen
C	190±16		5,755±180	356±20	577±25	56.75±6.0	396±78	424±40
C + IGF	199±9		5,950±108	393±28	589±25	51.23±4.7	320±59	635±32[a]
AH-130	150±13[c]	1,269±255	6,131±577	388±73	470±57[b]	46.02±5.7[a]	269±60	861±95[c]
AH-130 + IGF	155±9[c,e]	1,390±377	5,981±473	370±26	498±43[a,d]	44.88±5.1[b]	229±80[a]	811±78[c,d]

Data are expressed as means ± SD, n = 6 for each group. Body weight (g) in the AH-130 hosts (*day 4*) was exclusive of the tumor. [a]$P < 0.05$; [b]$P < 0.01$; [c]$P < 0.001$ vs. controls [d]$P < 0.05$; [e]$P < 0.01$ vs. treated controls. C, controls; IGF, insulin-like growth factor; WAT, white adipose tissue.

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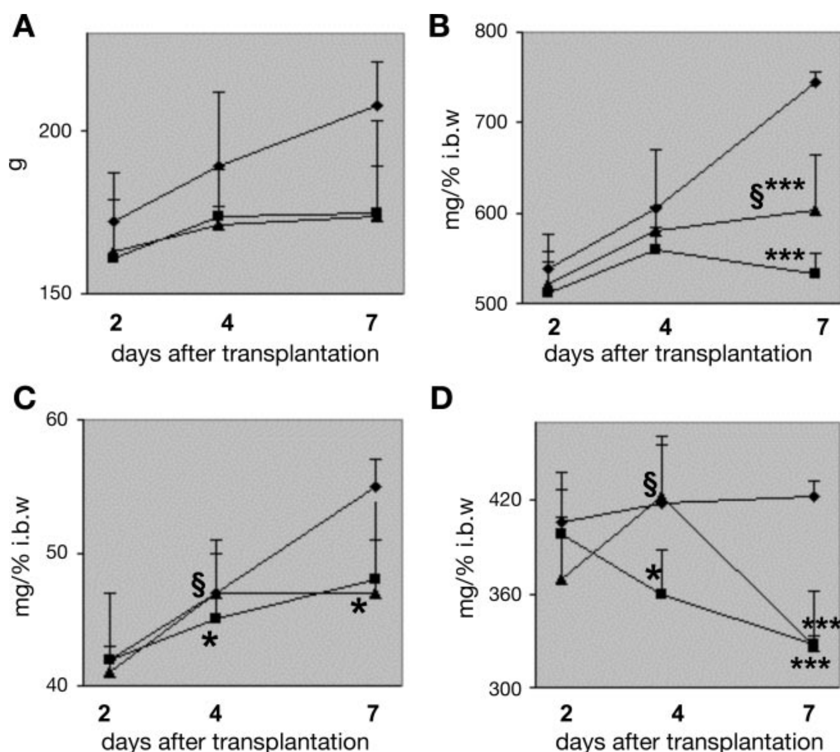

Fig. 5. Body (*A*) and muscle (*B*: gastrocnemius; *C*: soleus; *D*: heart) weight in the AH-130 hosts treated with pentoxifylline (PTX). ◆, C; ■, AH-130; ▲, AH-130 + PTX. i.b.w., Initial body weight. Data are means ± SD ($n = 6$). *$P < 0.05$; ***$P < 0.001$ vs. controls. §$P < 0.05$ vs. untreated tumor bearers.

trocnemius, in this experiment the depletion in AH-130 hosts became significant on *day 7* and was effectively reduced by treatment with PTX (Fig. 5; cf. also Ref. 21). PTX does not induce any change in food intake during the whole experimental period (*day 7* cumulative food intake: 112 ± 10 and 115 ± 12 g in untreated and treated AH-130 hosts, respectively).

To achieve a more pronounced protective effect, we also administered PTX in association with FRT, a β-adrenergic agonist that prevents cachexia in the AH-130 model system (12). When PTX was combined with FTR, no significant changes of body and muscle weight were observed in control rats, whereas in the AH-130 hosts, a significant protection against muscle wasting was observed at both *days 4* and *7* after transplantation (Table 3 and Fig. 6).

The partial prevention of muscle depletion by PTX plus FRT, but not that induced by PTX alone, was associated with significant reduction of MuRF1 mRNA levels that become comparable to those observed in untreated controls (Fig. 7). By contrast, neither atrogin-1 nor IGF-1 mRNA levels were affected by both treatments in tumor-bearing rats (Figs. 7 and 8; IGF-1 mRNA = 830 ± 132, 406 ± 150, and 310 ± 131 arbitrary units in control, AH-130, and AH-130 + PTX, respectively) and in controls (atrogin-1 expression: 103% of untreated controls).

DISCUSSION

The present study reports for the first time that the expression of IGF-1 in the skeletal muscle is reduced in cancer cachexia, at least in the AH-130 model system. This observation is consistent with other recent reports suggesting that downregulation of IGF-1 is crucially involved in the development of skeletal muscle wasting (14, 35).

IGF-1 serves the function of a circulating hormone as well as of a tissue growth factor acting through autocrine or paracrine mechanisms. Whereas circulating IGF-1 isoforms seem to have negligible effects on muscle, locally synthesized IGF-1 is believed to be most relevant to the control of the skeletal muscle mass (14). Two isoforms are predominantly expressed in the skeletal muscle, IGF-1Ea, the most abundant isoform, and IGF-1Eb, also termed mechanogrowth factor, which is mainly produced by damaged or loaded skeletal muscles (52). IGF-1 plays a critical role in muscle homeostasis and regeneration, promoting the proliferation and differentiation of satellite cells and their fusion with existing muscle fibers in hypertrophic processes and in the repair of damaged fibers (32, 44). Particularly relevant in this regard is the observation that

Table 3. *Effect of treatment with PTX + FRT in tumor-bearing rats*

	Body Weight, g	Tissue weight, mg/100 g initial body weight		
		EDL	Soleus	Heart
Day 2				
C	121±5	39.4±1	38.8±4.5	409±33
C + PTX + FRT	124±6	42.9±2.3[a]	42.3±5.7	430±45
AH-130	121±3	40.5±1.9	39.8±3.3	365±33[d]
AH + PTX + FRT	118±8	43.2±1.4[a]	43.1±3	424±22
Day 4				
C	137±10	46.7±2.4	42.8±2.3	402±16
C + PTX + FRT	132±7	45.9±2.4	45.4±3.5	474±66
AH-130	123±16	40.5±1.8[b,e,g]	40.6±3.2	387±44[d]
AH + PTX + FRT	118±5	46.2±1.4	40.9±4.1	429±21
Day 7				
C	151±15	52.3±4.1	50.8±5.6	430±58
C + PTX + FRT	155±4	59.9±3.7[a]	52.5±4.3	485±51
AH-130	112±4[c,f]	38.7±3.7[c,f]	32.4±12.4[b,e]	358±77[e]
AH + PTX + FRT	114±4[c,f]	43.8±3.2[b,f]	40.7±4.4	416±20

Data are expressed as means ± SD, $n = 8$ for each group at each time point. [a]$P < 0.05$; [b]$P < 0.01$; [c]$P < 0.001$ vs. controls; [d]$P < 0.05$; [e]$P < 0.01$; [f]$P < 0.001$ vs. treated controls. [g]$P < 0.001$ vs. treated tumor bearers.

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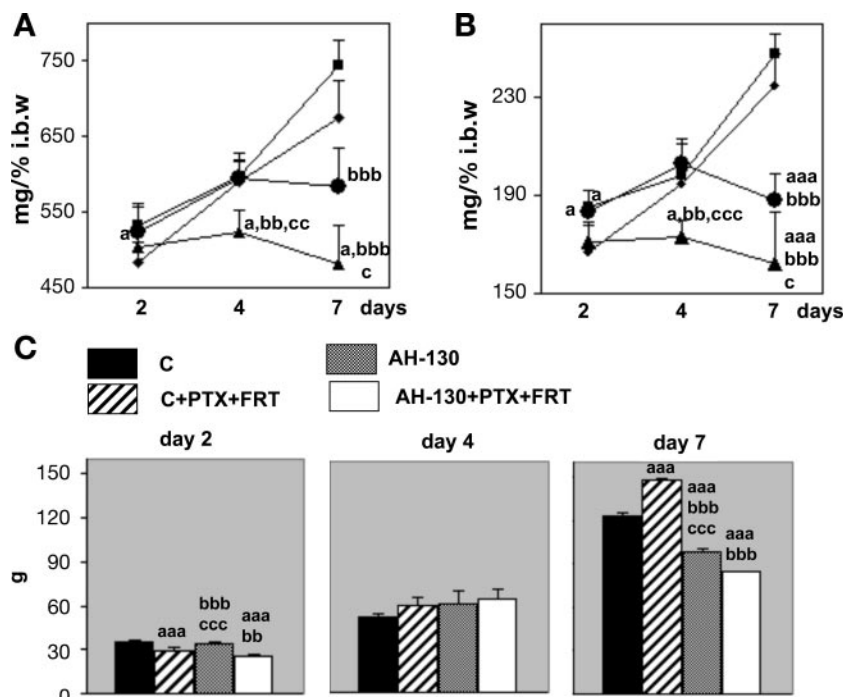

Fig. 6. Muscle weight (*A*: gastrocnemius; *B*: tibialis) and cumulative food intake (*C*) in the AH-130 hosts treated with PTX + formoterol (FRT). ■, controls; ◆, C + PTX + FRT; ▲, AH-130; ●, AH-130 + PTX + FRT. Data are means ± SD (n = 8). $^{a}P < 0.05$; $^{aaa}P < 0.001$ vs. controls. $^{bb}P < 0.01$; $^{bbb}P < 0.001$ vs. treated controls. $^{c}P < 0.05$; $^{ccc}P < 0.001$ vs. treated tumor bearers.

transgenic mice overexpressing an IGF-1 isoform driven by the myosin light chain promoter (mIGF-1) exhibit skeletal muscle hypertrophy (44). Expression of mIGF-1 preserves muscle fiber integrity even at advanced ages, suggesting that this factor may prolong the regenerative potential of the skeletal muscle (44). More recently, expression of mIGF-1 has been shown to counteract muscle wasting in *mdx* dystrophic mice, in an experimental model of amyotrophic lateral sclerosis, and in mice administered angiotensin II, by improving both muscle mass and strength and enhancing the levels of phosphorylated (active) Akt/PKB (6, 27, 54). In addition, local overexpression of IGF-1 by means of gene transfer by electroporation is able to prevent skeletal muscle atrophy induced by hindlimb suspension or by treatment with glucocorticoids (2, 51). Contrasting results, however, have been reported by Criswell et al. (24), showing that transgenic mice overexpressing IGF-1 in the skeletal muscle are not protected against unloading-induced atrophy.

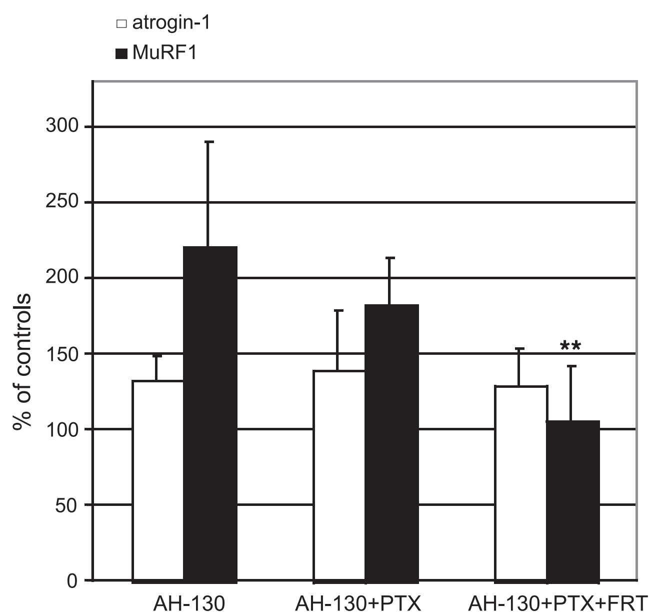

Fig. 7. Atrogin-1 and MuRF1 mRNA levels in the skeletal muscle of tumor-bearing rats treated with PTX or PTX + FRT. Data are means ± SD (n = 6) expressed as percentages of controls (C = 1.11 ± 0.21 and 0.62 ± 0.19 a.u. for atrogin-1 and MuRF1, respectively). $^{**}P < 0.01$ vs. AH-130.

Perturbations of the IGF-1 signaling pathway have been recently proposed to play a major role in the pathogenesis of skeletal muscle atrophy (14). Occupancy of the insulin and IGF-1 receptors activate their tyrosine kinase domain that catalyzes phosphorylation of a number of intracellular substrates, including insulin responsive sequence (IRS)-1 and IRS-2 (cf. Ref. 49). Tyrosine phosphorylation of IRS factors generates docking sites for several SH2-containing proteins. Among these, a major role is played by class I phosphatidylinositol 3-kinase, which in turn, with the partnership of phosphoinositide-dependent kinase-1, phosphorylates and activates Akt (61). Active Akt has been found significantly reduced in different models of skeletal muscle atrophy, such as unloading and denervation (50), whereas transgenic mice overexpressing Akt are protected against denervation-induced muscle atrophy (7). Hyperexpression of Akt in skeletal muscle cells or its conditional activation in the skeletal muscle of adult rats results in a hypertrophic phenotype (39, 47). Not only does activation of Akt stimulate protein synthesis through regulation of glycogen synthase kinase-3β and mTOR (mammalian target of rapamycin) kinases (7), but it also may downregulate protein breakdown. In particular, active Akt may phosphorylate and sequester in the cytoplasm the transcription factors Foxo-1 and Foxo-3, thus inhibiting the transcription of several genes among which is *atrogin-1* (50, 55). In the present study, however, the reduction of IGF-1 mRNA levels was associated with enhanced expression of the “atrogenes” atrogin-1,

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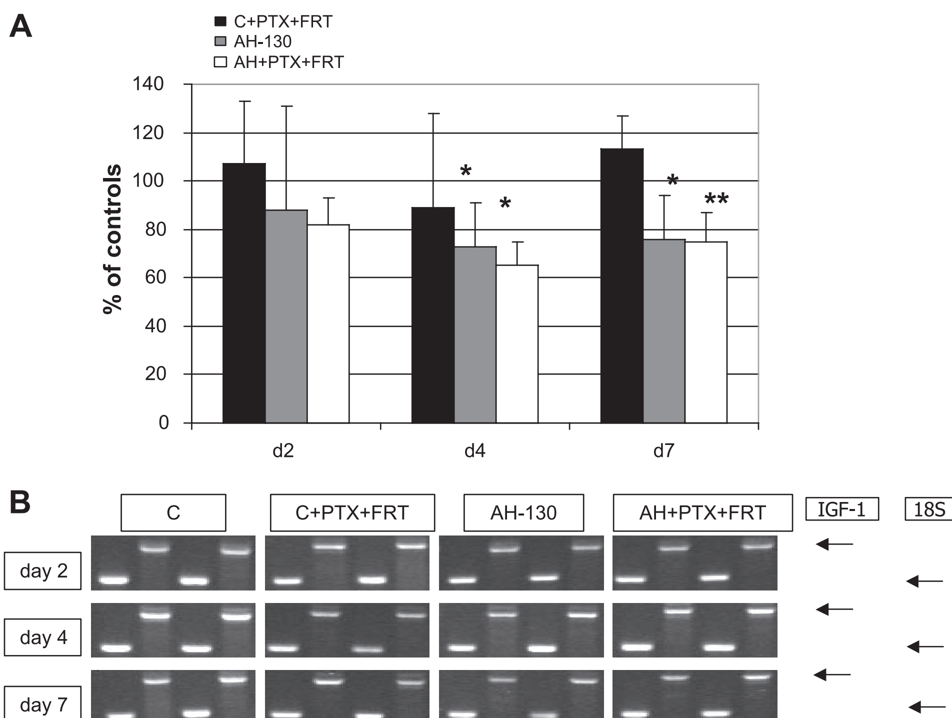

Fig. 8. IGF-1 mRNA levels in the gastrocnemius of AH-130 hosts treated with PTX + FRT. *A*: densitometric analysis. Data are means ± SD (n = 8) expressed as percentages of controls (C = 1.53 ± 0.42 a.u.). $^*P <$ 0.05; $^{**}P < 0.01$ vs. controls. *B*: representative RT-PCR pattern.

MuRF1, and ubiquitin only in *day 7* tumor hosts (cf. Refs. 41, 42). It is unlikely that these changes are related to decreased food intake. In fact, it was previously shown by Lecker et al. (41) that in this experimental model, changes in atrogin-1 mRNA specifically reflect effects of the disease process and not any associated decrease in food intake.

In the present work, IGF-1 mRNA levels in the gastrocnemius muscle of rats bearing the AH-130 hepatoma decreased early after tumor transplantation (*day 2*), although these decreases did not always reach significance, and even more thereafter (*days 4* and *7*). Our results are consistent with the decreased IGF-1 expression demonstrated in other situations of muscle atrophy such as unloading and chronic heart failure (5, 10, 36). Moreover, similar results have been reported for an experimental model of sepsis in which the autocrine production of muscle IGF-1 was impaired by LPS administration to rats (31) and in rats treated with glucocorticoids (51).

The expression of IGF-1R in the skeletal muscle of the AH-130 bearers showed a biphasic pattern, being initially reduced (*day 4*) and then markedly increased (*day 7*). The decrease observed at *day 4* parallels that of IGF-1 mRNA levels, whereas the late increase (*day 7*) may reflect an adaptive response to the reduced IGF-1 levels. Accordingly, high levels of IGF-1R mRNA associated with reduced IGF-1 expression also have been reported in the skeletal muscle of patients with chronic heart failure (36).

Bearing in mind that IGF-1 is decreased in the AH-130 hosts at both the muscle level and systemically, the possibility to correct muscle protein wasting by parenteral administration of recombinant IGF-1 was investigated. We have previously reported that insulin replacement in the AH-130 hosts is able to prevent the onset of muscle depletion (58). By contrast, the present study demonstrates that IGF-1 administration was totally ineffective in this regard, and it did not induce any change in control animals. This observation is consistent with a previous report showing that systemic administration of IGF-1 has not been able to correct muscle wasting induced in rats by angiotensin II infusion (10). Recently published data have indeed shown that exogenous IGF-1 has a limited bioavailability and that this problem can be circumvented by complexing IGF-1 with the IGF binding protein IGF-BP3 to delay its clearance from the circulation (see Ref. 38 and references therein). This could explain why no significant effects were observed in both control ant tumor-bearing rats after IGF-1 administration. On the other hand, it has been suggested that autocrine IGF-1 is probably more relevant to the regulation of muscle mass than the circulating isoform (cf. Refs. 36, 44). This hypothesis is supported by recent results showing that local overexpression of IGF-1 is able to prevent experimental skeletal muscle atrophy (2, 51).

Muscle wasting in rats bearing the AH-130 hepatoma can be prevented to varying extents by different pharmacological agents (cf. Refs. 20, 22). In this regard, anti-TNF-α treatments (specific antibodies or PTX; Refs. 18, 20) and β_2-adrenergic agonists (clenbuterol, FRT; Refs. 12, 19) have proven particularly effective. To investigate whether their protective effect is associated with restoration of normal levels of IGF-1 gene expression, rats bearing the AH-130 hepatoma were administered PTX or a combination of PTX and FRT. Both schedules significantly prevented the development of muscle atrophy, although PTX alone did so to a lesser extent. On the basis of the present observations, it is likely that one of the mechanisms by which PTX + FRT exerts its preventive effect is the reduced expression of MuRF1 ubiquitin ligase. By contrast, neither treatment was able to increase IGF-1 gene expression. In this regard, it could be hypothesized that the lack of changes of IGF-1 expression in the muscle of treated rats does not necessarily imply that the IGF-1 signaling pathway was not normalized. On the other hand, PTX and PTX + FRT did not even restore normal levels of atrogin-1 in the skeletal muscle of

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AH-130 hosts. This is quite an unexpected finding, given that several reports in the literature have shown that recovery of muscle mass in different experimental models of atrophy is paralleled by normalization of atrogin-1 expression (48, 54, 62). However, this result, as well as our other observation that muscle wasting in *day 4* tumor hosts is not associated with increased expression of atrogin-1, suggests that changes of muscle mass and atrogin-1 mRNA levels are not necessarily tightly coupled. This possibility also is consistent with a recent report showing that restoration of normal atrogin-1 expression induced in fasted or diabetic rats by treatment with IGF-1 is not associated with recovery of muscle mass (25). In addition, the reduction of protein degradation exerted by GSK-3β inhibitors in muscles isolated from burned rats has been reported to occur without changes in atrogin-1 mRNA levels (30). Finally, inhibition of protein wasting by PTX + FRT is paralleled by restoration of MuRF1 mRNA levels, without changes of atrogin-1 expression. This suggests that muscle depletion in the AH-130 model of cachexia would derive, at least in part, from activation of a transcription factor-dependent mechanism. Indeed, Cai et al. (13) showed that muscle wasting induced by NF-κB activation is associated with modulations of MuRF1 rather than atrogin-1 expression. Although we have shown that NF-κB is not hyperactivated in the muscle of AH-130 hosts (23), the DNA-binding activity of AP-1, another typical target of cytokine bioactivity, is significantly enhanced (23) and probably plays a role in increasing MuRF1 mRNA levels.

We do not have information about the state of activation of insulin/IGF-1 signal transduction pathway in the present tumor-bearing animals. This may be modulated by different conditions such as malnutrition, alterations of the hormonal homeostasis, and increased levels of proinflammatory cytokines (1), not necessarily via downregulation of insulin/IGF-1 expression. TNF-α, in particular, has been proposed to be involved in the development of the insulin resistance that often complicates the management of neoplastic patients (43, 46), whereas the peripheral insulin resistance that develops in diet-induced obesity is less severe in TNF-α knockout mice than in their normal counterparts (60). In addition, reduced IGF-1 release has been demonstrated in C_2C_{12} murine myocyte cultures exposed to TNF-α (31). Inhibition by TNF-α of insulin/IGF-1 signaling, mainly depending on serine phosphorylation of IRS-1 and IRS-2 (11, 34), has been demonstrated in different cell types such as adipocytes, hepatocytes, or C_2C_{12} myocytes (26, 53). Consistent with these observations, we have previously shown that the hypoinsulinemia induced in the host rat by the AH-130 hepatoma can be partially corrected by treatment with anti-TNF-α antibodies (18). In the present study, however, treatment of AH-130 bearers with PTX did not increase muscle IGF-1 mRNA, suggesting that TNF-α is not involved in the downregulation of IGF-1 expression, at least not in this model system. This observation is consistent with a previous report by Colson et al. (16) showing that inhibition of TNF-α synthesis by PTX did not restore normal serum levels of IGF-1 in endotoxin-treated rats. By contrast, TNF-α has been shown to inhibit IGF-1 mRNA expression in C_2C_{12} myocyte cultures as well as in rat gastrocnemius (28, 31, 33). Our results, however, do not rule out the possibility that PTX administration may correct muscle atrophy by improving IGF-1 signaling.

In conclusion, the present study shows that protein wasting in rats bearing the AH-130 tumor is associated with reduced IGF-1, both in blood plasma and at the muscle level, further supporting the hypothesis that perturbations of this signaling pathway may be relevant to the pathogenesis of cancer cachexia. Despite the role played by TNF-α in AH-130-induced cachexia, this cytokine does not seem to be involved in the downregulation of IGF-1 expression. In addition, the protection against muscle wasting exerted by pharmacological treatments is not associated with restoration of normal IGF-1 mRNA levels in muscle. Finally, these results are of great interest because they are the first demonstration that the IGF-1 system is perturbed in the skeletal muscle of cachectic rats. Further studies, however, are needed to investigate the state of activation of the IGF-1 signal transduction pathway and to clarify the mechanisms by which the reduced IGF-1 gene expression may participate in muscle wasting.

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