Prostaglandin-dependent muscle wasting during infection in the broiler chick (*Gallus domesticus*) and the laboratory rat (*Rattus norvegicus*)

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Systemic infection with *Escherichia coli* significantly decreased feed intake, slowed growth of the whole body and skeletal muscles, and severely inhibited muscle protein accumulation in both chicks and rats. Treatment with naproxen (6-methoxy- α -methyl-2-naphthaleneacetic acid), an inhibitor of prostaglandin production, decreased weight losses of body and muscle, and significantly inhibited muscle protein wasting in infected chicks and rats. *E. coli* infection increased net protein degradation by 44.8% ($P < 0.05$) and prostaglandin E_2 production by 148% ($P < 0.05$) in isolated extensor digitorum communis muscle from chicks on day 2 after infection. Naproxen treatment significantly decreased net protein degradation and prostaglandin E_2 production in infected chicks to values seen in muscles of healthy controls. Quantitatively and qualitatively similar results were seen in isolated rat epitrochlearis muscle.

INTRODUCTION

A marked whole-body wasting and protein loss from skeletal muscle are characteristics of infection and various other pathological states [1]. Infectious disease leads to increased muscle breakdown, urinary nitrogen loss, and detectable losses of body protein [1,2]. The relative importance of changes in muscle protein synthesis and degradation in muscle wasting during infection have been addressed by several authors [3–5]. Many hormones and factors have been thought to be involved in the regulation of muscle protein metabolism during infection, including insulin, glucagon, growth hormone, thyroid hormone, glucocorticoids [1], and, more recently, prostaglandins [5–7].

Prostaglandins (PGs) are cyclopentane derivatives formed from polyunsaturated fatty acids and released by most animal cells in response to a variety of physiological and pathological stimuli [8]. Recently, the possibility that prostaglandins might be important in the regulation of muscle protein turnover in normal or pathological conditions has come to light. Skeletal muscle in humans and rats can produce metabolites of arachidonic acid, including PGI_2 , thromboxane B_2 , PGE_2 and $PGF_{2\alpha}$ [9–11]. When added to muscles *in vitro*, PGE_2 stimulated muscle protein degradation and $PGF_{2\alpha}$ activated protein synthesis [11,12]. For example, when rat muscles were treated with PGE_2 , protein synthesis was not affected, but protein degradation increased by about 22% [11]. By contrast, $PGF_{2\alpha}$ had no effect on protein degradation, but increased protein synthesis [10,11].

More recently, several authors have attempted to clarify the role of prostaglandins in the regulation of muscle protein turnover *in vivo*. For example, injection of live *Escherichia coli* into rats caused fever, and increased both PGE_2 production and protein degradation in skeletal muscle [7]. When the rats were pre-treated with indomethacin, an inhibitor of prostaglandin production, the increased protein degradation and prostaglandin

synthesis owing to infection were inhibited [7]. Indomethacin has also been reported to prevent muscle protein loss and impaired contractile force owing to *S. pneumoniae* infection [13]. These studies suggested that the rapid onset of muscle wasting that takes place during infection *in vivo* is signalled by a prostaglandin-dependent mechanism [7,13], although it remains unclear as to which stimulus might be responsible for enhanced PGE_2 release. However, the role of PGE_2 in regulation of muscle proteolysis is the subject of continuing controversy [14–17]. Further evidence is therefore required to confirm whether PGE_2 is important in regulation of muscle proteolysis. The objective of the study reported here was to test for a prostaglandin-dependent mechanism in muscle wasting induced by a systemic infection in two species: the broiler chick (*Gallus domesticus*) and laboratory rat (*Rattus norvegicus*), using an experimental *E. coli* infection technique previously developed in our laboratory (Tian & Baracos [17a]).

EXPERIMENTAL

Experimental design

Chicks or rats were assigned to four treatment groups: control, infected, naproxen control, naproxen infected, such that the mean initial body weight and S.E.M. of each group were similar. All experiments used at least six animals per treatment, and experiments were repeated at least twice. For each animal allocated to a control treatment, two animals were allocated to be infected, because of anticipated mortality. Naproxen (6-methoxy- α -methyl-2-naphthaleneacetic acid) [18] was administered in the drinking water (1.6 mg/100 g body wt. per day). Preliminary experiments showed this was a minimum effective dose to block fever induced by the injection of *E. coli* endotoxin (V. E. Baracos, unpublished work). In preliminary experiments the water consumption of healthy and infected birds was monitored in order

to verify the ingestion of the naproxen-treated water. Addition of this agent did not affect the water consumption of treated rats or chicks. Since feed and water intake of infected subjects fell markedly, in those experiments where naproxen was administered after infection, this agent was injected intraperitoneally (1.6 mg/100 g body wt.).

Animals and diet

The experiments described here were carried out in accordance with the guidelines of the Canadian Council on Animal Care. Male broiler chicks (1 day old) of the Hubbard strain, vaccinated for Marek's disease, were obtained from Co-op Hatchery (Edmonton, Alberta, Canada). Chicks were housed (6–18/pen) in electrically heated batteries (33–35 °C) with raised wire-mesh floors and continuous lighting. The chicks were fed *ad libitum* on a broiler starter diet based on wheat and soybean meal containing 11700 kJ (2795 kcal) of metabolizable energy/kg and 23.3% (w/w) crude protein. Female Sprague–Dawley rats (9 weeks old) were housed in individual wire cages and maintained on Wayne Laboratory chow (containing 24% crude protein) (Continental Grain Co., Chicago, IL, U.S.A.) and on a 12 h light/dark cycle. Feed and water were provided *ad libitum* throughout the period of study. Feed intake was measured on a per-pen basis (chicks) or individual basis (rats), and individual body weights were measured daily. Animals from each treatment group were killed on day 2 after the *E. coli* injection, and individual muscles were obtained: extensor digitorum communis (EDC) and sartorius muscles from chicks, and extensor digitorum longus (EDL) and soleus muscles from rats. Muscles were weighed and stored frozen at –30 °C until analysed.

Experimental infection

Infection was induced in chicks (on day 8 *ex ovo*) and rats (70 days old) by intraperitoneal injection of *E. coli* (Tian & Baracos [17a]). The *E. coli* used was of a single strain obtained from an outbreak of coliform septicemia in turkeys. Cell stocks were stored frozen in sheep blood at –70 °C. A single intraperitoneal injection containing 3×10^8 (chicks) or 8.1×10^8 (rats) colony-forming units was given to each animal in a volume less than 1.0 ml. The injection was given when birds weighed approx. 100 g and rats were approx. 250 g, so that both species received 3×10^9 cells/kg live wt. Control subjects were sham-injected with sterile phosphate-buffered non-pyrogenic saline (Travenol).

Analysis of protein

Frozen muscles were thawed and dissolved with 1.0 M-NaOH at 55 °C for 4–12 h. Muscle protein content was measured by a modification of the Bradford method, in accordance with the changes suggested by Read & Northcote [19]. Bovine serum albumin was used as protein standard.

Measurement of net protein degradation and PGE₂ production

At 2 days after infection, chicks or rats from each treatment were dissected to obtain muscles for incubation *in vitro*: EDC muscles (chicks) and epitrochlearis muscles (rats). Muscles were incubated in 3 ml of Krebs–Ringer bicarbonate medium (119 mM-NaCl, 4.8 mM-KCl, 1.25 mM-MgSO₄, 25 mM-NaHCO₃, 1.24 mM-NaHPO₄, 1.0 mM-

CaCl₂) containing 2 mM-Hepes/NaOH (pH 7.4) and 0.3 µg of chloramphenicol/ml. The medium was supplemented with glucose (chick muscle 15 mM; rat muscle 5 mM) and insulin (chick 0.1 unit/ml; rat 0.1 unit/ml). Muscles were incubated for 30 min at 36 °C, then transferred to fresh medium of the same composition and incubated for another 3 h. Rates of protein synthesis and degradation, and net degradation were determined as previously described [6,20]. In this approach, the rate of protein synthesis is determined as the rate of incorporation of [³H]phenylalanine into tissue protein. Net protein degradation is determined as the rate of net phenylalanine release into the incubation medium. Protein degradation is calculated as the sum of rates of synthesis and net degradation. Incubated muscles are in negative protein balance, apparently owing to depressed rates of protein synthesis [20]. In our chick muscle preparation used here, this represents the net degradation of only 0.03% of total protein/h, and rates of protein synthesis compare favourably with those observed *in vivo* [20].

PGE₂ production was estimated by radioimmunoassay of the incubation medium as described previously [6]. The cross-reactivity of the anti-PGE₂ antibody (ICN Immunobiologicals) with PGE₁ is 150%, but with PGF_{2α} less than 2%. Since skeletal muscle is not known to synthesize PGE₁ [9], this cross-reactivity would not appear to pose a problem for the determination of PGE₂. The effective range of the assay was 12.5–875 pg/sample.

Data analysis

Results are expressed as means ± standard error of the group mean. The results were analysed with one-way analysis of variance.

RESULTS

Initially, control experiments were carried out to assess the effects of naproxen on growing Sprague–Dawley rats (Table 1). This agent had no significant effect on feed intake, faecal and urinary nitrogen losses, and muscle protein content, when administered for a period of 5 days in the drinking water. In addition, naproxen had no effect on protein synthesis or degradation determined in isolated epitrochlearis muscle (results not shown). In addition, naproxen administered to healthy rats and chicks had no significant effect on growth, feed intake or muscle protein metabolism (see below).

Effect of naproxen administration before infection

In order to test whether prostaglandins might be involved in signalling muscle protein catabolism after infection, animals were infected under conditions of naproxen pretreatment. The data shown in Figs. 1 and 2 and Tables 2–5 are from experiments where naproxen was administered from 24 h before infection.

Whole-body and muscle growth

Healthy young broiler chicks and naproxen-treated control chicks showed rapid rates of growth during the experimental period. After injection of *E. coli*, the growth of infected chicks continued at a low rate, such that the body-weight gain of infected birds was less than that of controls by 59% ($P < 0.05$) on day 2 after injection (Table 2). Rats grew more slowly than chicks, and *E. coli* injection significantly decreased the body weight in rats

over a 2-day interval after infection ($P < 0.05$) (Table 2). The administration of naproxen alone had no effect on the body-weight gain of rats or chicks. The body-weight gain of infected chicks or rats treated with naproxen was significantly improved compared with infected subjects (Table 2).

As shown in Table 3, the weights of two different muscles in the chicks were decreased on day 2 after infection. The weights of EDC and sartorius muscles were depressed by 22.6% and 29.3% ($P < 0.05$) (Table 3); at the same time body weight was decreased by 17% (Table 2). In the rats, *E. coli* injection decreased muscle weight by 11.2% (NS) in soleus and by 10% ($P < 0.05$) in EDL (Table 3); these changes were similar to the depression in whole body weight (-10.2%). Naproxen alone had no effect on muscle weight; however, naproxen-treated infected subjects had muscle weights which were intermediate between those of control and infected subjects ($P > 0.05$).

Protein content

Table 4 depicts the protein content of muscles shown in Table 3. The total protein content and percentage

protein of the muscles were significantly lower than those of healthy controls in both infected chicks and rats. The changes in total protein contents seen in muscles of infected birds and rats were more dramatic than the changes in muscle weight (Tables 3 and 4). Total muscle protein was depressed by *E. coli* injection by 37.2% ($P < 0.05$) in chick EDC muscle, 53.8% ($P < 0.05$) in chick sartorius, 46.4% ($P < 0.05$) in rat EDL, and 23.7% ($P < 0.05$) in rat soleus. This was associated with a decrease in muscle protein content/g of tissue, which was depressed ($P < 0.05$, $n = 7$) in all muscles tested (EDC -18% , sartorius -36% , soleus -13% , EDL -40%). Naproxen alone had no significant effect on total muscle protein or protein content/g wet wt.

Table 3. Effect of naproxen pretreatment on muscle weight after *E. coli* infection in chicks and rats

Chicks and rats were injected with *E. coli* (3×10^9 colony-forming units/kg body wt.) or saline. Naproxen was given continuously in the drinking water from the day before infection. Muscles were dissected 2 days after infection. ^{abcd} Means within in a column not sharing a common superscript are significantly different, $P < 0.05$ ($n \geq 7$).

Table 1. Effect of naproxen pretreatment on nitrogen balance and muscle protein content in rats

Naproxen was administered in the drinking water (76 mg/l; approx. 1.6 mg/kg body wt. per day). Rats were housed individually in metabolic cages for collection of faeces and urine, and determination of feed intake. The period of observation was 5 days; data shown represent the cumulative 5-day intake and nitrogen loss. There were no significant differences between treatments for any parameter measured ($n = 8$).

Parameter	+ Naproxen	- Naproxen
Feed intake (g)	56 ± 1.4	51 ± 4.4
Faecal N (mg)	457 ± 16	425 ± 22
Urinary N (mg)	1139 ± 62	1071 ± 38
Protein content (mg)		
(a) Soleus muscle	3.21 ± 0.13	3.24 ± 0.14
(b) EDL	4.18 ± 0.17	4.22 ± 0.15

Animal and treatment	Muscle wt. (mg)	
	EDC	Sartorius
Chick		
Control	23.0 ± 0.6 ^a	359.1 ± 16.0 ^a
Infected	17.8 ± 0.8 ^b	253.9 ± 7.1 ^b
Naproxen control	24.6 ± 1.0 ^a	356.8 ± 16.9 ^a
Infected + naproxen	20.3 ± 1.3 ^{ab}	313.4 ± 14.9 ^{ab}
Rat		
Control	75.6 ± 2.7 ^{ad}	100.9 ± 2.8 ^a
Infected	67.1 ± 2.9 ^{ab}	90.8 ± 2.7 ^b
Naproxen control	77.5 ± 2.3 ^{cd}	106.4 ± 1.3 ^a
Infected + naproxen	69.0 ± 1.9 ^{ad}	96.7 ± 2.5 ^{ab}

Table 2. Effect of naproxen pretreatment on body weight in infected chicks and rats

Chicks and rats were injected with *E. coli* (3×10^9 colony-forming units/kg body wt.) or saline. Naproxen was given continuously in the drinking water from the day before infection. All animals were killed at 2 days after infection. ^{abc} Means within in a column not sharing a common superscript are significantly different, $P < 0.05$ ($n \geq 7$).

Animal and treatment	Initial body wt. (g)	Final body wt. (g)	Weight gain (g)	Final/initial body wt.
Chick				
Control	103.1 ± 2.2	141.0 ± 3.2 ^a	37.9 ± 2.0 ^a	1.37 ± 0.02 ^a
Infected	101.9 ± 2.7	117.4 ± 2.4 ^b	15.5 ± 4.2 ^b	1.16 ± 0.04 ^b
Naproxen control	103.4 ± 2.8	143.4 ± 4.2 ^a	40.0 ± 3.0 ^a	1.39 ± 0.03 ^a
Infected + naproxen	102.0 ± 2.2	129.8 ± 3.7 ^{ab}	27.8 ± 1.1 ^c	1.27 ± 0.02 ^{ab}
Rat				
Control	248.9 ± 5.4	251.6 ± 4.5 ^a	2.7 ± 1.16 ^a	1.01 ± 0.004 ^a
Infected	249.4 ± 6.7	225.9 ± 5.9 ^b	-23.5 ± 1.3 ^b	0.91 ± 0.004 ^b
Naproxen control	248.9 ± 4.1	250.0 ± 4.2 ^a	1.1 ± 0.3 ^a	1.00 ± 0.001 ^a
Infected + naproxen	248.9 ± 4.1	237.4 ± 4.7 ^{ab}	-11.5 ± 3.7 ^c	0.95 ± 0.015 ^c

Table 4. Effect of naproxen pretreatment on muscle protein content in *E. coli* infected chicks and rats

Chicks and rats were injected with *E. coli* (3×10^9 colony-forming units/kg body wt.) or saline. Naproxen was given continuously in the drinking water from the day before infection. Muscles were dissected 2 days after infection.

^{abc}Means within a column not sharing a common superscript are significantly different, $P < 0.05$ ($n \geq 7$).

Animal and treatment	Muscle total protein (mg)	
	EDC	Sartorius
Chick		
Control	2.58 ± 0.9^a	20.51 ± 0.97^a
Infected	1.62 ± 0.07^b	9.46 ± 0.61^b
Naproxen control	2.53 ± 0.09^a	21.13 ± 2.11^a
Infected + naproxen	2.01 ± 0.11^c	19.72 ± 1.59^a
	Muscle total protein (mg)	
	EDL	Soleus
Rat		
Control	6.74 ± 0.57^a	5.23 ± 0.12^a
Infected	3.61 ± 0.11^b	3.99 ± 0.08^b
Naproxen control	6.55 ± 0.09^a	5.22 ± 0.15^a
Infected + naproxen	5.38 ± 0.18^a	4.87 ± 0.22^a

However, administration of naproxen to infected rats or chicks increased total muscle protein towards values seen in healthy controls. In chick EDC and sartorius, the loss of protein owing to infection was inhibited by 41 % and 93 % ($P < 0.05$) respectively. In rat EDL and soleus, the

Table 5. Effect of naproxen pretreatment on muscle protein turnover and PGE₂ production during *E. coli* infection

Chicks and rats were injected with *E. coli* (3×10^9 colony-forming units/kg body wt.) or saline. Naproxen was given continuously in the drinking water from the day before infection. Muscles were dissected 2 days after infection. Muscles were incubated in 3.0 ml of Krebs-Ringer bicarbonate medium for 3 h. ND, not determined. ^{ab} Means within a column not sharing a common superscript are significantly different, $P < 0.05$ ($n \geq 7$).

Muscle and treatment	Protein synthesis (nmol of Phe/mg of tissue)	Net protein degradation (nmol of Phe/mg of tissue)	PGE ₂ production (pg/mg of tissue)
Chick EDC			
Control	ND	0.38 ± 0.03^a	55.6 ± 6.8^a
Infected	ND	0.55 ± 0.05^b	138.0 ± 20.4^b
Naproxen control	ND	0.36 ± 0.04^a	71.6 ± 6.9^a
Infected + naproxen	ND	0.43 ± 0.04^a	41.6 ± 5.9^a
Rat epitrochlearis			
Control	0.136 ± 0.006	0.191 ± 0.010^a	21.6 ± 1.2^a
Infected	0.125 ± 0.004	0.257 ± 0.005^b	55.4 ± 4.4^b
Naproxen control	0.128 ± 0.008	0.205 ± 0.012^a	29.8 ± 3.1^a
Infected + naproxen	0.129 ± 0.005	0.197 ± 0.008^a	20.1 ± 2.0^a

Fig. 1. Effect of naproxen pretreatment on feed intake during *E. coli* infection in chicks

Broiler chicks were housed in battery cages, 10–22 birds/pen. Feed intake *ad libitum* for each group was determined daily. Chicks were fed on broiler starter ration containing 23.3 % crude protein and 11700 kJ (2795 kcal) of metabolizable energy/kg. Values given represent averages for a group (pen) of birds. Chicks were injected with *E. coli* (3×10^9 colony-forming units/kg body wt.) or saline. Naproxen was offered in the drinking water from the day before infection. Group: ▨, control; ■, infected; □, naproxen control; ▤, infected + naproxen.

response to infection was inhibited by 57 % and 71 % ($P < 0.05$) respectively. In general a greater degree of inhibition was seen in the oxidative rat soleus and chick sartorius than in the glycolytic EDC and EDL muscles.

PGE₂ production and net protein degradation

Table 5 shows net protein degradation and PGE₂ production in isolated chick EDC muscle and rat epitrochlearis muscle. Infected animals showed increased rates ($P < 0.05$) of net protein degradation (+44.8 % in chicks; +32.6 % in rats) and PGE₂ production (+148 % in chicks; +156 % in rats) on day 2 after injection. Naproxen alone had no significant effect on either basal PGE₂ production or net protein degradation in either chicks or rats. However, naproxen treatment significantly ($P < 0.05$) inhibited PGE₂ production and net protein degradation in muscles from infected chicks, to values seen in healthy controls (Table 5). Neither infection nor naproxen had any detectable effect on protein synthesis in rat epitrochlearis muscle, suggesting that increased rates of protein degradation

Fig. 2. Effect of naproxen pretreatment on feed intake during *E. coli* infection in rats

Rats were housed in individual cages. Feed intake *ad libitum* for each rat was determined daily. Rats were fed on laboratory chow containing 24% crude protein. Means within a day not sharing a common superscript are different ($P < 0.05$; $n > 7$). Rats were injected with *E. coli* (3×10^9 colony-forming units/kg body wt.) or saline. Naproxen was offered in the drinking water from the day before infection.

may be primarily responsible for the net catabolism of muscle in infected rats.

Feed intake

Feed intake of healthy control chicks and rats was constant during 3 days of the study. Feed intake of infected birds was depressed by 36% on day 1 and by 42% on day 2 after infection (Fig. 1). Feed intake of infected rats was depressed by 64% on day 1 and by 48% on day 2 after infection ($P < 0.05$) (Fig. 2). Naproxen pretreatment had no significant effect on feed intake in either healthy or infected chicks or rats.

Effect of naproxen administration after infection

To determine whether naproxen might influence muscle wasting after this process had already been initiated, a series of experiments was conducted where naproxen was administered 6 or 24 h after *E. coli*. When infected chicks were treated with naproxen after infection, the effect of naproxen treatment was qualitatively similar to the effect of naproxen administered after infection (see above). Naproxen treatment after infection improved body weight and muscle tissue weight measured 4 days

Table 6. Effect of naproxen treatment at 24 h after infection on muscle content in *E. coli*-infected chicks

Chicks are injected with *E. coli* (3×10^9 colony-forming units/kg body wt.) or saline. Naproxen was injected intraperitoneally at 12 h after infection; thereafter naproxen was offered in the drinking water. Muscles were dissected 2 days after infection. ^{abc} Means within a column not sharing a common superscript are significantly different, $P < 0.05$ ($n \geq 7$).

Treatment	Muscle total protein (mg)	
	EDC	Sartorius
Control	6.94 ± 0.2^a	86.6 ± 3.9^a
Infected	2.88 ± 0.3^b	29.0 ± 2.4^b
Naproxen control	7.22 ± 0.4^a	73.7 ± 4.3^a
Infected + naproxen	4.00 ± 0.8^b	48.8 ± 7.6^c

after infection. As illustrated in Table 6, naproxen treatment at 24 h after infection increased the protein content of sartorius muscles ($P < 0.05$) and EDC muscles (NS), compared with infected birds.

DISCUSSION

Protein balance of the whole body and of individual tissues is dictated by the relative rates of protein synthesis and degradation. In catabolic states such as infection, many factors have been suggested to activate the net catabolism of skeletal muscle, including circulating hormones, depressed feed intake, inactivity, and factors associated with host defence responses [1]. It remains to be clarified whether, or when, each of these factors is of importance. Our recent work has concentrated on the role of factors originating from mononuclear phagocytes in muscle catabolism, including lymphokines and prostaglandins [5,6]. The present results support the suggestion that prostaglandins might be important in regulation of muscle protein turnover [6,7,10–13,21] and identify their function in two species.

Naproxen is an inhibitor of prostaglandin biosynthesis [18]. In the present study, naproxen administered at a minimum effective dose had no effect on protein balance or muscle protein metabolism in healthy individuals. PGE₂ production by muscles of infected chicks or rats was elevated, as reported by others [7,16]. The administration of naproxen decreased this enhanced PGE₂ production to values seen in healthy controls. Under conditions of naproxen pretreatment, inhibition of PGE₂ production was associated with a significant decrease in body weight loss and muscle protein loss owing to infection. The increased net protein degradation observed in both incubated chick and rat muscles was also abolished by naproxen pretreatment. When naproxen was administered after infection, similar results were observed, although the degree of improvement was less. These results suggest that prostaglandins may be important for the initiation and maintenance of muscle catabolism.

Our results agree with those of Fagan & Goldberg [7], who treated rats with indomethacin before injection of *E. coli* or *E. coli* endotoxin, and observed decreased PGE₂ production and net protein degradation in rat muscle

in vitro. Ruff & Secrist [13] similarly treated rats with indomethacin before injection of *S. pneumoniae*, and prevented the loss of muscle protein and contractile force after infection. The results of the present study and those cited above do not agree with those of Hasselgren *et al.* [14,15]. In their experiments, indomethacin administered *in vitro* partially inhibited PGE₂ production, but did not significantly decrease accelerated protein degradation in muscles of septic rats. Several possible explanations arise for this discrepancy from the present results. The addition of indomethacin (3 μ M) to incubation medium decreased PGE₂ production weakly, by only 20% in controls and by 50% in muscles of septic rats [14]. This is in contrast with our observations [6] and those of Rodemann & Goldberg [11], where indomethacin abolished PGE₂ production *in vitro* at low concentrations (2.8 μ M). Since indomethacin is extremely insoluble, one possibility is that in the studies of Hasselgren *et al.* [14,15] this agent was not solubilized at an effective concentration. In addition, if addition of indomethacin could influence protein degradation only after a time lag of several hours, an effect of indomethacin might not be evident during a 2 h incubation. A further complicating factor is that cycloheximide (0.5 μ M) was used in the muscle incubations *in vitro* by Hasselgren *et al.* [14,15] to inhibit protein synthesis; this allows direct determination of rates of protein degradation. This agent has been found to inhibit muscle PGE₂ production [22]. Since 70% inhibition of PGE₂ production can be demonstrated in the presence of 1 μ M-cycloheximide [22], it seems unlikely that Hasselgren *et al.* [14,15] would have been able to observe prostaglandin-dependent muscle protein catabolism in their system. The same authors also treated rats with indomethacin (3 mg/kg) *in vivo*, 45 min before induction of sepsis and again after 3 h [14]. Since they did not report whether indomethacin had achieved an effective concentration *in vivo*, these results are difficult to interpret. When Fagan & Goldberg [7] treated rats with indomethacin after injection of *E. coli* endotoxin, the increased net proteolysis was not affected, although the augmented PGE₂ production of muscles was clearly abolished. The reason for this discrepancy with our results is not clear; this point requires further investigation.

The involvement of prostaglandins in regulation of muscle protein turnover requires further study. It is not clear whether muscle tissue is the major source of PGE₂ in infected animals, or whether cells such as activated mononuclear phagocytes make a significant contribution. Although the action of PGE₂ on muscle may be direct, inhibition of prostaglandin synthesis systemically might indirectly influence muscle catabolism. For example, naproxen treatment would inhibit fever, and the elevated metabolic energy expenditure associated with it [23]. This would serve to lower the net use of muscle protein as a metabolic fuel. It remains to be clarified which other instances of muscle protein loss might also involve metabolites of arachidonic acid. In related work (A. B. Strelkov, A. L. A. Fields & V. E. Baracos, unpublished work), a naproxen-sensitive component of cancer-induced muscle wasting has been observed. In addition, we have noted that muscle protein loss after traumatic injury may be decreased by administration of naproxen

(B. D. Fisher & V. E. Baracos, unpublished work). Finally, since inhibition of prostaglandin synthesis did not entirely block weight and protein loss associated with infection, further effort is required to determine the underlying basis of the naproxen-insensitive protein degradation. For example, the muscle wasting seen here may be related, to some extent, to the large and rapid fall in voluntary feed intake by infected chicks and rats.

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