

# Exercise in Maintenance Hemodialysis Patients Induces Transcriptional Changes in Genes Favoring Anabolic Muscle

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## ABSTRACT

Exercise training increases exercise capacity of maintenance hemodialysis patients, but the cellular mechanisms responsible for this effect are unclear. We studied the effects of different forms of exercise training (endurance, strength, or a combination where patients underwent about one-half each of the endurance and strength training of the first two groups) on mRNA levels of genes in muscle that may contribute to increased exercise capacity. Before exercise training, muscle mRNA of insulin-like growth factor (IGF)-IEa, IGF-I receptor, IGF-II, IGF-binding protein (IGFBP)-2, and IGFBP-3 were lower in hemodialysis patients than normal controls. After approximately 21 weeks of exercise training, muscle mRNA increased significantly for all of these genes, as well as for IGF-IEc. Muscle mRNA for myostatin, a protein that inhibits skeletal muscle protein synthesis and muscle hypertrophy, decreased. The responses of mRNA to the different types of exercise training generally showed similar significant changes or trends. Muscle IGF-I protein also increased with exercise. Anthropometry, but not dual energy x-ray absorptiometry or bioelectrical impedance, showed a decrease in body fat and an increase in fat-free mass. In conclusion, exercise training in hemodialysis patients induces changes in skeletal muscle mRNA and increases muscle IGF-I protein, which may promote protein anabolism.

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Maintenance hemodialysis (MHD) patients frequently describe malaise and display muscle weakness, sarcopenia, and intolerance to all but the mildest forms of exercise.<sup>1–6</sup> These factors can contribute to the impaired rehabilitation and constricted lifestyle and quality of life of these individuals.<sup>1,3–5</sup> Many studies indicate that exercise training by MHD patients improves their exercise capacity.<sup>1–6</sup> In healthy humans and mammals, improvement in exercise capacity is associated with the expression in skeletal muscle of many proteins, including those involved with increased enzyme activity and myosin heavy chains.<sup>7–10</sup> There are increased quantities of growth factor proteins that promote synthesis or suppress degradation of proteins. There are almost no studies concerning the

cellular mechanisms that are responsible for improved exercise capacity in exercising MHD patients.

This study was undertaken in MHD patients to examine, in right vastus lateralis muscle, the effects of various types of exercise training on mRNA levels for various growth factors that stimulate or sup-

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press protein synthesis or inhibit protein degradation. Normal sedentary adults served as control subjects.

## RESULTS

Eighty MHD patients completed baseline testing, including a muscle biopsy, and then underwent randomization to one of the four groups: Endurance training (ET), strength training (ST), endurance plus strength training (EST) or no training (NT). Seventeen patients (six ET, two ST, five EST, and four NT) dropped out because of illness, noncompliance, moving away, or possibly reluctance to undergo another muscle biopsy. Of these, two ET, one ST, and three EST patients dropped out before (five patients) or only after 1 wk of exercise training. As a result of a laboratory accident, one or more of the muscle biopsy specimens from 12 patients were destroyed. This left data from 51 patients who completed the study.

Characteristics of the 51 MHD patients and 20 normal control subjects are shown in Tables 1 and 2. MHD patients exercised for  $21.5 \pm 0.7$  wk. No patient had insulin-dependent diabetes. The normal individuals had normal serum creatinine levels, and none had a history of kidney disease or hypertension. Mean age and serum albumin were similar in the two groups; hemoglobin and hematocrit were only slightly lower in the MHD patients, which probably reflects the effects of erythropoietin treatment.

During the course of exercise training, the ET patients increased the duration of their ET exercises from  $18 \pm 1$  to  $35 \pm 3$  min, an increase of 96%. Mean change in work rate was  $16 \pm 9$  W (35% increase). The product of these data provides a measure of the total work completed. At baseline (week 1 of training), these individuals completed  $155 \pm 28$  kJ of work. At the end of training, defined as the last week completed, these individuals completed  $330 \pm 73$  kJ work, an improvement of 113% in leg-cycling work. The ST patients increased the total weight lifted per week from  $4001 \pm 660$  kg at week 1 to  $15,463 \pm 2073$  kg during the last week of training for a 286% increase in total training volume (kg lifted per repetition  $\times$  repetitions  $\times$  sets). By comparison, the EST patients, between the first and last week of training, increased the duration of their ET exercises from  $10 \pm 0$  to  $18 \pm 2$  min (80%), the work rate from  $42 \pm 12$  to  $72 \pm 16$  watts (71%), and the total work from  $103 \pm 53$  to  $245 \pm 63$  kJ (138%). These individuals also increased the total weight lifted from  $2976 \pm 1069$  kg during week 1 to  $7423 \pm 1607$  kg during the final week, a 149% rise. It should be noted that these data are based on sample sizes of six, seven, and five individuals for the ET, ST, and EST groups, respectively. Regrettably, training data files of other patients are not available.

Serum C-reactive protein (CRP), TNF- $\alpha$ , and IL-6 concentrations in the MHD patients did not change with exercise training or in the NT group (Table 2). Serum CRP and IL-6, obtained from all four pre-exercise MHD groups combined, did not differ from the initially obtained normal values. Pre-exercise serum TNF- $\alpha$  was significantly greater than normal

**Table 1.** Characteristics of MHD patients and normal volunteers

Characteristic	MHD Exercisers			MHD Nonexercisers	Normal Control Subjects
	ET	ST	EST		
No. of participants	10	15	12	14	20
Gender (M/F)	6/4	9/6	7/5	9/5	15/5
Race/ethnicity					
black	6	5	5	4	6
Hispanic	2	9	7	5	10
other <sup>a</sup>	2	1	0	5	4
Age (yr; mean $\pm$ SEM)	$45.9 \pm 4.1$	$46.0 \pm 2.7$	$42.7 \pm 3.8$	$41.3 \pm 3.3$	$42.2 \pm 2.6$
Duration of MHD (mo; mean $\pm$ SEM [range])	$45.9 \pm 14.1$ (6 to 129)	$51.9 \pm 12.4$ (6 to 187)	$38.3 \pm 5.8$ (15 to 81)	$51.4 \pm 21.0$ (6 to 296)	—
Duration of exercise training (wk; mean $\pm$ SEM)	$20.7 \pm 0.7$	$21.2 \pm 1.3$	$22.4 \pm 1.3$	—	—
Interval between initial and end-muscle biopsies (wk; mean $\pm$ SEM)	$24.6 \pm 1.1$	$26.1 \pm 1.9$	$24.9 \pm 1.4$	$22.6 \pm 1.2$	$9.6 \pm 0.2$

<sup>a</sup>White and Asian.

**Table 2.** Clinical measurements at baseline and at end of exercise training<sup>a</sup>

Parameter	ET		ST		EST		NT		Normal Control Subjects	
	Before	End	Before	End	Before	End	Before	End	Before	End
Blood										
hemoglobin (g/dl)	11.3 ± 1.3	12.9 ± 0.4	12.9 ± 0.3	12.5 ± 0.2	13.4 ± 0.4	13.0 ± 0.4	13.0 ± 0.4	12.5 ± 0.5	14.1 ± 0.2	13.6 ± 0.5
hematocrit (vol%)	38.2 ± 2.1	39.4 ± 1.1	38.6 ± 0.9	37.4 ± 0.6	40.5 ± 1.0	39.2 ± 1.3	39.1 ± 1.1	37.4 ± 1.3	41.4 ± 0.7	40.2 ± 1.5
Serum										
bicarbonate (mEq/L)	31.3 ± 1.2	30.4 ± 0.7	32.9 ± 0.7	31.8 ± 0.8	29.8 ± 1.1	29.8 ± 0.8	31.4 ± 0.6	32.5 ± 0.6	29.0 ± 0.6	29.0 ± 0.7
albumin (g/dl)	3.7 ± 0.1	3.8 ± 0.1	3.9 ± 0.1	3.8 ± 0.1	3.8 ± 0.1	3.8 ± 0.1	3.9 ± 0.1	3.9 ± 0.1	3.9 ± 0.1	3.8 ± 0.1
CRP (mg/L)	4.5 ± 1.5	2.5 ± 0.6	3.5 ± 0.8	4.2 ± 1.3	4.6 ± 1.4	5.8 ± 2.1	2.1 ± 0.4	2.8 ± 0.8	2.5 ± 0.7	3.6 ± 1.4
TNF-α (pg/ml)	3.8 ± 0.5	4.0 ± 0.4	3.6 ± 0.3	4.0 ± 0.5	3.8 ± 0.5	3.9 ± 0.5	3.3 ± 0.2	3.3 ± 0.3	1.5 ± 0.3 <sup>b</sup>	1.3 ± 0.2
IL-6 (pg/ml)	6.6 ± 1.7	3.9 ± 0.7	5.7 ± 1.3	4.7 ± 1.2	4.5 ± 0.9	4.3 ± 0.5	3.8 ± 0.8	3.6 ± 0.8	3.7 ± 0.6	4.6 ± 0.9
Kt/V <sub>urea</sub>	1.60 ± 0.08	1.85 ± 0.25	1.55 ± 0.12	1.37 ± 0.10	1.65 ± 0.11	1.56 ± 0.18	1.64 ± 0.04	1.77 ± 0.09	—	—
DPI (g/d)	78.0 ± 9.8	66.9 ± 6.9	74.1 ± 8.6 <sup>e</sup>	70.1 ± 10.5	79.8 ± 10.4	70.7 ± 5.7	69.3 ± 6.0 <sup>f</sup>	64.7 ± 5.8	95.9 ± 11.1 <sup>c</sup>	—
DEI (kcal/d)	1993 ± 245	1660 ± 152	1717 ± 257 <sup>e</sup>	1719 ± 287	1824 ± 175	1887 ± 150	1629 ± 172 <sup>f</sup>	1646 ± 134	2370 ± 171 <sup>d</sup>	—
DPI (g/kg per d)	1.03 ± 0.11	0.91 ± 0.10	1.12 ± 0.18 <sup>e</sup>	1.08 ± 0.22	1.18 ± 0.20	1.04 ± 0.11	1.04 ± 0.08 <sup>f</sup>	0.94 ± 0.07	1.35 ± 0.16	—
DEI (kcal/kg per d)	26.2 ± 3.0	22.4 ± 1.9	25.8 ± 5.0 <sup>e</sup>	26.9 ± 6.0	26.5 ± 3.2	27.2 ± 2.2	24.5 ± 2.4 <sup>f</sup>	24.4 ± 2.1	32.9 ± 2.2 <sup>c</sup>	—

<sup>a</sup>Data are means ± SEM. There were no significant differences between pre- and postexercise values for any outcome measure. DEI, dietary energy intake; DPI, dietary protein intake.<sup>b</sup>Significantly different from the pre-exercise values in the four groups of MHD patients combined: \**P* < 0.001, \**P* < 0.05, \**P* < 0.01.<sup>c</sup>Data from only 13 patients were available for this calculation.<sup>d</sup>Data from only 12 patients were available for this calculation.

(*P* < 0.001). Dietary energy and protein intakes, before exercise, expressed in kcal/d or g/d or kcal/kg per d or g/kg per d, were significantly lower for the four groups combined (*P* < 0.05 to 0.01; Table 2) except for protein intake, expressed in g/kg per d. Dietary energy or protein intakes did not change with exercise training in any group or in the three exercising groups combined.

Skeletal muscle IGF-IEa mRNA in the four groups of MHD patients, before the onset of exercise training, was significantly lower than in the normal control subjects (Table 3). With exercise training, muscle IGF-IEa mRNA tended to increase in all groups of MHD patients, and this increase was significant in the ST and EST groups and in all three exercising MHD groups combined (Table 3). Skeletal muscle IGF-IEc mRNA also tended to be lower than normal in the MHD groups, although these values were not significantly different (Table 3). With exercise training, IGF-IEc tended to increase in all three groups, and this increase was significant in the EST group and in all three exercising groups combined (Table 3). The IGF-I receptor (IGF-IR) mRNA before exercise training in the four MHD groups combined was significantly lower than in the normal group. By the end of exercising, IGF-IR mRNA increased significantly in the three exercising groups combined.

IGF-II mRNA in the MHD patients before exercise training was significantly lower than the normal values (Table 3). By the end of exercise training, IGF-II mRNA had risen significantly in the EST group as well as in the three exercising groups combined. Before starting exercise training, IGF-IIR mRNA was not different from normal in the combined four groups of MHD patients. IGF-IIR mRNA did not change significantly with exercise training in any group. At the end of training, IGF-IIR levels were not different from normal in any group.

IGF binding protein-2 (IGFBP-2) mRNA in the MHD patients, before commencing exercising, was lower than normal (Table 3). With exercise training, IGFBP-2 mRNA increased significantly only in the ET group and in all exercising groups combined. IGFBP-3 mRNA in the MHD patients, before exercising, was not significantly lower than normal values. With exercise training, IGFBP-3 mRNA rose significantly in the three groups of exercising patients combined.

Before commencement of exercise training, there were no significant differences in the myostatin mRNA levels between the four groups of MHD patients combined and the normal control subjects (Table 3). After exercise training, there was some decrease in myostatin mRNA in each of the three exercising groups (ET 22%; ST 23%; EST 39%) and some increase in the NT group (29%). This decrease was statistically significant (*P* < 0.05) for all three groups combined. The fall in myostatin mRNA in the ET, ST, and EST groups combined was also significantly different from the change in the NT group (*P* < 0.05).

In the 29 MHD patients who had mid-training muscle biopsies, mRNA was also assayed for IGF-IEa, IGF-IEc, IGF-IR, IGF-II, IGF-IIR, IGFBP-2, IGFBP-3, and myostatin. In general, the same changes in mRNA levels present in the final

**Table 3.** Effect of various of exercise regimens on mRNA levels of various growth factors at baseline and at the end of exercise training<sup>a</sup>

mRNA	ET (n = 10)	ST (n = 15)	EST (n = 12)	NT (n = 14)	ET + ST + EST (n = 37)	Normal Control Subjects (n = 20)
Before IGF-IEa $\times 10^4$	16.4 $\pm$ 3.3	17.5 $\pm$ 2.4	14.9 $\pm$ 2.9	18.5 $\pm$ 3.1	16.3 $\pm$ 1.6	23.2 $\pm$ 2.0 <sup>b</sup>
End IGF-IEa	23.6 $\pm$ 4.7	28.1 $\pm$ 4.7 <sup>c</sup>	30.1 $\pm$ 5.5 <sup>d</sup>	20.8 $\pm$ 3.4	27.6 $\pm$ 2.8 <sup>e</sup>	27.9 $\pm$ 2.6
Before IGF-IEc $\times 10^{5f}$	3.3 (1.7, 8.4)	5.0 (2.8, 10.0)	3.8 (2.2, 4.8)	5.6 (3.3, 10.7)	4.2 (1.9, 6.9)	5.7 (3.6, 13.4)
End IGF-IEc	8.5 (5.3, 26.4)	8.4 (3.8, 20.4)	9.9 (6.8, 25.0) <sup>d</sup>	6.5 (3.0, 18.0)	8.6 (4.5, 22.4) <sup>e</sup>	9.2 (5.1, 19.3)
Before IGF-IR $\times 10^4$	8.3 $\pm$ 1.4	10.8 $\pm$ 1.1	9.7 $\pm$ 1.4	12.4 $\pm$ 1.3	9.8 $\pm$ 0.7	14.4 $\pm$ 1.4 <sup>g</sup>
End IGF-IR	8.9 $\pm$ 1.4	12.5 $\pm$ 1.5	13.8 $\pm$ 1.3	11.0 $\pm$ 1.6	11.9 $\pm$ 0.9 <sup>c</sup>	14.8 $\pm$ 0.8
Before IGF-II $\times 10^3$	6.7 $\pm$ 1.8	8.3 $\pm$ 1.4	7.5 $\pm$ 1.5	8.9 $\pm$ 1.9	7.6 $\pm$ 0.9	12.9 $\pm$ 1.6 <sup>g</sup>
End IGF-II	13.9 $\pm$ 4.7	13.9 $\pm$ 3.2	16.0 $\pm$ 3.1 <sup>c</sup>	10.4 $\pm$ 1.8	14.6 $\pm$ 2.0 <sup>e</sup>	13.8 $\pm$ 1.6
Before IGF-IIR $\times 10^2$	2.0 $\pm$ 0.2	2.8 $\pm$ 0.3	2.5 $\pm$ 0.5	2.8 $\pm$ 0.3	2.5 $\pm$ 0.2	3.0 $\pm$ 0.3
End IGF-IIR	2.5 $\pm$ 0.4	3.0 $\pm$ 0.3	3.5 $\pm$ 0.5	3.0 $\pm$ 0.3	3.0 $\pm$ 0.2	3.1 $\pm$ 0.3
Before IGFBP-2 $\times 10^2$	2.2 $\pm$ 0.6	3.1 $\pm$ 0.6	3.2 $\pm$ 0.7	3.0 $\pm$ 0.5	2.9 $\pm$ 0.4	4.4 $\pm$ 0.5 <sup>b</sup>
End IGFBP-2	5.1 $\pm$ 1.8 <sup>c</sup>	4.8 $\pm$ 1.0	5.3 $\pm$ 1.1	2.9 $\pm$ 0.5	5.1 $\pm$ 0.7 <sup>d</sup>	4.4 $\pm$ 0.5
Before IGFBP-3 $\times 10^4$	13.9 $\pm$ 2.5	16.8 $\pm$ 2.1	20.2 $\pm$ 3.8	19.7 $\pm$ 2.7	17.1 $\pm$ 1.6	21.6 $\pm$ 2.1
End IGFBP-3	18.4 $\pm$ 2.1	23.8 $\pm$ 3.7	25.1 $\pm$ 3.9	18.1 $\pm$ 2.2	22.7 $\pm$ 2.0 <sup>c</sup>	24.3 $\pm$ 2.8
Before myostatin $\times 10^4$	22.3 $\pm$ 4.4	19.4 $\pm$ 4.7	18.8 $\pm$ 3.7	15.0 $\pm$ 3.6	19.1 $\pm$ 2.3	18.4 $\pm$ 1.5
End myostatin	17.4 $\pm$ 4.4	15.0 $\pm$ 3.3	11.5 $\pm$ 2.4	19.4 $\pm$ 3.6	13.8 $\pm$ 1.7 <sup>c</sup>	20.9 $\pm$ 1.7

<sup>a</sup>Data are means  $\pm$  SEM unless otherwise stated. ET, ST, and EST patients had muscle biopsies before commencing exercise training and the day after the last exercise training day. NT patients had biopsies separated by the same interval of time but with no exercise training carried out between the initial and final biopsies. Normal control subjects did not undergo exercise training and had two biopsies separated by an interval of 9.6  $\pm$  0.2 wk.

Significantly different from the corresponding pre-exercise (before) values: <sup>c</sup> $P < 0.05$ , <sup>d</sup> $P < 0.01$ , <sup>e</sup> $P < 0.001$ .

Significantly different from the corresponding pre-exercise values in the four groups of MHD patients combined: <sup>b</sup> $P < 0.05$ , <sup>g</sup> $P < 0.01$ .

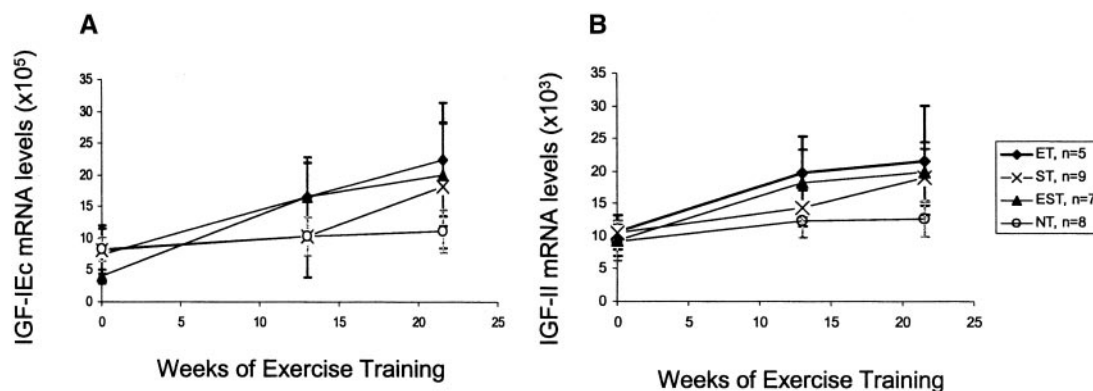
<sup>f</sup>Only the distribution of IGF-IEc values was not parametric. This was observed before exercise training in the ET and normal groups and at end of training in the ET, EST, and NT groups. Hence, the median values and 25th and 75th percentiles for IGF-IEc are shown for all groups.

muscle specimens were already present as trends in the mid-training biopsies (data shown only in Figure 1). By coincidence, the mean IGF-IEc values in the patients who underwent three muscle biopsies tended to be greater than in the patients who underwent two biopsies. We are unable to identify a cause for this discrepancy (e.g., there were no differences between the two groups with regard to age, gender, presence or absence of diabetes, or proportions of patients undergoing each type of exercise intervention).

Serum IGF-I concentrations before exercise training were not different than in the normal group (Table 4). Serum IGF-I did not change significantly during exercise in any of the exercise training groups. Before exercise training, serum IGF-II was

significantly greater than normal values in all four groups of MHD patients combined. Serum IGF-II levels did not change in any group after exercise training. Muscle IGF-I was greater than normal in the four groups of MHD patients combined (Table 4). Muscle IGF-I rose significantly with exercise training in the ET and ST groups and in the three exercising groups combined and by a borderline level of significance ( $P = 0.07$ ) in the EST group (Table 4). Muscle IGF-II did not differ from the normal values in the MHD patients, before training, and did not change in any group of MHD patients during the study.

Possible gender differences in the response of the gene transcripts to exercise were examined. When all three groups of exercising MHD patients combined were analyzed together,

**Figure 1.** Time course of IGF-IEc mRNA levels (A) and IGF-II mRNA levels (B) in right vastus lateralis muscle of MHD patients undergoing various types of exercise training or no exercise training. Lines indicate mean values for each group of MHD patients.

**Table 4.** Effect of various exercise regimens on serum and skeletal muscle protein levels of IGF-I and IGF-II at baseline and at the end of exercise training<sup>a</sup>

Protein	ET (n = 10)	ST (n = 15)	EST (n = 12)	NT (n = 14)	ET + ST + EST (n = 37)	Normal Control Subjects (n = 20)
Serum (ng/ml)						
before IGF-I	197.7 ± 39.8	248.6 ± 25.5	251.9 ± 26.3	233.4 ± 23.8	235.9 ± 17.1	198.4 ± 14.4
end IGF-I	191.7 ± 30.0	196.2 ± 25.5	242.8 ± 42.1	231.4 ± 25.4	210.6 ± 19.0	228.7 ± 23.1
before IGF-II	912 ± 98	1070 ± 73	1192 ± 93	1149 ± 64	1067.1 ± 51.5	812.7 ± 47.2 <sup>b</sup>
end IGF-II	987 ± 91	1039 ± 103	1073 ± 92	1133 ± 78	1036.9 ± 57.5	708 ± 42
Skeletal muscle						
before IGF-I (pg/mg wt wet wt)	117.9 ± 24.7	115.1 ± 14.0	125.8 ± 9.7	152.0 ± 17.1	119.3 ± 8.0	95.1 ± 6.5 <sup>c</sup>
end IGF-I (pg/mg wt wt wet wt)	167.6 ± 17.1 <sup>d</sup>	161.5 ± 12.5 <sup>d</sup>	166.2 ± 15.2	145.4 ± 17.4	164.7 ± 8.0 <sup>e</sup>	110.5 ± 14.9
before IGF-II (ng/mg wt wet wt)	8.0 ± 0.4	7.4 ± 0.4	8.0 ± 0.5	8.2 ± 0.4	7.8 ± 0.2	7.7 ± 0.3
end IGF-II (ng/mg wt wt wet wt)	6.7 ± 0.7	7.9 ± 0.3	7.8 ± 0.5	8.2 ± 0.4	7.5 ± 0.3	7.4 ± 0.3

<sup>a</sup>Data are means ± SEM.Significantly different from the corresponding pre-exercise values in the four groups of MHD patients combined: <sup>c</sup>*P* < 0.05, <sup>b</sup>*P* < 0.001.Significantly different from the corresponding pre-exercise (before) values: <sup>d</sup>*P* < 0.05, <sup>e</sup>*P* < 0.001.

the men, as compared with women, displayed a significantly greater increase in muscle mRNA for IGF-IEc and IGFBP-3 (*P* < 0.05 for each comparison) during exercise training. The changes in the muscle gene transcripts for IGF-IEa, IGF-IR, IGF-II, and IGFBP-2, although not statistically significant in the men *versus* the women, tended to be greater in the men, whereas the fall in mRNA for the antigrowth factor myostatin, which again was not statistically significant in men *versus* women, tended to be substantially greater in women. There was a trend in the NT female MHD patients for the mRNA levels to increase more between the first and last muscle biopsies for IGF-IEa, IGF-II, and myostatin. IGF-IR mRNA increased to a statistically significantly greater degree (*P* < 0.05) in the NT female *versus* male MHD patients. IGFBP-3 mRNA tended to fall more in these NT women.

No MHD patient or normal individual manifested edema. There were no significant differences in the pre-exercise anthropometric measurements in the four MHD patients combined *versus* the normal control subjects when the two genders were considered together. In the male patients considered separately, the same anthropometric similarities and differences were found except for a lower proximal thigh muscle area in the four MHD groups combined at baseline ( $187.7 \pm 7.5 \text{ cm}^2$ ) as compared with the normal men ( $215.3 \pm 11.2 \text{ cm}^2$ ; *P* = 0.044). The number of female patients studied was considered to be too small for separate statistical comparisons. After exercise training, there was a significant decrease in triceps and subscapular skinfold thicknesses in all three exercise groups combined (Table 5). Most striking, after exercise, anthropometry-derived percentage of body fat and total body fat decreased and fat-free mass (FFM) increased significantly in all exercising patients combined.

The dual-energy x-ray absorptiometry (DEXA) measure-

ments showed that in the total group of MHD patients at baseline, there was a decrease in FFM and total mass in the left leg, right leg, and both legs combined and in bone mineral concentration as compared with the normal control subjects (Table 6). With exercise training in the three exercising MHD groups combined, there was a significant decrease in bone mineral concentration (Table 6). Bioelectrical impedance measurements showed no difference in total body FFM or fat mass in the combined four groups of MHD patients, before exercise, as compared with the normal values (Table 7). There also was no change in total body FFM or fat mass, expressed as kilograms of weight or as percentage of total body mass, between the pre- and end of study measurements in the ET, ST, EST, or NT groups or in the three exercising groups combined (Table 7).

When the 43 MHD patients who did not have diabetes were analyzed separately, the identical muscle mRNA, before exercise training, were found to be significantly different from the control subjects, as was observed for the entire group of 51 MHD patients with and without diabetes. Eight MHD patients had non-insulin-dependent diabetes; they all were assigned to one of the three exercising groups. In general, they showed the same trends in mRNA levels as the MHD patients without diabetes, except that the patients with diabetes, in comparison with normal individuals (none of whom had diabetes), had significantly lower pre-exercise IGF-IIR and myostatin mRNA and higher postexercise IGF-IEc mRNA. In the MHD patients with diabetes as compared with the MHD patients without diabetes, muscle IGF-II mRNA before exercise training was higher ( $13.4 \pm 1.7$  *versus*  $6.9 \pm 0.8$ ; *P* = 0.003) and myostatin mRNA was lower (median [25th and 75th percentiles]  $8.2$  [4.2, 12.2] *versus*  $16.4$  [7.5, 33.7]; *P* = 0.049). Muscle IGF-I peptide concentrations in the MHD patients without and with diabetes, before exercising, were  $131.6 \pm 8.7$  and  $110.7 \pm 12.4 \text{ pg/mg}$



**Table 5.** Effect of various exercise regimens on anthropometry of MHD patients at baseline and at the end of exercise training<sup>a</sup>

Parameter	ET (n = 10)	ST (n = 15)	EST (n = 12)	NT (n = 14)	ET + ST + EST (n = 37)	Normal Control Subjects (n = 20)
Before weight (kg)	76.2 ± 5.0	75.2 ± 7.8	71.4 ± 4.8	66.1 ± 3.8	74.2 ± 3.7	72.5 ± 2.7
End weight (kg)	74.8 ± 4.3	75.8 ± 7.8	71.0 ± 4.9	67.7 ± 4.0	74.0 ± 3.6	
Before relative body weight (%)	103.8 ± 5.1	107.0 ± 8.6	103.5 ± 4.9	96.1 ± 4.5	105.0 ± 4.0	98.6 ± 2.6
End relative body weight (%)	102.3 ± 4.9	108.8 ± 8.5	103.6 ± 5.3	97.3 ± 4.6	105.4 ± 4.0	
Before FFM (kg)	52.5 ± 3.5	52.0 ± 4.0	49.6 ± 2.5	47.7 ± 2.3	51.4 ± 2.0	53.6 ± 2.3
End FFM (kg)	53.1 ± 2.9	52.7 ± 3.8	51.6 ± 3.1	48.6 ± 2.2	52.4 ± 1.9 <sup>b</sup>	
Before body fat (%)	30.8 ± 2.3	28.5 ± 2.5	29.4 ± 2.7	27.0 ± 2.2	29.4 ± 1.4	26.2 ± 1.4
End body fat (%)	28.4 ± 2.8	28.0 ± 2.4	26.6 ± 2.5	27.0 ± 2.3	27.7 ± 1.4 <sup>c</sup>	
Before total body fat (kg)	23.7 ± 2.5	23.1 ± 4.3	21.8 ± 3.2	18.4 ± 2.1	22.8 ± 2.1	18.9 ± 1.2
End total body fat (kg)	21.7 ± 2.8	23.1 ± 4.7	19.4 ± 2.9	19.1 ± 2.4	21.5 ± 2.1 <sup>b</sup>	
Before BMI (kg/m <sup>2</sup> )	26.9 ± 1.9	28.7 ± 2.5	26.2 ± 1.5	24.9 ± 1.1	27.4 ± 1.2	24.9 ± 0.6
End BMI (kg/m <sup>2</sup> )	26.6 ± 1.8	27.7 ± 2.5	26.0 ± 1.5	25.1 ± 1.2	26.9 ± 1.2	
Before waist-to-hip ratio	0.89 ± 0.02	0.92 ± 0.02	0.89 ± 0.03	0.90 ± 0.02	0.90 ± 0.01	0.88 ± 0.02
End waist-to-hip ratio	0.88 ± 0.02	0.95 ± 0.02	0.90 ± 0.03	0.89 ± 0.02	0.92 ± 0.01	
Before TSF (mm)	16.1 ± 2.5	15.2 ± 2.5	18.2 ± 2.8	15.7 ± 2.4	16.4 ± 1.5	14.0 ± 1.3
End TSF (mm)	15.6 ± 3.2	13.7 ± 2.5	14.5 ± 2.7	15.1 ± 2.3	14.5 ± 1.6 <sup>b</sup>	
Before BSF (mm)	9.6 ± 1.9	8.5 ± 1.5	11.5 ± 2.7	8.8 ± 1.6	9.8 ± 1.2	7.7 ± 0.9
End BSF (mm)	8.3 ± 1.8	9.4 ± 2.2	9.0 ± 1.7	8.5 ± 1.7	8.9 ± 1.1	
Before SSSF (mm)	20.5 ± 1.8	20.5 ± 3.4	18.1 ± 3.3	17.0 ± 2.3	19.7 ± 1.8	18.2 ± 1.4
End SSSF (mm)	17.9 ± 2.1	19.9 ± 3.3	16.1 ± 2.5	18.1 ± 2.8	18.1 ± 1.6 <sup>b</sup>	
Before mid-calf area (cm <sup>2</sup> )	81.8 ± 5.6	81.1 ± 7.8	83.1 ± 4.2	79.5 ± 4.4	81.9 ± 3.7	87.4 ± 3.3
End mid-calf area (cm <sup>2</sup> )	80.4 ± 5.4	85.5 ± 8.1	84.0 ± 4.4	78.0 ± 5.0	83.7 ± 3.8	
Before mid-arm MA (cm <sup>2</sup> )	67.2 ± 6.3	58.5 ± 7.2	53.9 ± 4.9	52.4 ± 3.1	59.4 ± 3.7	59.2 ± 3.3
End mid-arm MA (cm <sup>2</sup> )	66.7 ± 4.8	60.9 ± 7.0	59.8 ± 5.6	56.3 ± 3.5	62.1 ± 3.6	
Before proximal thigh MA (cm <sup>2</sup> )	200.9 ± 18.5	182.2 ± 11.5	207.7 ± 20.9	183.1 ± 8.8	195.9 ± 9.6	206.2 ± 10.0
End proximal thigh MA (cm <sup>2</sup> )	198.2 ± 11.4	179.7 ± 12.8	196.6 ± 15.8	182.5 ± 9.9	190.5 ± 7.8	
Before mid-thigh MA (cm <sup>2</sup> )	158.6 ± 14.4	144.0 ± 11.9	149.0 ± 8.7	143.1 ± 7.5	149.7 ± 6.6	159.9 ± 6.5
End mid-thigh MA (cm <sup>2</sup> )	157.6 ± 11.0	148.7 ± 14.5	158.3 ± 10.1	144.5 ± 8.1	154.4 ± 7.1	

<sup>a</sup>Data are means ± SEM. There were no significant differences for any outcome measure between the normal values and the pre-exercise measurements in the four groups of MHD patients combined. BMI, body mass index; BSF, biceps skinfold thickness; MA, muscle area; SSSF, subscapular skinfold thickness; TSF, triceps skinfold thickness.

Significantly different from the corresponding pre-exercise (Pre-Ex) values: <sup>b</sup>*P* < 0.05, <sup>c</sup>*P* < 0.01.

wet muscle tissue, respectively (NS). There was no clear association between the results of the postexercise testing and the frequency or duration of hospitalization, possibly because patients were required to continue to exercise train for a minimum of 2 wk after their last hospitalization before undergoing their final exercise testing.

## DISCUSSION

This study indicates that in right vastus lateralis muscle of sedentary MHD patients, there were decreased mRNA levels for a number of factors that may stimulate protein anabolism, suppress protein degradation, and promote accrual of protein. In particular, the mRNA for IGF-IEa, IGF-IR, IGF-II, and IGFBP-2 were decreased. Exercise training was associated with an increase in muscle mRNA for many of these growth factors, namely, IGF-IEa, IGF-IEc, IGF-IR, IGF-II, IGFBP-2, and IGFBP-3. Moreover, myostatin mRNA fell significantly. As might be expected, there was no change in the mRNA for any

growth factor in the NT patients. As a result of these gene transcription changes, most of the mRNA levels were closer to normal at the end of exercise training. The only exceptions were IGFBP-2 mRNA, which after exercise training tended to be greater than normal, and myostatin mRNA, which tended to be less than normal. These findings indicate that in clinically stable, sedentary MHD patients, long-term exercise training induces a pattern of changes in gene transcription that would be likely to promote protein synthesis and reduce protein degradation.

Muscle IGF-I peptide was significantly increased whereas muscle IGF-II protein was normal in the MHD patients before they exercised. The significant further increase in muscle IGF-I protein with exercise in the ET and ST groups and in the three exercising groups combined (Table 4) is consistent with the rise in the skeletal muscle mRNA levels for IGF-IEa and IGF-IEc. These results provide further evidence that exercise training engenders an anabolic response in sedentary MHD patients. The absence of a rise in muscle IGF-II despite the rise in IGF-II mRNA with exercising may reflect posttranscriptional

**Table 6.** Effect of various exercise regimens on DEXA of MHD patients at baseline and at the end of exercise training<sup>a</sup>

Parameter	ET (n = 10)	ST (n = 13) <sup>b</sup>	EST (n = 12)	NT (n = 14)	ET + ST + EST (n = 37)	Normal Control Subjects (n = 20)
Before BMC (kg)	2.37 ± 0.12	2.12 ± 0.10	2.07 ± 0.17	2.14 ± 0.09	2.17 ± 0.08	2.46 ± 0.16 <sup>d</sup>
End BMC (kg)	2.33 ± 0.11	2.09 ± 0.10	2.06 ± 0.17	2.12 ± 0.08	2.15 ± 0.08 <sup>c</sup>	
Before total fat (kg)	21.5 ± 0.34	16.2 ± 0.25	20.0 ± 0.28	16.6 ± 0.22	19.0 ± 0.16	16.7 ± 0.12
End total fat (kg)	20.7 ± 0.34	16.4 ± 0.26	19.3 ± 0.32	17.8 ± 0.24	18.6 ± 0.17	
Before total fat (%)	27.3 ± 3.0	23.5 ± 2.6	28.3 ± 2.6	24.3 ± 2.5	26.2 ± 1.6	23.8 ± 1.6
End total fat (%)	26.8 ± 3.4	23.7 ± 2.6	27.1 ± 2.8	25.1 ± 2.6	25.7 ± 1.6	
Before FFM (kg)	52.1 ± 0.28	47.3 ± 0.26	48.0 ± 0.33	47.7 ± 0.27	48.9 ± 0.17	52.8 ± 0.22
End FFM (kg)	51.4 ± 0.27	47.7 ± 0.27	48.5 ± 0.32	48.4 ± 0.26	49.1 ± 0.16	
Before left leg fat (kg)	3.42 ± 0.7	2.30 ± 0.3	3.07 ± 0.4	2.55 ± 0.3	2.88 ± 0.3	2.69 ± 0.2
End left leg fat (kg)	3.30 ± 0.7	2.36 ± 0.3	2.84 ± 0.4	2.70 ± 0.4	2.79 ± 0.3	
Before left leg FFM (kg)	7.96 ± 0.4	6.96 ± 0.5	7.45 ± 0.5	7.17 ± 0.4	7.41 ± 0.3	8.34 ± 0.4 <sup>d</sup>
End left leg FFM (kg)	7.77 ± 0.4	7.07 ± 0.4	7.68 ± 0.5	7.29 ± 0.4	7.48 ± 0.3	
Before left leg total mass (kg)	11.82 ± 0.9	9.62 ± 0.6	10.88 ± 0.7	10.10 ± 0.6	10.68 ± 0.4	11.50 ± 0.5 <sup>d</sup>
End left leg total mass (kg)	11.49 ± 0.8	9.80 ± 0.6	10.88 ± 0.7	10.36 ± 0.7	10.65 ± 0.4	
Before right leg fat (kg)	3.65 ± 0.8	2.39 ± 0.3	3.15 ± 0.4	2.70 ± 0.3	3.01 ± 0.3	2.92 ± 0.2
End right leg fat (kg)	3.43 ± 0.7	2.48 ± 0.3	3.0 ± 0.5	2.81 ± 0.4	2.93 ± 0.3	
Before right leg FFM (kg)	8.12 ± 0.4	7.08 ± 0.5 <sup>d</sup>	7.64 ± 0.5	7.38 ± 0.5	7.57 ± 0.3	8.53 ± 0.4 <sup>d</sup>
End right leg FFM (kg)	8.01 ± 0.4	7.18 ± 0.5	7.82 ± 0.5	7.44 ± 0.4	7.64 ± 0.3	
Before right leg total mass (kg)	12.22 ± 1.0	9.84 ± 0.6	11.16 ± 0.7	10.43 ± 0.7	10.97 ± 0.5	11.92 ± 0.5 <sup>d</sup>
End right leg total mass (kg)	11.88 ± 0.9	10.03 ± 0.7	11.20 ± 0.8	10.62 ± 0.7	10.96 ± 0.4	
Before both legs fat (kg)	7.08 ± 1.4	4.69 ± 0.6	6.21 ± 0.8	5.22 ± 0.6	5.89 ± 0.6	5.62 ± 0.4
End both legs fat (kg)	6.73 ± 1.4	4.85 ± 0.6	5.85 ± 0.9	5.51 ± 0.8	5.73 ± 0.6	
Before both legs FFM (kg)	16.08 ± 0.8	14.03 ± 0.9	15.09 ± 1.0	14.55 ± 0.9	14.98 ± 0.3	16.86 ± 0.9 <sup>d</sup>
End both legs FFM (kg)	15.78 ± 0.8	14.25 ± 0.9	15.49 ± 1.0	14.72 ± 0.9	15.11 ± 0.5	
Before both legs total mass (kg)	24.04 ± 1.9	19.46 ± 1.2	22.05 ± 1.5	20.53 ± 1.3	22.07 ± 1.0	23.42 ± 1.0 <sup>d</sup>
End both legs total mass (kg)	23.37 ± 1.7	19.83 ± 1.2	22.07 ± 1.5	20.98 ± 1.4	21.61 ± 0.8	

<sup>a</sup>Data are means ± SEM. BMC, bone mineral concentration.<sup>b</sup>Data from two obese people are not available.<sup>c</sup>Significantly different from the corresponding pre-exercise (before) values:  $P < 0.05$ .<sup>d</sup>Significantly different from the corresponding pre-exercise values in the four groups of MHD patients combined:  $P < 0.05$ .**Table 7.** Effect of various exercise regimens on bioelectrical impedance of MHD patients at baseline and at the end of exercise training<sup>a</sup>

Parameter	ET (n = 10)	ST (n = 15)	EST (n = 12)	NT (n = 14)	ET + ST + EST (n = 37)	Normal Control Subjects (n = 20)
Before FFM (kg)	55.1 ± 3.4	53.0 ± 3.3	51.9 ± 2.9	51.1 ± 3.2	53.3 ± 1.9	56.1 ± 2.5
End FFM (kg)	53.3 ± 3.2	53.1 ± 3.5	52.1 ± 3.0	51.7 ± 3.2	52.8 ± 1.9	
Before FFM (%)	73.3 ± 4.2	74.5 ± 3.9	73.8 ± 3.3	77.9 ± 3.1	74.0 ± 2.2	77.2 ± 1.8
End FFM (%)	72.6 ± 4.5	74.1 ± 4.1	74.4 ± 3.5	77.1 ± 2.8	73.8 ± 2.3	
Before fat (kg)	21.2 ± 4.3	22.2 ± 6.2	19.5 ± 3.9	15.0 ± 2.5	21.0 ± 3.0	16.4 ± 1.2
End fat (kg)	21.2 ± 4.4	22.7 ± 6.1	19.0 ± 4.2	16.0 ± 2.4	21.1 ± 3.0	
Before fat (%)	26.7 ± 4.2	25.5 ± 3.9	26.2 ± 3.3	22.1 ± 3.1	26.0 ± 2.2	22.8 ± 1.8
End fat (%)	27.4 ± 4.5	25.9 ± 4.1	25.6 ± 3.5	23.1 ± 2.9	26.2 ± 2.3	

<sup>a</sup>Data are means ± SEM. There are no significant differences for any outcome measure between normal values and the corresponding baseline (before) measurements in all four groups of MHD patients combined. There are no significant differences for any outcome measure between the pre- and postexercise values in any group of exercising MHD patients or in the three groups of MHD patients combined.

or posttranslational factors. It is also possible that the IGF-II protein levels rose transiently during exercise training and then fell back to normal by the time of the biopsy.

Skeletal muscle IGF-I promotes skeletal muscle protein synthesis and hypertrophy, preserves architecture, and suppresses protein degradation.<sup>11</sup> It seems that circulating IGF-I has little effect on muscle hypertrophy,<sup>12</sup> and most skeletal

muscle IGF-I is synthesized *in situ*.<sup>13</sup> In this regard, Nindl *et al.*<sup>14</sup> described a decrease in serum total IGF-I, ternary IGF-I (IGF-I, IGFBP-3, and the acid labile substance), and the IGF-I/IGFBP-3 ratio during resistance training in 10 maintenance dialysis patients. In this study, serum IGF-I was normal and serum IGF-II was elevated before exercising, and neither peptide changed with exercise in our MHD patients (Table 4).

Similar to our findings, MacDonald *et al.*<sup>15</sup> described normal serum IGF-I and IGFBP-3 in 17 MHD patients; however, muscle IGF-I was low in their patients, whereas we observed increased muscle IGF-I that rose further with exercise (Table 4). The low muscle IGF-I may have been due to the wasted state of their patients, as indicated by anthropometry and body composition measurements, whereas our patients did not seem wasted. This discrepancy might be explained by the fact that our MHD patients were a more selected group of healthier individuals because they were required to undergo exercise training in our study.

Three isoforms of IGF-I are recognized in humans: IGF-IEa, IGF-IEb, and IGF-IEc. These isoforms are generated by splice variants of the IGF-I gene.<sup>16</sup> IGF-IEa and IGF-IEc are present in skeletal muscle, whereas IGF-IEb is found primarily in liver and is also detected in muscle. These reductions in mRNA for IGF-IEa, IGF-IR, and IGF-II may predispose to the myofiber atrophy and sarcopenia that are described in MHD patients<sup>17–19</sup> and reduce their ability to remodel muscle protein. Although IGF-IEc mRNA was not significantly reduced in the MHD patients, the same trend toward lower mRNA values was observed for this growth factor as for IGF-IEa mRNA (Table 3). IGF-IEc, also called mechanogrowth factor, is of special interest for exercise training, because it may play a particularly important role in promoting skeletal muscle physical capacity and hypertrophy.<sup>20</sup>

Myostatin is inhibitory to skeletal muscle protein synthesis and muscle hypertrophy.<sup>21</sup> The finding that myostatin fell with exercise training is again consistent with a protein synthetic response to exercise training. These results are consistent with the findings of Sun *et al.*,<sup>22</sup> who described an increase in IGF-I mRNA and a decrease in myostatin mRNA in plantaris muscle of rats with chronic renal failure after the rats were subjected to a chronically increased workload on that muscle.

The dietitian-performed anthropometry indicated that the MHD patients who underwent exercise training experienced a decrease in triceps and subscapular skinfold thicknesses, percentage of body fat, and total body fat mass and an increase in FFM (Table 5). The DEXA and bioelectrical impedance studies showed no changes in body composition, except for reduced bone mineral density with exercise training observed by DEXA. The reason for this discrepancy is not known. Because DEXA is often considered a more reliable measure of body composition and because the DEXA and bioelectrical impedance measurements were in agreement, we conclude that there probably were no changes in FFM or fat mass in these patients. Although no patient or normal individual had evidence of edema, MHD patients cannot regulate their water balance well; therefore, it is possible that undetected increases or reductions in total body water may have obscured our ability to detect the effects of exercise on muscle mass by DEXA or bioelectrical impedance measurements.

The increase in skeletal muscle mRNA levels for growth factors in our exercising MHD patients is similar to those reported with exercising normal humans and various animals<sup>7–10</sup>; however, in normal humans and animals, the type of

training that they undergo (*e.g.*, endurance *versus* strength) influences the specific muscle mRNA levels that increase with exercise,<sup>7–10</sup> and skeletal muscle hypertrophy tends to occur only with resistance training.<sup>7–10</sup>

These distinctions were not observed in our MHD patients. After exercise training, there was a significant increase in mRNA levels for one growth factor each with ET and ST and with three growth factors for EST (Table 3). The mRNA that increased with ET was for IGFBP-2, which might not be considered a growth factor. Moreover, in groups for which the mRNA levels did not change significantly, the mean values for mRNA usually moved in the same direction, as was found for the significant changes in mRNA.

There are several possible explanations for the similar responses in all three groups of exercising MHD patients. First, because of the severe degree of deconditioning and comorbidity of the MHD patients, most patients were not able to exercise vigorously; this may have obscured the ability of the various exercise groups to develop different responses to exercising. Second, the patients undergoing ST or EST might have shown more muscle hypertrophy if their training had been based on a high percentage of the maximum weight exertion performed one time (1-RM) rather than the 5-RM, which reduced their resistance load. We chose this lower resistance technique as a safety measure to reduce the risk for tendon rupture and bone fractures that have been reported in MHD patients who were subjected to high-resistance loads.<sup>6,23,24</sup> Also, for this latter reason and because they were deconditioned and our focus was on developing exercise capacity rather than muscle hypertrophy, the ST and EST patients generally spent their first 8 wk performing less intensive exercise training and increased the intensity of their resistance training slowly. Our objective was more focused on improving strength than muscle hypertrophy *per se*; therefore, we did not use such techniques as multiple sets of multiple exercises with a relatively short rest interval and eight to 12 repetitions to muscle failure, which are more effective at recruiting type IIx fibers (previously identified as type IIb or fast-twitch glycolytic fibers) in human skeletal muscle (see Sale<sup>25</sup> for motor unit recruitment patterns). Similarly, the ET patients, in general, did not exercise very vigorously (see also the Concise Methods section).

Third, many MHD patients seem to have a myopathy that might impair the response to exercise training.<sup>2,26–28</sup> Fourth, there may be metabolic factors in advanced renal failure that inhibit or facilitate certain mRNA responses so that the effects of different types of exercise training are obscured.<sup>29</sup> In this regard, all exercising groups manifested evidence of chronic inflammation as indicated by the significantly elevated serum TNF- $\alpha$  and the NS trend toward raised serum CRP and IL-6.

Finally, because the sample sizes in the various exercising groups were small, there is the possibility of a type B statistical error (see the Concise Methods section). Against this possibility is the finding that the trends toward changes in mRNA levels with exercising were virtually the same in all groups. Indeed, when the responses of all three exercising groups were



pooled, a significantly increased mRNA was observed for every growth factor measured, except for myostatin, a growth inhibitor that decreased significantly, and for the IGF-II receptor, which did not change.

Our results indicated a greater increase in muscle gene transcripts for IGF-IEc and IGFBP-3 in men *versus* women in all three exercise training groups combined. Bamman *et al.*<sup>30</sup> described a greater increase in strength and in myofiber hypertrophy in older men *versus* older women who were undergoing similar resistance (strength) training. These differences could not be attributed to different expressions of IGF-I or myogenin mRNA, to serum IGF-I or dehydroepiandrosterone sulfate concentrations, or to changes in serum testosterone levels.

A limitation of this study is the relatively small numbers of patients in each group, which prevented a rigorous comparison of possible differences in responses to exercise training in the three exercising groups. Also, the uneven number of dropouts in the various groups could reflect a treatment bias; that most of the people who dropped out did so before commencing exercise training or were in the NT group makes this a less likely possibility. Finally, all muscle mRNA and peptide measurements were from the right vastus lateralis muscle, which may not reflect the growth factor mRNA or peptide responses in other skeletal muscles.

The exercise training regimens in this study may have also reduced muscle protein degradation. Muscle mRNA increased for some growth factors that suppress protein degradation as well as stimulate protein synthesis. Such growth factors include IGF-I and IGF-II.<sup>31</sup> In addition, the increased mRNA for the IGF-I receptor or IGFBP might have inhibited protein degradation. In this regard, we have observed a significant reduction in the 14-kD fragment of actomyosin in our patients undergoing ET and EST but not ST or NT.<sup>32</sup> Du *et al.*<sup>33</sup> described this 14-kD fragment as an indicator of the degradation rate of actomyosin protein in skeletal muscle *via* the caspase-3 system.

It is somewhat puzzling that our body composition measurements did not show clear evidence for increased muscle mass with exercise training; however, a rise in muscle mRNA levels and growth factor proteins with exercise training may increase protein synthesis and reduce degradation of selected proteins without engendering muscle hypertrophy. In normal mammals, ET or ST increases the synthesis of many skeletal muscle proteins that are associated with enhanced physical capacity.<sup>9</sup> These changes include increased mitochondria number, particularly of the subsarcolemmal type; vascular endothelial growth factor; capillary density; fibronectin; carnitine palmitoyltransferase; and GLUT4, among others.<sup>7,8,10,34,35</sup> There is a redistribution of myosin heavy-chain isoforms and of the activities of many other enzymes involved with energy generation.<sup>7,8,10</sup> Many of these changes are associated with an increase in skeletal muscle mRNA levels for the respective proteins and also for a number of growth factors, including IGF-I and IGF-II.<sup>7,8,10,34,35</sup> These structural and biochemical changes in skeletal muscle are thought to contribute to the increased

physical exercise capacity that occurs with ET or ST. To our knowledge, this study is the first to show that similar changes in skeletal muscle mRNA in MHD patients for certain growth factors can occur in response to ET and/or ST. It is possible that these changes in skeletal muscle mRNA observed with exercise training in this study may contribute to structural and biochemical changes in muscle that might improve the exercise capacity of MHD patients.

## CONCISE METHODS

### Patients

Inclusion criteria were clinically stable MHD patients who were aged 25 to 65 yr and had been undergoing MHD thrice weekly for at least 6 mo. Exclusion criteria were no history of hospitalization or systemic infection for at least 3 mo; active cancer other than basal cell carcinoma; severe heart, lung, or liver disease; poorly controlled hypertension; acute or chronic inflammatory disease including tuberculosis or acquired immunodeficiency disease; insulin-dependent diabetes; severe osteoporosis, neuropathy, or musculoskeletal disease; amputations involving the lower extremities; or a joint infirmity that would prevent participants from exercising. Healthy adults who were not disqualified by these exclusionary criteria and who were of similar age range, gender distribution, and racial/ethnic mix as the MHD patients served as control subjects.

Only MHD patients and normal control subjects who gave a history of a sedentary lifestyle and no recent regular manual labor, sports activity, or exercise training were studied. The dorsal pedal artery: brachial artery BP ratio was  $\geq 0.90$  in all MHD patients and normal subjects. This study was approved by the Human Subjects Committee at Harbor-UCLA Medical Center; informed written consent was obtained from each participant.

### Exercise Training Regimens

Patients first underwent exercise testing, including an exercise electrocardiogram and a muscle biopsy. They were then randomly assigned to one of four treatment arms: ET, ST, EST, or NT. Exercise training was performed thrice weekly immediately before the onset of a hemodialysis treatment for ST and during the first 60 min of each hemodialysis session for ET. Exercise training was always performed under the direct supervision of an experienced exercise trainer, who operated under the direction of one of the authors (T.W.S.). The planned term of exercise training was 18 wk; exercise was sometimes extended as a result of scheduling problems or because occasionally patients who became ill had their exercise training period extended to allow several weeks of continuous exercise before their postexercise tests were conducted. Normal control subjects underwent baseline testing but did not undergo exercise training. All study participants were strongly encouraged not to perform sports activity or exercise training, other than what was assigned by their treatment arm, and not to increase their physical activity at work.

Exercise was preceded by 5 to 10 min of warmup and stretching. ET was performed with a SCIFIT PRO II Recumbent Cycle Ergometer (Sci-Fit Pro, Tulsa, OK), which maintains a constant work rate even

when pedaling rate changes. During weeks 1 through 4, patients exercised at approximately 50% of their peak oxygen consumption. Some patients exercised only in 4:1 work/rest intervals because of debility. Initial exercise duration, including interval training, was 20 min, which was extended to 30 min as tolerated. Attempts were made to convert interval training to continuous training. During weeks 5 through 8, duration of exercise was advanced to 40 min with the same intensity. During the remainder of training, exercise duration was kept at or progressively increased to 40 min, and work intensity was increased as tolerated.

ST was performed with a Life Fitness leg extension/leg curl combination and a Life Fitness leg press/calf extension combination apparatus (Life Fitness, Schiller Park, IL). During weeks 1 through 4, patients performed one set of each exercise at 12 to 15 repetitions at 70% of the 5-RM,<sup>6</sup> resting 1 min between sets. During weeks 5 through 8, patients performed two sets of this exercise, with resistance increasing as tolerated. During the remaining weeks, patients increased their exercise, as tolerated, to three sets of six to eight repetitions and resistance to 80% of a newly determined 5-RM score. With EST, ET and ST were each conducted for approximately one half of the time but with the same intensity expended by patients undergoing ET or ST and with the same progression of training (*i.e.*, EST patients performed a combination of approximately one half of the ET work effort and also one half of the ST work effort).

### Randomization Procedure

Patients who completed baseline testing were assigned in random order to one of the four exercise groups: ET, ST, EST, or NT. Randomization was performed using a computer-generated set of random numbers stratified by exercise training center and gender and blocked in sets of four.

### Skeletal Muscle Biopsy

Biopsies of the right vastus lateralis muscle were performed as described previously<sup>36</sup> on MHD patients at baseline, before commencing exercise training, and at the end of the study before cessation of exercise training and on nonexercising MHD patients at the same time intervals. After the study began, the protocol was revised so that the last 29 MHD patients underwent an additional muscle biopsy  $13.0 \pm 1.1$  wk after the first biopsy. Muscle biopsies were always performed between 9:00 a.m. and 11:00 a.m. on the day after a hemodialysis treatment in patients who were fasted from 10:00 p.m. the previous night.<sup>36</sup> For the mid- and end-exercise biopsies, patients continued exercise training up through the day before the muscle biopsy was performed. Normal control subjects underwent biopsy twice under identical conditions to the patients, except that they were not receiving hemodialysis or exercising. Individuals were fasted from 10:00 p.m. the night before the biopsy until the end of the procedure.<sup>36</sup>

### Real-Time PCR Amplification of mRNA for IGF-I (the Splice Variants IGF-IEa and IGF-IEc), IGF-II, IGF-IR, IGF-IIR, IGFBP-2, IGFBP-3, and Myostatin

Total RNA was extracted from skeletal muscle and quantified, and first-strand cDNA was synthesized as described previously with Random Primer p[dN]6 and AMV Reverse Transcriptase.<sup>36</sup> Real-time

PCR was performed with the ABI Prism Sequence Detection System 7000, as described previously,<sup>36</sup> to facilitate SYBR-green fluorescence quantification (Applied Biosystems, Foster City, CA). Glyceraldehyde-3-phosphate dehydrogenase mRNA levels were assayed as internal controls. Primers with the following oligonucleotides were developed for IGF-I, IGF-IR, IGF-II, IGF-IIR, IGFBP-2, IGFBP-3, and myostatin as described previously<sup>36</sup> or as follows: IGFBP-2, forward 5'-AGCAGGTTGCAGACAATG-3' and reverse 5'-AGCTCCTTCATACCCGACT-3'; IGFBP-3, forward 5'-GAACCTCTCCTCCGAGTCC-3' and reverse 5'-CTTGTCACAGTTGGGAATGT-3'.

### Protein Assay for Muscle IGF-I and IGF-II

Acid/ethanol extraction was used to separate IGF-I and IGF-II from muscle samples as described previously.<sup>37</sup> The DSL-5600 IRMA and DSL-2600 kits (Diagnostic System Laboratories, Webster, TX) and accompanying protocols were used, respectively, for the IGF-I and IGF-II assays.<sup>36</sup>

### Protein Assay for Serum IGF-I and IGF-II

Acid/ethanol solution (12.5%/87.5% vol/vol) was used to extract serum IGF-I and IGF-II.<sup>37</sup> A Nichols Institute Diagnostics IGF-I 100T kit (San Clemente, CA) was used for the serum IGF-I assay. The serum IGF-II assay followed the same procedure as specified in the DSL-2600 kit.<sup>36</sup>

### CRP, IL-6, and TNF- $\alpha$ Assays

CRP, IL-6, and TNF- $\alpha$  were measured as described previously.<sup>36</sup> The Wako Diagnostics (Richmond, VA) CRP-UL kit was used for the CRP assay. The R&D Systems (Minneapolis, MN) IL-6 and TNF- $\alpha$  kits were used for the IL-6 and TNF- $\alpha$  assays, respectively.

### Nutrient Intake, Clinical Laboratory Measurements, and Anthropometry

Nutrient intake was determined and serum for IGF-I, IGF-II, CRP, IL-6, and TNF- $\alpha$  measurements were obtained at the time of the first and last muscle biopsies. Blood obtained immediately before a mid-week hemodialysis was measured for serum creatinine, bicarbonate, and albumin and for blood hemoglobin and hematocrit in the Davita Central Laboratory. Harbor-UCLA General Clinical Research Center research nutritionists assessed nutrient intake by 3-d dietary diaries and dietary interviews and performed anthropometry as described previously, except that mid-arm muscle circumference was calculated without adjustment for bone mass.<sup>38–40</sup> Proximal-thigh and mid-thigh muscle areas were measured as follows<sup>38,39</sup>:

Thigh muscle circumference = thigh circumference – (3.1416  $\times$  thigh skinfold)

Thigh muscle area

$$= \frac{[\text{thigh circumference} - (3.1416 \times \text{thigh skinfold})]}{4 \times 3.1416}$$

Mid-calf muscle areas were assessed as described previously.<sup>40</sup> Skin-fold thicknesses were measured in the biceps, triceps, and subscapular area, and total body fat was calculated as described previously.<sup>38–41</sup> Lean body mass or FFM was calculated from anthropometry as the

difference between body weight and total body fat. DEXA measurements for assessment of body composition were performed with Dual Energy X-Ray Absorptiometry (Model QDR; Hologic, Bedford, MA).

Bioelectrical impedance was measured with a RJL BIA Quantum instrument (model 101Q; RJL Systems, Mt. Clemens, MI), which applies an 800- $\mu$ A current at a frequency of 50 KHz. Patients reclined on an examination table, with limbs not touching each other. Measurements were made on the side of the body that did not contain a hemodialysis access. The measurements were performed according to the National Institutes of Health.<sup>42</sup> Electrodes were placed between the distal prominences of the radius and ulna, the distal end of the third metacarpal, between the median and lateral malleoli at the ankle, and at the distal end of the third metatarsal. FFM was calculated from the measurements of resistance made at 50 kHz by using previously validated predictive equations as supplied by the manufacturer.<sup>43</sup>

### Power Analysis

The *a priori* power analysis for this clinical trial was based on the heterogeneity of changes in muscle myostatin mRNA in our previous study in MHD patients after they had undergone a mean of 8.9 wk of ET.<sup>28</sup> On the basis of these data, we estimated that in this study there would be at least an 80% power to detect a significant decrease in myostatin mRNA between the NT group and an exercise group if there were at least 17 individuals in each group and if the NT patients have no change in myostatin mRNA and the exercise group has at least a 50% change,

### Statistical Analyses

Statistical analyses to assess differences between pre- and postexercise testing values were performed with repeated measures two-way ANOVA (using groups and time) with the Sigma STAT software (version 3.0; SPSS, Chicago, IL). When statistically significant differences ( $P < 0.05$ ) were detected among all four groups of patients, significant differences for pre- versus postexercise values within individual groups were tested with Tukey test. Significant differences for pre- versus postexercise values for all three groups of exercising MHD patients combined were tested using the paired *t* test. The pre-exercise values in all four groups of patients combined were compared with the measurements in the normal control subjects with a nonpaired *t* test. Data are expressed as the means  $\pm$  SE or, for nonparametric sets, as the median and interquartile (25th and 75th percentiles) range of values. Statistical significance was taken as  $P < 0.05$ .

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### DISCLOSURES

None.

### REFERENCES

- Painter P: The importance of exercise training in rehabilitation of patients with end-stage renal disease. *Am J Kidney Dis* 24[Suppl 1]: S2–S9, 1994
- Kouidi E, Albani M, Natsis K, Megalopoulos A, Gigis P, Guiba-Tziampiri O, Tourkantonis A, Deligiannis A: The effects of exercise training on muscle atrophy in haemodialysis patients. *Nephrol Dial Transplant* 13: 685–699, 1998
- Johansen KL: Physical functioning and exercise capacity in patients on dialysis. *Adv Ren Replace Ther* 6: 141–148, 1999
- Koufaki P, Mercer TH, Naish PF: Effects of exercise training on aerobic and functional capacity of end-stage renal disease patients. *Clin Physiol Funct Imaging* 22: 115–124, 2002
- Mercer TH, Crawford C, Gleeson NP, Naish PF: Low-volume exercise rehabilitation improves functional capacity and self-reported functional status of dialysis patients. *Am J Phys Med Rehabil* 81: 162–167, 2002
- Storer TW, Casaburi R, Sawelson S, Kopple JD: Endurance exercise training during haemodialysis improves strength, power, fatigability and physical performance in maintenance hemodialysis patients. *Nephrol Dial Transplant* 20: 1429–1437, 2005
- Hawley JA: Adaptations of skeletal muscle to prolonged, intense endurance training. *Clin Exp Pharmacol Physiol* 29: 218–222, 2002
- Flück M, Hoppeler H: Molecular basis of skeletal muscle plasticity – from gene to form and function. *Rev Physiol Biochem Pharmacol* 146: 159–216, 2003
- Putman CT, Xu X, Gillies E, MacLean IM, Bell GJ: Effects of strength, endurance and combined training on myosin heavy chain content and fibre-type distribution in humans. *Eur J Appl Physiol* 92: 376–384, 2004
- Booth FW, Baldwin KW: Muscle plasticity: Energy demand and supply processes. In: *Handbook of Physiology*, edited by Rowell LB, Shepherd JT, New York, Oxford University Press, 1996, pp 1075–1123
- Musaro A, McCullagh K, Paul A, Houghton L, Dobrowolny G, Molinaro M, Barton ER, Sweeney HL, Rosenthal N: Localized IGF-I transgene expression sustains hypertrophy and regeneration in senescent skeletal muscle. *Nat Genet* 27: 195–200, 2001
- Sjogren K, Liu JL, Blad K, Skrtic S, Vidal O, Wallenius V, LeRoith D, Törnelli J, Isaksson OG, Jansson JO, Ohlsson C: Liver-derived IGF-I is the principal source of IGF-I in blood but is not required for postnatal body growth in mice. *Proc Natl Acad Sci U S A* 96: 7088–7092, 1999
- Florini J, Ewton D, Magri K: Hormones, growth factors, and myogenic differentiation. *Annu Rev Physiol* 53: 201–216, 1991
- Nindl BC, Headley SA, Tuckow AP, Pandorf CE, Diamandi A, Khosravi MJ, Welles R, Jones M, Germain M: IGF-I system responses during 12 weeks of resistance training in end-stage renal disease patients. *Growth Horm IGF Res* 14: 245–250, 2004
- Macdonald JH, Phanish MK, Marcora SM, Jibani M, Bloodworth LL, Holly JM, Lemmey AB: Muscle insulin-like growth factor status, body composition, and functional capacity in hemodialysis patients. *J Ren Nutr* 14: 248–252, 2004
- Hameed M, Harridge SD, Goldspink G: Sarcopenia and hypertrophy: A role for insulin-like growth factor-I in aged muscle? *Exerc Sport Sci Rev* 30: 15–19, 2002

17. Keshaviah PR, Nolph KD, Moore HL, Prowant B, Emerson PF, Meyer M, Twardowski ZJ, Khanna R, Ponferrada L, Collins A: Lean body mass estimation by creatinine kinetics. *J Am Soc Nephrol* 4: 1475–1485, 1994
18. Woodrow G, Oldroyd B, Turney JH, Tompkins L, Brownjohn AM, Smith MA: Whole body and regional body composition in patients with chronic renal failure. *Nephrol Dial Transplant* 11: 1613–1618, 1996
19. Heimbürger O, Qureshi AR, Blazer WS, Berglund L, Stenvinkel P: Hand-grip muscle strength, lean body mass, and plasma proteins as markers of nutritional status in patients with chronic renal failure close to start of dialysis therapy. *Am J Kidney Dis* 36: 1213–1225, 2000
20. Hameed M, Orrell RW, Cobbold M, Goldspink G, Harridge SD: Expression of IGF-I splice variants in young and old human skeletal muscle after high resistance exercise. *J Physiol* 547: 247–254, 2003
21. McPherron AC, Lee SJ: Double muscling in cattle due to mutation in the myostatin gene. *Proc Natl Acad Sci U S A* 94: 12456–12461, 1997
22. Sun DF, Chen Y, Rabkin R: Work-induced changes in skeletal muscle IGF-I and myostatin gene expression in uremia. *Kidney Int* 70: 453–459, 2006
23. Morein G, Goldschmidt Z, Pauker M, Seelenfreund M, Rosenfeld JB, Fried A: Spontaneous tendon ruptures in patients treated by chronic hemodialysis. *Clin Orthop Relat Res* 124: 209–213, 1977
24. Ryuzaki M, Konishi K, Kasuga A, Kumagai H, Suzuki H, Abe S, Saruta T, Takami H, Tashiro M: Spontaneous rupture of the quadriceps tendon in patients on maintenance hemodialysis: Report of three cases with clinicopathological observations. *Clin Nephrol* 32: 144–148, 1989
25. Sale DG: Influence of exercise and training on motor unit activation. *Exerc Sport Sci Rev* 15: 95–151, 1987
26. Diesel W, Emms M, Knight BK, Noakes TD, Swanepoel CR, van ZylSmit R, Kaschula RO, Sinclair-Smith CC: Morphologic features of the myopathy associated with chronic renal failure. *Am J Kidney Dis* 22: 677–684, 1993
27. Moore GE, Parson BD, Stray-Gunderson J, Painter PL, Brinker KR, Mitchell JH: Uremic myopathy limits aerobic capacity in hemodialysis patients. *Am J Kidney Dis* 22: 277–287, 1993
28. Kopple JD, Cohen AW, Wang H, Fournier M, Qing D, Tang Z, Casaburi R, Lewis M, Storer T: Effect of exercise training on mRNA levels for growth factors in skeletal muscle of hemodialysis patients. *J Ren Nutr* 16: 312–324, 2006
29. Raj DS, Dominic EA, Pai A, Osman F, Morgan M, Pickett G, Shah VO, Ferrando A, Moseley P: Skeletal muscle, cytokines, and oxidative stress in end-stage renal disease. *Kidney Int* 68: 2338–2344, 2005
30. Bamman MM, Hill VJ, Adams GR, Haddad F, Wetzstein CJ, Gower BA, Ahmed A, Hunter GR: Gender differences in resistance-training-induced myofiber hypertrophy among older adults. *J Gerontol A Biol Sci Med Sci* 58: 108–116, 2003
31. Ding H, Kopple JD, Cohen A, Hirschberg R: Recombinant human insulin-like growth factor-I accelerates recovery and reduces catabolism in rats with ischemic acute renal failure. *J Clin Invest* 91: 2281–2287, 1993
32. Workeneh BT, Rondon-Berrios H, Zhang L, Hu Z, Ayehu G, Ferrando A, Kopple JD, Wang H, Storer T, Fournier M, Lee SW, Du J, Mitch WE: Development of a diagnostic method for detecting increased muscle protein degradation in patients with catabolic conditions. *J Am Soc Nephrol* 17: 3233–3239, 2006
33. Du J, Wang X, Miereles C, Bailey JL, Debigare R, Zheng B, Price SR, Mitch WE: Activation of caspase-3 is an initial step triggering accelerated muscle proteolysis in catabolic conditions. *J Clin Invest* 113: 115–123, 2004
34. Prior BM, Yang HT, Terjung RL: What makes vessels grow with exercise training? *J Appl Physiol* 97: 1119–1128, 2004
35. Radom-Aizik S, Hayek S, Shahar I, Rechavi G, Kaminski N, Ben-Dov I: Effects of aerobic training on gene expression in skeletal muscle of elderly Men. *Med Sci Sports Exerc* 1680–1696, 2005
36. Wang H, Casaburi R, Taylor WE, Aboellail H, Storer TW, Kopple JD: Skeletal muscle mRNA for IGF-IEa, IGF-II, and IGF-I receptor is decreased in sedentary chronic hemodialysis patients. *Kidney Int* 68: 352–361, 2005
37. Daughaday E, Rotwein P: Insulin like growth factors I and II. Peptide, messenger ribonucleic acid and gene structure, serum and tissue concentrations. *Endocr Rev* 10: 68–91, 1989
38. Lohman TG, Roche AF, Martorell R, eds.: *Anthropometric Standardization Reference Manual*. Abridged Ed. Champaign, Human Kinetics Books, 1988, pp 47–49, pp 63–65
39. American Dietetic Association: *Manual of Clinical Dietetics*. 6th Ed. Chicago, American Dietetic Association, 2000, p 17
40. Kopple JD, Greene T, Chumlea WC, Hollinger D, Maroni BJ, Merrill D, Scherch LK, Schulman G, Wang SR, Zimmer GS: Relationship between nutritional status and glomerular filtration rate: Results from the MDRD study. *Kidney Int* 57: 1688–1703, 2000
41. Durnin JV, Womersley J: Body fat assessed from total body density and its estimation from skinfold thickness: Measurements on 481 men and women aged from 16 to 72 years. *Br J Nutr* 32: 77–97, 1974
42. Bioelectrical impedance analysis in body composition measurement: National Institutes of Health Technology Assessment Conference Statement. *Am J Clin Nutr* 64[Suppl]: 524S–532S, 1996
43. Kotler DP, Burastero S, Wang J, and Pierson RN: Prediction of body cell mass, FFM, and total body water with bioelectrical impedance analysis: Effects of race, sex, and disease. *Am J Clin Nutr* 64[Suppl]: 489S–497S, 1996