Effects of uremia and inflammation on growth hormone resistance in patients with chronic kidney diseases

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Resistance to the anabolic action of growth hormone may contribute to the loss of strength and muscle mass in adult patients with chronic kidney disease. We tested this hypothesis by infusing growth hormone in patients to levels necessary to saturate hormone receptors. This led to a significant decrease of plasma potassium and amino acid levels in control and hyperkalemic patients with chronic kidney disease. These effects were completely or partially blunted in patients with elevated C-reactive protein levels. In forearm perfusion studies, growth hormone caused a further decrease in the negative potassium and protein balance of hemodialysis patients without inflammation but no effect was seen in patients with inflammation. Only IL-6 levels and age were found to be independent correlates in these growth hormone-induced variations in plasma potassium and blood amino acids. This shows that although a resistance to pharmacologic doses of growth hormone is not a general feature of patients with chronic kidney disease, there is a subgroup characterized by blunted growth hormone action. Our results support the hypothesis that uremia with inflammation, but not uremia per se, inhibits downstream growth hormone signaling contributing to muscle atrophy.

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Loss of muscle with protein–calorie malnutrition is relatively common in patients with chronic renal diseases (CKD) and is strongly associated with a negative clinical outcome.[1–5] Growth hormone (GH) and insulin-like growth factor-I (IGF-I) are potent regulators of anabolism in normal adults.[6,7] Although unproven in humans, an emergent hypothesis is that a resistance to the anabolic drive by GH/IGF-I may contribute to loss of energy, strength, and skeletal and muscle mass in adult patients with CKD.[6,7] Most of the anabolic actions of GH are mediated by IGF-I, which is produced in many different tissues.[8,9] Findings in experimental uremia show that the mechanisms which account for the development of GH resistance include reduced IGF-I synthesis[8,9] sensitivity,[10,11] bioavailability,[12] as well as impaired GH signal transduction.[13] In accordance with these findings, previous elegant clinical studies have shown that the response to IGF-1 is impaired in dialysis patients.[14] However, when IGF-I has been used in malnourished dialysis patients an anabolic effect has been observed.[15] In addition, several clinical studies have reported that GH has a salutary effect on body composition[8,9] and muscle protein synthesis[16–18] in patients with CKD. Nonetheless, the GH response appears to be variable.[19–21] Besides uremia *per se*, the effectiveness of GH might be also attenuated by other factors often found in CKD patients, such as inflammation,[22] metabolic acidosis,[23,24] and low nutrient intake.[23,24] A recent 3-month trial of GH revealed a significant increase in IGF-1 levels and markers of bone turnover, but not lean body and fat mass, in malnourished hemodialysis (HD) patients.[19] Conversely, Kopple *et al.*[20] observed that GH induced a strong and sustained anabolic effect in HD patients with protein–energy malnutrition. The reasons for the diverging clinical effects of GH are not understandable. Identification of variables predicting the response to GH would offer substantial clinical benefits.

Although chronic GH actions are IGF-I mediated, it is now recognized that GH has direct actions via the GH

receptor expressed in many tissues, including muscle.[25] GH can directly stimulate different biological pathways, including lipolysis (by peroxisome proliferator-activated receptor-α signaling),[26] protein synthesis (by the stimulation of mammalian target of rapamycin)[27] and tissue potassium uptake (by enhanced activity of Na/K ATPase pump).[28] However, the effects of GH *per se* on potassium and amino acid (AA) metabolism in CKD patients have never been explored. This study was designed to provide these data and to examine whether the acute anabolic effects of GH can be predicted by clinical or biochemical findings.

Previous studies have shown that GH promotes nitrogen retention and increases lean body mass and accumulation of total body potassium.[7] The present clinical investigation explores the acute GH effects on two selected endpoints of the anabolic GH hormone action, such as AA and potassium metabolism, in patients with advanced CKD and controls. We hypothesized that the GH response is blunted in uremia. We tested this postulate via an interventional design comprising the single administration of recombinant human GH e.v. for 300 min. First, we monitored blood AA levels and plasma potassium, and the GH response was analyzed in relation to several clinical and biochemical parameters in CKD patients. We observed that the GH response was overall preserved, but that a blunted GH effect was present in CKD patients with elevated C-reactive protein (CRP) plasma levels. As a next step, we planned a study on the effects of GH on the forearm balance of AA and potassium in maintenance HD displaying or not an inflammatory response.

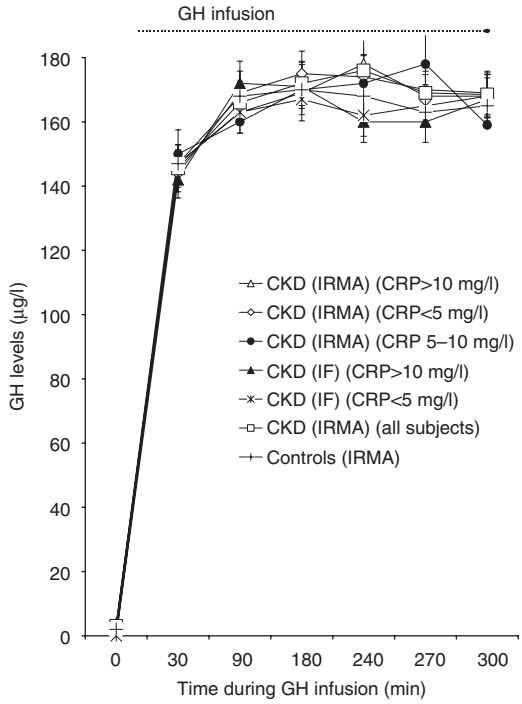

Figure 1 | Time-related increase in plasma growth hormone (GH) levels during the GH infusion in patients and controls. Systemic plasma IRMA and immunofunctional (IF) GH levels were raised to a similar extent in both patients and controls, with steady-state plasma GH concentrations reached after 90 min from the start of the infusion ($P<0.01$ or less vs basal levels).

RESULTS

Baseline and GH-induced changes in hormone and potassium levels

GH infusion raised systemic plasma immunoradiometric assay and immunofunctional levels of GH to a similar extent in both patients and controls (Figure 1), with steady-state plasma GH concentrations reached after 90 min from the start of the infusion (coefficient of variation of GH levels ranging from 3 to 5%). Owing to the short-term GH administration, glucose, lactate, insulin, as well as IGF-I levels were not significantly changed by GH (data not shown). These findings are in accordance with previous observations.[29,30]

In healthy controls, the GH infusion progressively decreased plasma potassium levels from a mean of 3.9 to 3.2 mEq/l (Δ plasma potassium = −0.7 mEq/l at 300 min; Figure 2a and b), with the occurrence of mild hypokalemia for any time beyond 150 min from the start of GH infusion.

Patients with CKD presented an increase in baseline plasma potassium, which ranged from 4.3 to 6.6 mEq/l. The GH infusion decreased plasma potassium from a mean of 5.5 to 4.8 mEq/l at 300 min (Δ plasma potassium = −0.7 mEq/l at 300 min; Figure 2a and b) reaching levels in the upper normal range at any time after 180 min. The overall response of plasma potassium to GH, as well as the estimated change in total extracellular potassium content in CKD patients were similar to controls (area under the curve 87 ± 17 and 90 ± 15 mEq/l for 300 min in patients and controls, respectively, P = NS; Figure 2b). When the subgroups were analyzed in relation to CRP levels, the decline in plasma potassium was similar to controls in CKD patients with CRP levels lower than 5 mg/l (low CRP) or between 6 and 10 mg/l (intermediate CRP) but it was blunted (−0.4 mEq/l at 300 min) in patients with plasma CRP > 10 mg/l (Figure 2b; likelihood ratio test = 12.15; $P<0.001$).

Effects of GH on blood amino-acid levels

Patients studied here presented baseline low arterial blood levels of essential amino acids (EAA) and higher levels of nonessential amino acids (NEAA) as compared to controls ($P<0.05$). Blood levels of branched-chain amino acids (BCAA) (335 ± 18 vs 289 ± 20 μmol/l) were lower (likelihood ratio test = 7.9; $P<0.01$) in the high vs low CRP group. Only as a trend, EAA (789 ± 42 vs 714 ± 63 μmol/l; $P=0.08$) were lower and NEAA higher (1855 ± 120 vs 1955 ± 88 μmol/l; P = NS), in the high vs low CRP group.

In healthy subjects AA levels declined by ∼11% ($P<0.025$) after 240 min from the start of GH infusion. This decline was statistically significant for EAA (−11%; $P<0.05$–0.01; Figure 3), NEAA (−9%; $P<0.05$; data not shown), and BCAA (−11%; $P<0.05$; Figures 3–4).

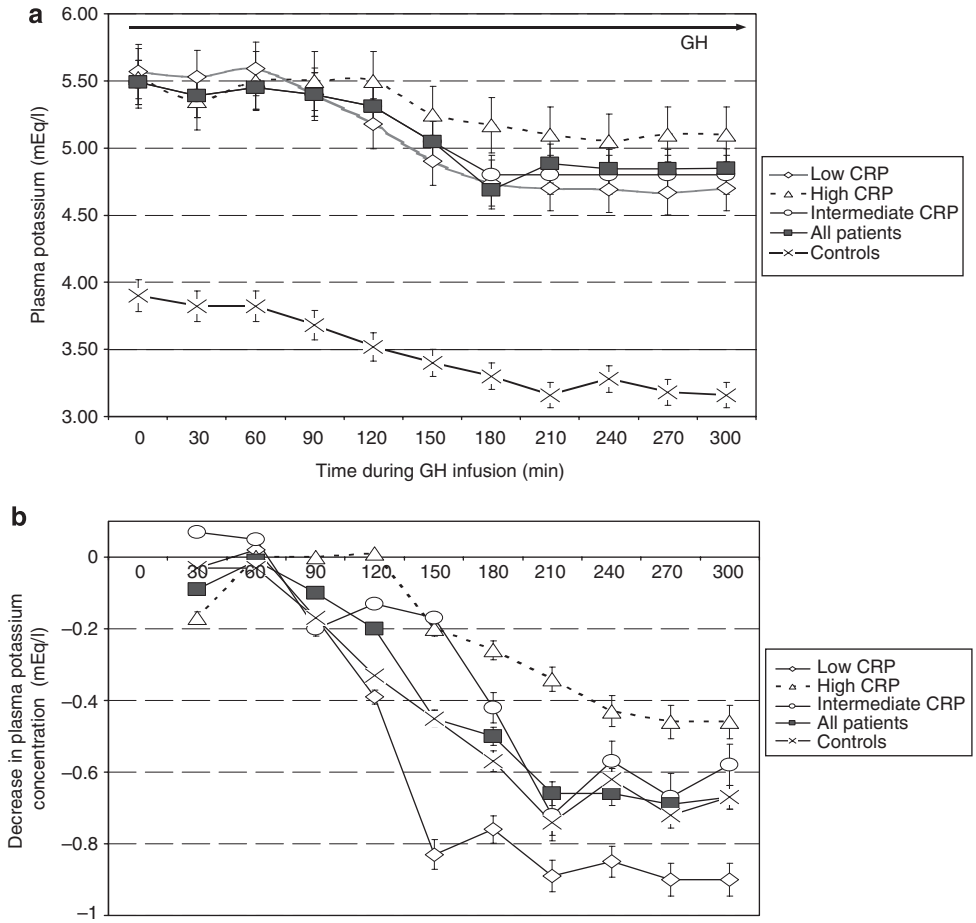

Figure 2 | Time-related decrease in plasma potassium concentration during GH infusion studies. Mean values at each time point are displayed. Similarly to controls, GH progressively decreased plasma potassium in CKD patients (**a**). However, when the subgroups were analyzed in relation to C-reactive protein (CRP) levels, the decline in plasma potassium was similar to controls in CKD patients with CRP levels lower than 5 mg/l (low CRP) or between 6 and 10 mg/l (intermediate CRP), but it was blunted in patients with plasma CRP >10 mg/l (high CRP) ($P<0.001$, in a mixed model analysis taking in account time, CKD and inflammation).

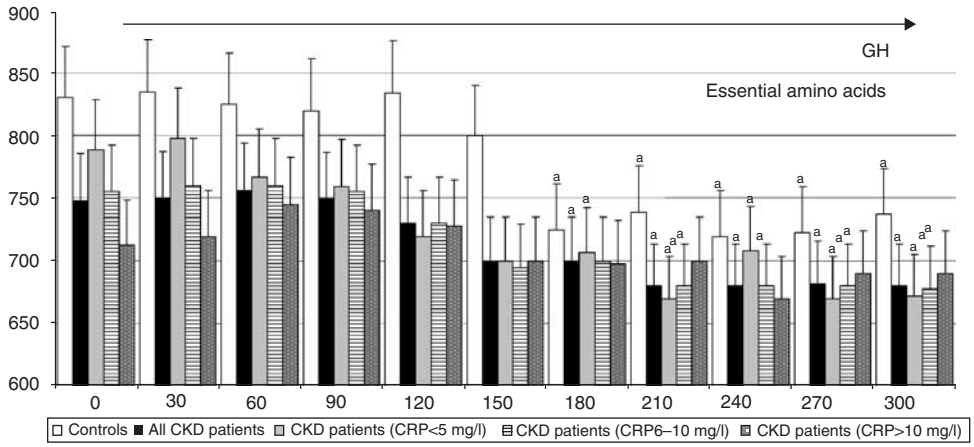

Figure 3 | Effects of GH infusion on blood essential amino acids (EAA) in CKD patients and controls. In healthy subjects EAA levels declined by ~11% after 240 min from the start of GH infusion. In CKD patients, EAA declined by ~9% ($P<0.05$ vs basal). In the subgroup analysis, arterial concentrations of EAA were not influenced by GH in patients with plasma CRP >10 mg/l (high CRP; $P<0.002$, by a mixed model analysis). Statistically different from basal levels: a = $P<0.05$–0.001.

The pattern of the GH-induced AA response in CKD patients pooled together was similar to controls. Overall, blood total AA declined by ~8% ($P<0.01$ vs baseline, $P=$ NS vs controls), NEAA by ~8% ($P<0.01$), and EAA by ~9.4% ($P<0.01$). However, the decrease in blood BCAA (−4%) was at the borderline of statistical significance ($P<0.07$ vs basal; Figure 4).

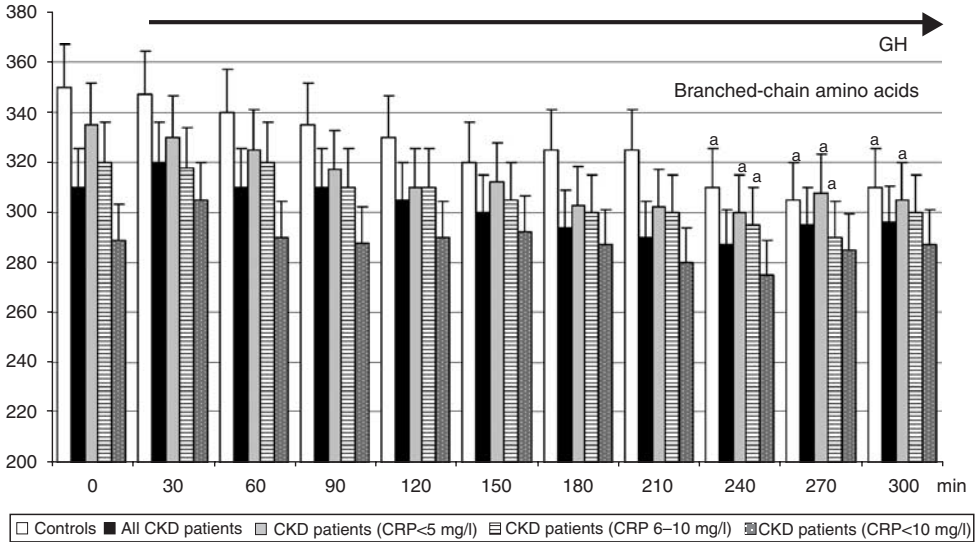

Figure 4 | Effects of GH infusion on blood branched-chain amino acids (BCAA) in CKD patients and controls. In healthy subjects BCAA levels declined by ~11% after 240 min from the start of GH infusion. In CKD patients, BCAA declined by ~4% (P = NS vs basal). In patients with plasma CRP > 10 mg/l (high CRP), arterial concentrations of BCAA were not influenced by GH ($P < 0.002$, by a mixed model analysis). Statistically different from basal levels: a = $P < 0.05$.

Table 1 | Results of the regression analyses modeling changes in plasma potassium and blood BCAA induced by GH in CKD patients (Protocol 1)

	Univariate				Multivariate			
	β	s.e.	*t*	*P*-value	β	s.e.	*t*	*P*-value
Dependent variable: GH-induced changes in plasma potassium								
Age	0.025	0.007	2.81	0.004	0.19	0.008	2.49	0.02
logIL-6	0.53	0.18	2.93	0.008	0.36	0.174	2.07	0.05
							Model r^2*=0.41;* P < *0.03*	
Dependent variable: GH-induced changes in blood BCAA								
Body weight	−0.77	−0.32	2.39	0.03				(a)
BMI	−2.16	0.93	2.22	0.03	−0.30	1.20	0.248	NS
Estimated GFR	−1.859	0.97	2.39	0.03	−0.85	1.23	0.687	NS
Serum albumin	−24.40	10.47	2.33	0.03	−14.59	10.04	1.45	NS
Log CRP	22.03	9.06	2.42	0.035				(a)
Log IL-6	17.35	5.26	3.32	0.001	12.42	5.607	2.21	0.035
							Model r^2*=0.50;* P < *0.01*	

β, β-coefficient; BCAA, branched-chain amino acids; BMI, body mass index; CRP, C-reactive protein; GFR, glomerular filtration rate; GH, growth hormone; IL-6, interleukin-6; s.e., standard error.
A positive sign indicates a direct association with a low response, a negative sign indicates a negative association with a low response.
(a), not included in the multiple regression model.

In the subgroup analysis, in patients with high CRP, blood levels of EAA (Figure 3) and BCAA (Figure 4) were not influenced by GH (likelihood ratio test $P < 0.002$ for both). By contrast, blood EAA and BCAA declined in response to GH similar to controls, in the low and intermediate CRP groups (likelihood ratio test, P = NS).

Determinants of GH-induced changes in potassium and amino-acid levels

At univariate analysis, GH-induced variations in plasma potassium at nadir (that is, at 210 min from the start of GH infusion) were related to age and log interleukin-6 (IL-6), but not to other considered variables (Table 1). By multiple regression analysis, these two factors accounted for 41% of variations in plasma potassium (Table 1).

At univariate analysis, variations in BCAA at nadir (that is, at the and of the study, 300 min) over basal values (Table 1) were related to body weight, body mass index, estimated glomerular filtration rate, serum albumin, and inflammatory markers, such as log-transformed CRP and IL-6. By multiple regression analysis, however, only log IL-6 was significantly related with GH-induced changes in BCAA levels (Table 1).

Effects of GH on net protein and potassium balance across the forearm

This protocol was based on different timing of sampling from the start of GH infusion (120–180 min, for the study of

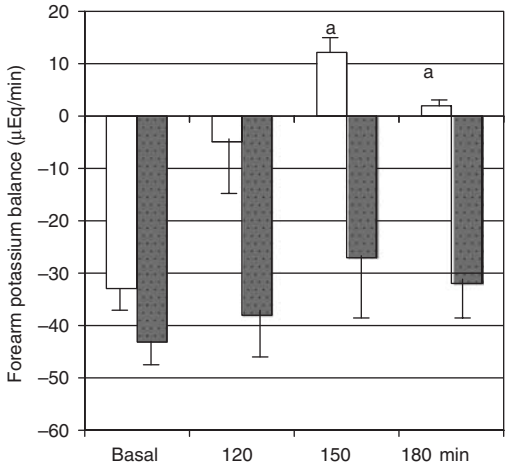

Figure 5 | Forearm potassium balance at the baseline and during GH infusion in hemodialysis (HD) patients with low (< 5 mg/l; □) or high (> 10 mg/l; ■) plasma C-reactive protein (CRP). GH infusion caused a positive shift in forearm potassium balance in HD patients with low CRP levels, while no effect was observed in subjects with high CRP. Data are expressed as mean ± s.e.m. Significance of difference vs baseline value: $^aP < 0.03$.

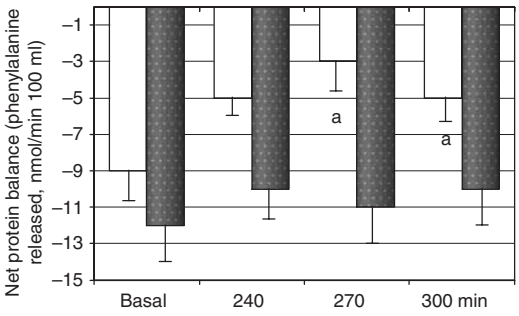

Figure 6 | Forearm net protein balance at the baseline and during GH infusion in hemodialysis (HD) patients with low (< 5 mg/l) (□) or high (> 10 mg/l) (■) plasma C-reactive protein (CRP). GH infusion decreased the negative forearm protein balance in HD patients with low CRP, while no effect was observed in subjects with elevated CRP. Data are expressed as mean ± s.e.m. Significance of difference vs baseline value: $^aP < 0.03$.

forearm potassium exchange; 240–300 min, for the study of muscle net protein balance). During the study, in patients not displaying an acute phase response, plasma potassium decreased from 5.3 ± 0.22 to 4.8 ± 0.24 mEq/l ($P < 0.02$; Δ potassium $= -0.5 \pm 0.11$ mEq/l at 300 min). Similar to what was obtained in undialyzed CKD patients, no significant change in plasma potassium and BCAA was observed in inflamed subjects (Δ potassium $= -0.2 \pm 0.21$ mEq/l, Δ BCAA $= -20 \pm 35$ μmol/l; $P =$ NS for both). At baseline, a forearm release of phenylalanine and potassium was observed in both groups of patients (Figures 5 and 6), indicating the occurrence of a negative potassium and protein balance. GH infusion caused a positive shift in the forearm potassium balance, together with less negative net protein balance, in noninflamed patients, while no effect was observed in subjects with elevated CRP.

DISCUSSION

The aim of this study was to learn more about the cause–effect relationship between GH action and uremia. We tested the hypothesis that the acute metabolic response to GH (an effect which is mediated by GH receptor) is impaired in patients with advanced CKD. To examine the effects of saturation of GH receptor, GH was raised and maintained in a steady high pharmacologic range in patients with CKD and in healthy controls. The effects of GH on potassium and AA metabolism were studied in association with several different parameters as potential predictors of the GH response. To explore those factors that may contribute to the uremia-related resistance to a pharmacological dose of GH, we measured circulating concentrations of cytokines as well as markers of acute phase response. These specific measures were chosen in light of studies suggesting a role for these factors in the development of resistance to GH.[31] Our data show that the GH acute anabolic response regarding two different GH receptor-downstream pathways, namely, potassium and AA metabolism, is overall preserved in patients with advanced CKD. However, this response is blunted in patients displaying evidence of microinflammation. Perhaps more important than group differences, when data from all patients were pooled, concentrations of inflammatory markers were negatively correlated with the GH response.

Basal potassium was mildly increased in patients studied here, in keeping with previous studies.[32,33] In accordance with changes observed in healthy volunteers, in CKD patients GH administration was associated with a significant decline in plasma potassium, which returned in the normal range after 3 h. The observed potassium-lowering effect of GH is similar to that observed for low-dose insulin (10 units plus glucose) or nebulized albuterol (20 mg).[34] However, this response occurs later (3 h vs ~30–40 min) than after insulin or albuterol. In addition this response is very variable, indicating that not all patients are GH-sensitive regarding the potassium-lowering effects.

Older subjects are responsive to the metabolic effects of GH, but this response is higher in younger individuals.[35,36] In our study, increasing age and plasma markers of inflammation were associated with decreased response to GH. In our study, the pattern of the GH response regarding AA was similar in patients and controls, with a small decline in plasma total EAA and NEAA in the 240–300 min period during the infusion. When patients were grouped separately according to CRP levels, the GH-induced decline in circulating BCAA and EAA was absent in the high CRP group, suggesting that the GH-induced AA intracellular removal is impaired in patients with an inflammatory response. To further explore these effects, we measured potassium and forearm muscle net protein balance in HD patients with or without an inflammatory response. We observed that the GH-induced stimulation of forearm

potassium and AA uptake was blunted in patients with evidence of inflammation, but it persisted in noninflamed subjects. According to these findings, inflammation antagonizes the effect of GH on protein and potassium metabolism in skeletal muscle of CKD patients.

Circulating GH-binding protein becomes saturated with 22 kDa GH (which constitutes approximately 90% of the circulating GH) at GH concentrations above 20–30 μg/l.[37] As both uremia and inflammation have been variably associated with low GH-binding protein expression,[38] we set out to obtain supraphysiological concentrations of GH, which were effectively achieved (150–160 μg/l). In addition, the measure of immunofunctional GH gave similar results in different groups. Therefore, the effects of variable expression of circulating GH-binding protein in different subgroups could be ruled out.

Our results could be due to reduced expression of GH receptor (which is unlikely, since the expression of GH receptor seems to be unaltered by uremia)[13] or reduced post-receptor signaling. Resistance to GH may stem from impaired GH stimulated JAK2-STAT5 phosphorylation, which may be the result of inflammation induced SOCS2 expression.[13,38–40] To our knowledge, this is the first study to demonstrate a relationship between circulating concentrations of cytokines and the biochemical processes that regulate the GH response in ESRD patients. These correlations are also in keeping with the effect of cytokines to decrease muscle protein synthesis.[41–44] Our study cannot, however, discern whether cytokines alter the GH response in an endocrine fashion or if the observed effects are reflective of inflammatory changes at the level of the skeletal muscle.[43–45] Regardless of the mode of action (that is, endocrine vs paracrine/autocrine), these findings suggest a role for inflammatory changes in the regulation of skeletal muscle protein balance.

In this paper, we examined only some of the GH-related pathways. Therefore, we must express caution regarding the extension of our results to all of the GH-dependent effects in uremia. In addition, findings reported in the present paper are limited to the acute GH effects, and cannot predict the true impact of GH after days or months of treatment.

In conclusion, a major finding of this study is that, although a resistance to GH is not a generalized feature of uremia, there is a subgroup of patients characterized by blunted acute GH action. Aging and increase in pro-inflammatory cytokines are independently associated with a lessened GH response. These factors may explain, at least in part, the heterogeneity in the response that CKD patients show to GH therapy.

MATERIALS AND METHODS

Experimental protocols

Characteristics of the CKD (stage IV) and HD patients are given in Table 2. Causes of CKD were chronic glomerulonephritis ($n=9$), hypertensive nephrosclerosis ($n=13$), tubulointerstitial nephritis

Table 2 | Clinical characteristics of patients and control subjects

	Controls	CKD subjects (Protocol 1)			HD subjects (Protocol 2)			
		All CKD subjects	CKD (CRP 2–5 mg/l)	CKD (CRP 6–10 mg/l)	CKD (CRP > 10 mg/l)	All HD subjects	HD (CRP 2–5 mg/l)	HD (CRP > 10 mg/l)
Number of subjects	6	23	8	6	9	10	5	5
Age (years)	58 ± 6	60 ± 2	60 ± 5	58 ± 2	63 ± 3	65 ± 3	65 ± 4	64 ± 3
Gender (M/F)	5/1	19/4	7/1	5/1	7/2	8/2	4/1	4/1
BMI (kg/m^2)	25 ± 1	20 ± 1	23 ± 1	23 ± 2	23 ± 1	24 ± 2	25 ± 2	23 ± 2
FFM (kg)	50 ± 2	47 ± 1	48 ± 1	45 ± 2	46 ± 2	47 ± 2	48 ± 1	46 ± 5
Fat mass (kg)	22 ± 1	19 ± 1	19 ± 2	20 ± 2	19 ± 2	20 ± 1	20 ± 1	19 ± 2
nPNA (g/kg)	1.0 ± 0.1	0.97 ± 0.03	0.94 ± 0.03	0.99 ± 0.03	0.96 ± 0.05	1.2 ± 0.1	1.3 ± 0.1	1.1 ± 0.1
SGA score	7 (6–7)	7 (4–7)	7 (4–7)	7 (6–7)	7 (4–7)	6 (5–7)	6 (6–7)	6 (5–7)
CRP (mg/l)	3 (2–4)	9 (2–25)[c]	4 (2–5)	8 (6–10)	12 (11–25)[b,c]	12 (2–40)[c]	4 (2–5)	20 (18–45)[c]
Estimated GFR ($ml/min \times 1.73\ m^2$)	101 ± 5	10 ± 1[e]	10 ± 1[e]	9 ± 1[e]	9 ± 1[e]	1 ± 1[e]	1 ± 0.4[e]	1 ± 1[e]
HCO_3 (mmol/l)	24 ± 1	23 ± 1	23 ± 1	23 ± 1	24 ± 1	23 ± 1	23 ± 1	24 ± 1
Albumin (g/100 ml)	4.0 ± 0.1	3.9 ± 0.03	4.0 ± 0.1	4.0 ± 0.1	3.7 ± 0.1	3.8 ± 0.2	3.8 ± 0.1	3.7 ± 0.3
Hemoglobin (g/100 ml)	14 ± 1	11 ± 0.2[c]	11 ± 0.2[c]	11 ± 0.3[c]	11 ± 0.2[c]	11 ± 0.2[c]	11 ± 0.2[c]	11 ± 0.2[c]
BUN (mg/100 ml)	12 ± 2	77 ± 4[e]	73 ± 8[e]	79 ± 5[e]	82 ± 3[e]	78 ± 7[e]	73 ± 7[e]	85 ± 6[e]
IGF-1 (ng/ml)	180 ± 15	172 ± 8	190 ± 10	180 ± 4	150 ± 7	178 ± 8	194 ± 10	182 ± 7
IL-6 (pg/ml)	3 (3–5)	12 (3–88)[c]	5 (3–7)	7 (5–12)	40 (10–88)[b,c]	21 (3–120)[b]	5 (3–5)	21 (12–120)[a,d]
TNF-α (pg/ml)	30 (25–32)	52 (32–160)[c]	42 (32–90)	60 (37–87)	56 (42–160)[b,c]	76 (45–212)[c]	68 (45–190)	84 (50–212)[c]
IL-1 (pg/ml)	23 (15–27)	78 (36–133)[c]	50 (36–66)[c]	77 (42–89)[c]	92 (50–133)[c]	84 (38–156)	67 (38–98)[c]	115 (56–156)[c]
Insulin (μU/ml)	7 ± 1	8 ± 1	6 ± 1	9 ± 1	8 ± 1	12 ± 2	12 ± 2	13 ± 2
GH (μg/l)	0.8 ± 0.16	3.2 ± 0.21[c]	2.3 ± 0.44[c]	3.4 ± 0.3[c]	3.9 ± 0.13[c]	3.9 ± 0.13[c]	3.7 ± 0.1[c]	4.1 ± 0.1[c]

BMI, body mass index; BUN, blood urea nitrogen; CKD, chronic kidney disease; CRP, C-reactive protein; FFM, fat-free mass; GFR, glomerular filtration rate; GH, growth hormone; HD, hemodialysis; IL-1, interleukin-1; nPNA, normalized protein nitrogen appearance; SGA, subjective global assessment; TNF-α, tumor necrosis factor-α.
Data are mean ± s.e.m. or median (range).
[a]Significance of difference vs CKD patients with CRP < 5 mg/l, $P<0.05$.
[b]Significance of difference vs CKD patients with CRP < 5 mg/l, $P<0.01$.
[c]Significance of difference vs control subjects, $P<0.05$.
[d]Significance of difference vs control subjects, $P<0.01$.
[e]Not tested.

($n=4$), and polycystic renal disease ($n=7$). Exclusion criteria were age >76 years, estimated glomerular filtration rate <7.0 ml/min, blood bicarbonate <22 mmol/l, or the occurrence of treatable causes of inflammation or of any illness requiring hospitalization within the previous 30 days. Persistent increases in CRP levels (>5 mg/l on three separate weekly sampling) associated with signs of vascular disease (coronary artery disease or peripheral artery disease) were present in 15 CKD and 5 HD patients. There were no differences among groups as for age, gender, body mass index, normalized protein nitrogen appearance, fat-free and fat mass, estimated glomerular filtration rate, blood bicarbonate, serum albumin, hemoglobin, IGF-1, insulin, and GH levels. Calorie intake was 30–32 kcal/kg as estimated by nutritional interviews, in CKD patients and about 28–30 kcal/kg in HD patients. Baseline GH levels were higher in patients as compared to controls ($P<0.05$). Control subjects were on a diet providing 31–35 kcal and 1–1.1 g of protein per kg/day, as also assessed by dietary histories and urea excretion. Routine laboratory tests, acid-base and electrolyte measurements were all normal. All studies were approved by the Ethical Committee of the Department of Internal Medicine of the University of Genoa. Patients were informed of the nature, purpose, procedure, and possible risks before their voluntary consent was obtained. Procedures were in accordance with the Helsinki declaration. Patients were taking drugs, including antihypertensive drugs, sodium bicarbonate, calcium carbonate, and erythropoietin, which were prescribed as appropriate for each individual. Angiotensin-converting enzyme inhibitors, angiotensin II receptor blockers, or β-blockers, if any, were discontinued at least 10 days before the study. Studies were performed in the postabsorptive state, and two different protocols were used (Figure 7).

Study protocol 1: acute effects of GH on plasma potassium and amino acids in CKD patients. A total of 23 CKD patients were studied (Table 2). At 0700 hours, teflon catheters were placed in an antecubital vein and a dorsal hand vein. Arterialized blood samples[46] were collected for the measuring plasma hormones, potassium, and AA from the dorsal hand vein at the baseline and at 30-min intervals during a primed continuous infusion of GH (0.6 U; 0.7 mU/min per kg for 300 min; (Genotropin, Pharmacia, Stockholm, Sweden; Figure 7). The patients were divided into three groups on the basis of inflammatory status: patients with CRP concentrations <5 mg/l (low CRP) were classified as having no inflammation, patients with CRP concentrations between 6 and 10 mg/l (intermediate CRP), and CRP >10 mg/l (high CRP) were classified as having inflammation.

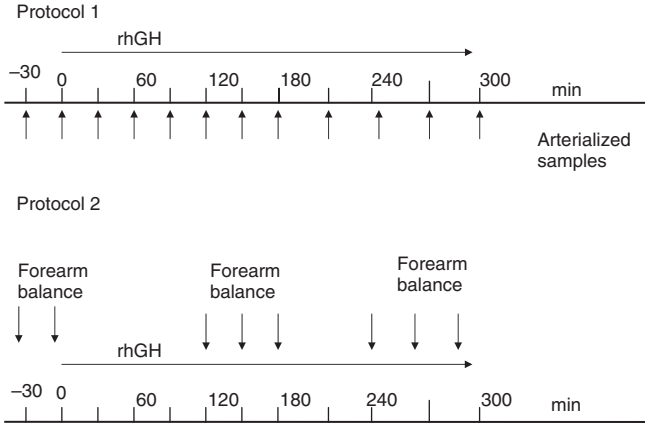

Figure 7 | The design of the two protocols. We studied the acute GH effects on two selected endpoints of hormone action, such as potassium and amino-acid metabolism, in patients with advanced CKD and controls. We tested the hypothesis that GH response is blunted in CKD patients via an interventional design comprising the single administration of recombinant human (rh)GH e.v. for 300 min. In Protocol 1, the GH-induced changes in blood amino acids and plasma potassium were analyzed in relation to several clinical and biochemical parameters in CKD patients. In Protocol 2, the forearm balance of phenylalanine and potassium was examined in the basal state and in response to GH in hemodialysis patients displaying or not an inflammatory response.

Study protocol 2: effects of GH on muscle net protein balance and potassium exchange. A total of 10 maintenance HD patients treated with bicarbonate HD for 18 months (range, 10–31 months) were studied (Table 2). Patients were separated according to low (2–5 mg/l) or high (>10 mg/l) CRP levels. Patients were studied after ~70 h from the last dialytic treatment. Forearm muscle potassium and net protein balance were studied in the postabsorptive state.[17,45,46] Catheters were inserted into a brachial artery and, in a retrograde fashion, into the ipsilateral deep forearm vein. Blood samples were taken simultaneously from the artery and the vein at 20-min intervals during a 60-min period. Blood flow across the forearm was determined immediately after each arterio-venous sampling by an indium-gallium strain gauge plethysmograph (Angiomed Instruments, Padua, Italy). The study was performed at the baseline and from 120 to 300 min from the start of a 300-min primed continuous infusion of GH (0.6 U; 0.7 mU/min per kg; Genotropin, Pharmacia) in the contralateral arm.

Assays

No tourniquet pressure was applied, and blood samples were collected in heparin (for AA and electrolyte analyses) or EDTA-coated (for hormones) syringes. Blood samples were accepted if the partial pressure of oxygen was >70 mm Hg. Blood acid-base and plasma electrolyte analysis of freshly separated plasma were performed immediately. AA were determined in whole blood.[17] Plasma potassium was measured by flame photometry (Corning 480 Flame Photometer, Corning, Medfield, MA, USA). Plasma IL-6, IL-1, and tumor necrosis factor-α were measured in plasma samples using enzyme-linked immunosorbent assay kits (Diaclone, Besancon, France), according to the manufacturer's directions. Serum CRP was measured by nephelometry (Behring, Deerfield, IL, USA). AA were determined in blood by an AA analyzer (Model 3A 30, Fisons Instr., Milan, Italy). Plasma insulin and IGF-I were determined by radioimmunoassay (Diagnostic Products, LA, USA and Medgenix, Fleurus, Belgium, respectively). Plasma GH was measured by a chemiluminescent immunoradiometric assay assay (Immulite, DPC, Los Angeles, CA, USA) and an immunofunctional assay.[47] By this technique only the GH molecules having both receptor interaction sites necessary for initiation of the signal transduction process in target cells are translated into an assay signal. All other serum chemical measurements were determined by routine clinical chemistry laboratory procedures. Glomerular filtration rate was estimated according to the abbreviated MDRD study equation.[48] Body composition was estimated by anthropometry.[49]

Calculations

The exchange of potassium and phenylalanine across the forearm and the total decrease in extracellular potassium was calculated by the Fick's principle: $(X_a-(X_v)) \times$ plasma flow, where X_a and X_v are the concentrations of potassium in arterial and venous plasma,

respectively. Plasma flow was calculated from blood flow and hematocrit. The forearm exchange of phenylalanine was measured and calculated in whole blood as reported.[17]

Statistical analysis

All data are presented as the mean ± standard error of the mean or median (range). To establish the differences in substrate concentrations between patients and controls, the unpaired *t*-test and one-way analysis of variance were used. Serum CRP, IL-1, IL-6, and tumor necrosis factor-α, were logarithmically transformed. A mixed model accounting for repeated measures with a first order autoregressive term and random intercept was used to analyze the time course differences between groups. A global heterogeneity test was run (likelihood ratio test) and *post hoc* comparisons were performed when the heterogeneity test resulted statistically significant (SPlus Professional Release 2, Math Soft Inc., Cambridge, MA, USA).

A repeated-measure analysis of variance was used to compare the overall changes the study. When ANOVA indicated statistical significance ($P<0.05$), a *post hoc* F-based test (Bonferroni) was performed between phases. Relationships between variables were sought by Pearson's correlation coefficient and multivariate linear regression.

DISCLOSURE

All the authors declared no competing interests.

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REFERENCES

1. Mitch WE. Proteolytic mechanisms, not malnutrition, cause loss of muscle mass in kidney failure. *J Ren Nutr* 2006; **16**: 208–211.
2. Bergstrom J, Lindholm B. Malnutrition, cardiac disease, and mortality: an integrated point of view. *Am J Kidney Dis* 1998; **32**: 834–841.
3. Kalantar-Zadeh K, Kopple JD, Block G *et al.* A malnutrition-inflammation score is correlated with morbidity and mortality in maintenance hemodialysis patients. *Am J Kidney Dis* 2001; **38**: 1251–1263.
4. Kopple JD, Greene T, Chumlea WC *et al.* Relationship between nutritional status and the glomerular filtration rate: Results from the MDRD study. *Kidney Int* 2000; **57**: 1688–1703.
5. Mitch WE. Uremic acidosis and protein metabolism. *Curr Opin Nephrol Hypertens* 1995; **4**: 488–492.
6. Fryburg DA, Louard RJ, Gerow KE. Growth hormone stimulates skeletal muscle protein synthesis and antagonizes insulin's antiproteolytic action in humans. *Diabetes* 1992; **41**: 424–429.
7. Umpleby AM, Russell-Jones DL. The hormonal control of protein metabolism. *Baillieres Clin Endocrinol Metab* 1996; **10**: 551–570.
8. Kaskel F. Chronic renal disease: a growing problem. *Kidney Int* 2003; **64**: 1141–1151.
9. Chen Y, Fervenza FC, Rabkin R. Growth factors in the treatment of wasting in kidney failure. *J Ren Nutr* 2001; **11**: 62–66.
10. Qing Peiyuan D, Ding HU, Vadgama J *et al.* Elevated myocardial cytosolic calcium impairs insulin-like growth factor-1-stimulated protein synthesis in chronic renal failure. *J Am Soc Nephrol* 1999; **10**: 84–92.
11. Powell DR, Zhao D, Durham SK *et al.* Decreased hepatic insulin-like growth factor (IGF)-I and increased IGF binding protein-1 and -2 gene expression in experimental uremia. *Endocrinology* 1997; **138**: 938–946.
12. Blum WF, Ranke MB, Kietzmann K *et al.* Growth hormone resistance and inhibition of somatomedin activity by excess of insulin-like growth factor binding protein in uremia. *Pediatr Nephrol* 1991; **5**: 539–544.
13. Sun DF, Zheng Z, Tummala P *et al.* Chronic uremia attenuates growth hormone-induced signal transduction in skeletal muscle. *J Am Soc Nephrol* 2004; **15**: 2630–2646.
14. Fouque D, Peng SC, Kopple JD. Impaired metabolic response to recombinant insulin-like growth factor-1 in dialysis patients. *Kidney Int* 1995; **47**: 876–883.
15. Fouque D, Peng SC, Shamir E *et al.* Recombinant human insulin-like growth factor-1 induces an anabolic response in malnourished CAPD patients. *Kidney Int* 2000; **57**: 646–654.
16. Ikizler TA, Wingard RL, Flakoll PJ *et al.* Effects of recombinant human growth hormone on plasma and dialysate amino acid profiles in CAPD patients. *Kidney Int* 1996; **50**: 229–234.
17. Garibotto G, Barreca A, Russo R *et al.* Effects of recombinant human growth hormone on muscle protein turnover in malnourished hemodialysis patients. *J Clin Invest* 1997; **99**: 97–105.
18. Pupim LB, Flakoll PJ, Yu C *et al.* Recombinant human growth hormone improves muscle amino acid uptake and whole-body protein metabolism in chronic hemodialysis patients. *Am J Clin Nutr* 20055; **82**: 1235–1243.
19. Kotzmann H, Yilmaz N, Lercher P *et al.* Differential effects of growth hormone therapy in malnourished hemodialysis patients. *Kidney Int* 2001; **60**: 1578–1585.
20. Kopple JD, Brunori G, Leiserowitz M *et al.* Growth hormone induces anabolism in malnourished maintenance haemodialysis patients. *Nephrol Dial Transplant* 2005; **20**: 952–958.
21. Feldt-Rasmussen B, Lange M, Sulowicz W *et al.* Growth hormone treatment during hemodialysis in a randomized trial improves nutrition, quality of life, and cardiovascular risk. *J Am Soc Nephrol* 2007; **18**: 2161–2171.
22. Cuneo RC, Salomon F, Wiles CM *et al.* Growth hormone treatment in growth hormone-deficient adults. I. Effects on muscle mass and strength. *J Appl Physiol* 1991; **70**: 688–694.
23. Cooney RN, Shumate M. The inhibitory effects of interleukin-1 on growth hormone action during catabolic illness. *Vitam Horm* 2006; **74**: 317–340.
24. Brungger M, Hulter HN, Krapf R. Effect of chronic metabolic acidosis on the growth hormone/IGF-1 endocrine axis: new cause of growth hormone insensitivity in humans. *Kidney Int* 1997; **51**: 216–221.
25. Robinson IC, Gevers E. Visualizing and manipulating growth hormone (GH) responses in muscle and other GH target tissues. *Horm Res* 2006; **66S1**: 35–41.
26. Ljungberg A, Linden D, Ameen C *et al.* Importance of PPAR alpha for the effects of growth hormone on hepatic lipid and lipoprotein metabolism. *Growth Horm IGF Res* 2007; **17**: 154–164.
27. Hayashi AA, Proud CG. The rapid activation of protein synthesis by growth hormone requires signaling through the mammalian target of rapamycin, mTOR. *Am J Physiol Endocrinol Metab* 2007; **292**: E1647–E1655.
28. Salomon F, Cuneo RC, Hesp R *et al.* Basal metabolic rate in adults with growth hormone deficiency and in patients with acromegaly: relationship with lean body mass, plasma insulin level and leucocyte sodium pump activity. *Clin Sci* 1992; **83**: 325–330.
29. Olivecrona H, Hilding A, Ekstrom C *et al.* Acute and short-term effects of growth hormone on insulin-like growth factors and their binding proteins: serum levels and hepatic messenger ribonucleic acid responses in humans. *J Clin Endocrinol Metab* 1999; **84**: 553–560.
30. Fryburg D, Gelfand G. Growth hormone acutely stimulates skeletal muscle but not whole-body protein synthesis in humans. *Metabolism* 1993; **42**: 1223–1227.
31. Haddad F, Zaldivar F, Cooper DM *et al.* IL-6-induced skeletal muscle atrophy. *J Appl Physiol* 2005; **98**: 911–917.
32. Widmer B, Gerhardt RE, Harrington JT *et al.* Serum electrolyte and acid base composition. The influence of graded degrees of chronic renal failure. *Arch Intern Med* 1979; **139**: 1099–1102.
33. Gennari FJ, Segal AS. Hyperkalemia: an adaptive response in chronic renal insufficiency. *Kidney Int* 2002; **62**: 1–9.
34. Allon M, Copckney C. Albuterol and insulin for treatment of hyperkalemia in hemodialysis patients. *Kidney Int* 1990; **38**: 869–872.
35. Vahl N, Moller N, Lauritzen T *et al.* Metabolic effects and pharmacokinetics of a growth hormone pulse in healthy adults: relation to age, sex, and body composition. *J Clin Endocrinol Metab* 1997; **82**: 3612–3618.
36. Hansen TK, Gravholt CH, ØRskov H *et al.* Dose dependency of the pharmacokinetics and acute lipolytic actions of growth hormone. *J Clin Endocrinol Metab* 2002; **87**: 4691–4698.
37. Baumann G, Amburn K, Shaw MA. The circulating growth hormone (GH)-binding protein complex: a major constituent of plasma GH in man. *Endocrinology* 1988; **122**: 976–984.

38. Greenstein J, Guest S, Tan JC *et al.* Circulating growth hormone binding protein levels and mononuclear cell growth hormone receptor expression in uremia. *J Ren Nutr* 2006; **16**: 141–149.
39. Nathan J, Lanning NJ, Carter-Su C. Recent advances in growth hormone signaling. *Rev Endocr Metab Disord* 2006; **7**: 225–235.
40. Chen Y, Sun D, Krishnamurthy VM *et al.* Endotoxin attenuates growth hormone induced hepatic insulin-like growth factor-1 expresion by inhibiting Jak2/Stat5 signal transduction and Stat5b DNA binding. *Am J Physiol Endocrinol Metab* 2007; **292**: E1856–E1862.
41. Honda H, Qureshi AR, Heimburger O *et al.* Serum albumin, C-reactive protein, interleukin-6, and fetuin a as predictors of malnutrition, cardiovascular disease, and mortality in patients with ESRD. *Am J Kidney Dis* 2006; **47**: 139–148.
42. Toth MJ, Matthews DE, Tracy RP *et al.* Age-related differences in skeletal muscle protein synthesis: relation to markers of immune activation. *Am J Physiol Endocrinol Metab* 2005; **288**: E883–E891.
43. Lang CH, Frost RA, Nairn AC *et al.* TNF-α impairs heart and skeletal muscle protein synthesis by altering translation initiation. *Am J Physiol Endocrinol Metab* 2002; **282**: E336–E347.
44. Greiwe JS, Cheng B, Rubin DC *et al.* Resistance exercise decreases skeletal muscle tumor necrosis factor in frail elderly humans. *FASEB J* 2001; **15**: 475–482.
45. Garibotto G, Sofia A, Procopio V *et al.* Peripheral tissue release of Interleukin-6 in patients with chronic kidney diseases: effects of end-stage renal disease and microinflammatory state. *Kidney Int* 2006; **70**: 384–390.
46. Forster HV, Dempsey JA, Thomson J *et al.* Estimation of arterial PO2, PCO2, pH and lactate from arterialized venous blood. *J Appl Physiol* 1971; **32**: 134–137.
47. Strasburger CJ. Laboratory assessment of GH. *Growth Horm IGF Res* 1998; **8**: S41–S46.
48. Levey AS, Bosch JP, Lewis JB *et al.* A more accurate method to estimate glomerular filtration rate from serum creatinine: a new prediction equation. Modification of Diet in Renal Disease Study Group. *Ann Intern Med* 1999; **130**: 461–470.
49. Heymsfield SB, Mc Manus C, Smith J *et al.* Anthropometric measurement of muscle mass: revised equations for calculating bone-free arm muscle area. *Am J Clin Nutr* 1982; **36**: 680–690.