November 01, 2009

# N-terminal propeptide of Type III procollagen as a biomarker of anabolic response to recombinant human GH and testosterone

Shalender Bhasin (1953-)
*Boston University School of Medicine*

E. Jiaxiu He
*University of Southern California*

Miwa Kawabuko
*University of Southern California*

E. Todd Schroeder
*University of Southern California*

Kevin Yarasheski
*Washington University School of Medicine in St. Louis*

***See next page for additional authors***

# N-Terminal Propeptide of Type III Procollagen as a Biomarker of Anabolic Response to Recombinant Human GH and Testosterone

Shalender Bhasin, E. Jiaxiu He, Miwa Kawakubo, E. Todd Schroeder, Kevin Yarasheski, Gregory J. Opiteck, Alise Reicin, Fabian Chen, Raymond Lam, Jeffrey A. Tsou, Carmen Castaneda-Sceppa, Ellen F. Binder, Stanley P. Azen, and Fred R. Sattler

Section of Endocrinology, Diabetes, and Nutrition (S.B.), Boston University School of Medicine, Boston Medical Center, Boston, Massachusetts 02118; Department of Medicine (F.R.S.), School of Dentistry (E.J.H.), Division of Biokinesiology (E.T.S.), and Department of Preventive Medicine (M.K., S.P.A.), University of Southern California, Los Angeles, California 90089; Merck Research Laboratories (G.J.O., A.R., F.C., R.L., J.A.T.), Rahway, New Jersey 07065; Washington University School of Medicine (K.Y., E.F.B.), St. Louis, Missouri 63110; Jean Mayer U.S. Department of Agriculture Human Nutrition Research Center on Aging (C.C.-S.), Tufts University, Boston, Massachusetts 02111

**Context:** Biomarkers that predict musculoskeletal response to anabolic therapies should expedite drug development. During collagen synthesis in soft lean tissue, N-terminal propeptide of type III procollagen (P3NP) is released into circulation. We investigated P3NP as a biomarker of lean body mass (LBM) and muscle strength gains in response to testosterone and GH.

**Design:** Community-dwelling older men received GnRH agonist plus 5 or 10 g testosterone gel plus 0, 3, or 5 $\mu$g recombinant human GH daily. P3NP levels were measured at baseline and wk 4, 8, 12, and 16. LBM and appendicular skeletal muscle mass (ASM) were measured by dual-energy x-ray absorptiometry.

**Results:** One hundred twelve men completed treatment; 106 underwent serum P3NP measurements. P3NP levels were higher at wk 4 than baseline ($6.61 \pm 2.14$ *vs.* $4.51 \pm 1.05$, $P < 0.0001$) and reached plateau by wk 4 in men receiving testosterone alone. However, wk 8 P3NP levels were higher than wk 4 levels in men receiving testosterone plus recombinant human GH. Increases in P3NP from baseline to wk 4 and 16 were significantly associated with gains in LBM ($r = 0.26$, $P = 0.007$; $r = 0.53$, $P < 0.001$) and ASM ($r = 0.17$, $P = 0.07$; $r = 0.40$, $P < 0.0001$). Importantly, for participants receiving only testosterone, P3NP increases at wk 4 and 16 were related to muscle strength gains ($r = 0.20$, $P = 0.056$ and $r = 0.36$, $P = 0.04$). In stepwise regression, change in P3NP explained 28 and 30% of the change in ASM and LBM, respectively, whereas change in testosterone but not IGF-I and age provided only small improvements in the models.

**Conclusion:** Early changes in serum P3NP levels are associated with subsequent changes in LBM and ASM during testosterone and GH administration. Serum P3NP may be a useful early predictive biomarker of anabolic response to GH and testosterone. **(*J Clin Endocrinol Metab* 94: 4224–4233, 2009)**

The past decade has witnessed substantial pharmaceutical and academic investment in the development of function promoting anabolic therapies that improve physical function and health-related outcomes (1). The leading function promoting anabolic molecules that are under development, androgens, myostatin inhibitors, and GH, are potential promyogenic agents that are expected to improve physical function primarily by increasing skeletal muscle mass. Considering the enormous resources required for conducting large efficacy trials, serum biomar-

ISSN Print 0021-972X ISSN Online 1945-7197
Printed in U.S.A.

doi: 10.1210/jc.2009-1434 Received July 7, 2009. Accepted August 21, 2009.
First Published Online October 16, 2009

Abbreviations: ASM, Appendicular skeletal muscle mass; BMI, body mass index; CV, coefficient of variation; HORMA, Hormonal Regulators of Muscle and Metabolism in Aging; LBM, lean body mass; MCID, minimal clinically important difference; P3NP, N-terminal propeptide of type III procollagen; rhGH, recombinant human GH.

kers that predict anabolic response to function promoting therapies would be of enormous value in the initial screening of candidate molecules. Indeed, biomarker discovery has been deemed a priority area of research by the National Institutes of Health. Here we describe the application of N-terminal propeptide of type III procollagen (P3NP) as a biomarker of anabolic response to supplementation with testosterone and GH in older persons.

Type III collagen in soft connective tissues, such as muscle and skin, is synthesized from larger procollagen III molecules that carry peptide extensions at both N- and C-terminal ends (2–4). The N- and C-terminal extensions of procollagen III are removed by specific proteinases during final stages of collagen synthesis and released into circulation in stoichiometric amounts (4). P3NP, a product of this proteolytic cleavage during collagen synthesis in connective tissue, can be measured in the human serum, and its circulating concentrations have been described in children, healthy adults, acromegalics, and athletes (5–9). P3NP levels are responsive to exercise, testosterone, and GH (6, 10–14) and are being explored as a marker of GH doping in sports (5, 6, 10, 12, 14–17). Based on the stoichiometric relationship between collagen synthesis and P3NP release during soft tissue generation (4), we hypothesized that changes in circulating P3NP concentrations would reflect gains in lean body mass (LBM) and appendicular skeletal muscle mass (ASM) during administration of anabolic therapies, such as testosterone and GH. Accordingly, we measured circulating P3NP concentrations at baseline and in response to administration of testosterone and recombinant human GH (rhGH) in community-dwelling older men, who participated in the Hormonal Regulators of Muscle and Metabolism in Aging (HORMA) trial (18, 19).

The HORMA trial, whose design and main findings have been published (18, 19), was a multicenter study of older, community-dwelling men who had levels of testosterone and IGF-I typical of their age and who received graded doses of testosterone gel either alone or in combination with placebo or one of two physiological doses of rhGH. Testosterone and rhGH administration were each associated with significant gains in LBM and ASM (18). We tested the hypothesis that increase in P3NP is associated with gains in LBM and ASM during testosterone and rhGH administration. We also determined whether early changes in P3NP at 4 wk were predictive of changes in LBM and ASM at the end of treatment (16 wk).

## Subjects and Methods

The protocol was approved by the Institutional Review Boards at University of Southern California, Tufts University, Charles Drew University, and Washington University. All participants provided written informed consent.

We recruited community-dwelling men, 65–90 yr old, who had serum IGF-I levels in the lower tertile for adults (<167 ng/ml; 21.9 nmol/liter; Quest Diagnostics, San Juan Capistrano, CA) and morning total testosterone level in the lower half (150–550 ng/dl; 5.21–19.1 nmol/liter) of adult male range (18, 19). The participants were required to have prostate-specific antigen of 4.0 ng/ml or less, hematocrit of 50% or less, and fasting blood glucose less than 126 mg/dl (6.99 mmol/liter).

### Study interventions

All participants were treated monthly starting on the day of randomization with a long-acting GnRH agonist (leuprolide acetate depot, 7.5 mg im; TAP Pharmaceuticals, Deerfield, IL) to suppress endogenous testosterone production. The participants were randomly assigned from computer-generated randomization tables to one of six treatment groups to receive either 5 g (groups A–C) or 10 g (groups D–F) of 1% testosterone transdermal gel (Solvay Pharmaceuticals, Marietta, GA) with or without 0, 3, or 5 $\mu$g/kg rhGH (groups A/D, B/E and C/F, respectively; Genentech, South San Francisco, CA). Testosterone gel was applied daily transdermally and rhGH was administered by sc injection each evening.

The 5- and 10-g doses of testosterone were chosen to produce a spectrum of serum levels via the Leydig cell clamp that would be in the low normal range typical of older men or mid- to high normal levels typical of younger men, respectively (20). The 3 $\mu$g/kg dose of rhGH was chosen because 3.3 but not 2.0 $\mu$g/kg · d has increased whole-body protein synthesis in GH-deficient adults (21). The 5 $\mu$g/kg · d dose was chosen to produce a greater anabolic stimulus but was expected to be low enough to minimize adverse effects that have occurred with higher doses (22, 23). Testosterone and rhGH were administered for 16 wk.

### Outcome measures

LBM and ASM were measured by dual-energy x-ray absorptiometry at baseline and during wk 16, as described (18). Composite strength was measured by the one-repetition maximum method (24, 25) for muscle groups of upper and lower extremities and reported as percent change from baseline (18). Screening testosterone was determined by RIA in the clinical laboratories of the participating centers. After study completion, stored samples were batch tested by liquid chromatography tandem mass spectrometry method with a sensitivity of 2 ng/dl and interassay coefficient of variation (CV) of 4–8% (26, 27). Serum IGF-I levels were measured by an immunoassay in University of Southern California General Clinical Research Center Endocrine Laboratory (18).

Serum P3NP concentrations were measured by a validated equilibrium RIA (28) by Pacific Biometrics, Inc. (Seattle, WA) using a UniQ PIIINP RIA kit (Orion Diagnostica, Espoo, Finland). The blood for P3NP measurement was collected in red top tubes, centrifuged within 20 min, and immediately frozen at −80 C. The samples were not thawed until the time of the assay. Two hundred microliters serum were incubated with 200 $\mu$l of iodinated P3NP tracer and 200 $\mu$l of diluted anti-P3NP antibody for 2 h at 37 C. Immune complexes were separated by addition of 500 $\mu$l second antibody for 30 min at room temperature (28). The sensitivity of the assay was 0.2 ng/ml. The reference range in adult men and women is 2.3–6.4 ng/ml. Intraassay CVs

were 23.0, 7.0, and 4.1% at P3NP concentrations of 2.8, 6.6, and 11.9 ng/ml, respectively, and interassay CVs were 6.5, 4.5, and 7.2% at P3NP concentrations of 2.7, 6.8, and 12.2 ng/ml, respectively (28).

### Statistical analyses

Analyses were conducted for 106 evaluable men who completed 16 wk of treatment and had complete P3NP data. Baseline characteristics were compared across groups using one-way ANOVA for continuous variables (*e.g.* age, hormones, body composition, and P3NP) and $\chi^2$ analyses for discrete variables (*e.g.* ethnicity/race). If the distribution of a continuous variable was nonnormal, then Kruskal-Wallis nonparametric ANOVA was used. Changes in P3NP levels at 4, 8, and 16 wk were compared across groups using one-way ANOVA and the Wald test for trend. When significant group differences were found, pairwise comparisons were made using the Tukey's procedure. Paired *t* tests were used to compare within-group 4-, 8-, and 16-wk changes from baseline.

Correlation analyses were conducted to assess the univariate relationship of temporal changes at 4, 8, and 16 wk in P3NP with the temporal changes in LBM, ASM, and testosterone levels and IGF-I levels. The distributions of temporal changes of LBM, ASM, testosterone, and IGF-I were stratified at the median into low and high responders. Student *t* tests were conducted to contrast the temporal changes in P3NP between low *vs.* high responders for LBM, ASM, testosterone, and IGF-I.

Separate stepwise linear regression analyses were conducted to identify the order of independent predictors of 16-wk change in LBM, ASM, or P3NP. In these analyses, we included independent variables that were associated with the dependent variable at $P < 0.20$ in univariate analyses. Thus, in models to identify predictors of 16-wk change in P3NP, candidate independent variables included age at baseline and 16 wk changes in LBM, ASM, and change in testosterone and IGF-I levels. In addition, logistic regression analysis was performed for each quartile of change in LBM at each time point (4, 8, and 16 wk) for high P3NP responders (based on the change in P3NP at wk 4, 8 or 16 being greater than the median response). Similar analyses were conducted for each quartile of change in ASM. Student *t* tests and logistic regression analyses were performed to compare the changes in P3NP at each time point between subgroups defined by low and high changes in testosterone and low and high changes in IGF-I.

We estimated the minimal clinically important difference (MCID) of change in P3NP concentrations that was associated with 1 kg gain in LBM and 0.5 kg gain in ASM. Rather than determining the MCID estimates from a single regression analysis using all 106 participants, we used the bootstrapping approach to obtain more robust estimates of MCIDs. We generated 1000 bootstrap samples, in which each bootstrap sample set contained 90% of the original sample (96 men) randomly drawn from the original 106 men without replacement. For each bootstrap sample, we estimated the two MCID estimates using regression analyses. The most reliable MCID estimates were the average of the 1000 bootstrap estimates, along with 95% confidence interval. Statistical analyses were carried out using the Statistical Analysis System 9.1 (SAS Institute Inc., Cary, NC).

The study was designed to test the primary hypotheses with 80% power (18). For secondary analyses described in this manuscript, we assessed the adequacy of the sample size by determining the detectable effect size or the detectable correlation coefficient at the 80% power level. For evaluating the temporal changes in P3NP levels between high and low responders based on LBM, ASM, and hormone levels, a sample size of 50 in each group has 80% power to detect an effect size of 0.57 using a two-group *t* test with a 0.05 two-sided significance level. For evaluating the temporal changes in P3NP levels between high quartile and low quartile responders based on LBM, ASM, and hormone levels, a sample size of 25 in each group has 80% power to detect an effect size of 0.81 using a two-group *t* test with a 0.05 two-sided significance level. The minimum detectable correlational relationship between change in P3NP and changes in LBM and ASM with sample size of 106 is 0.3, with 80% power at 0.05 two-sided significance level.

## Results

The baseline characteristics of the study population have been described (18, 19). Of 242 subjects who were screened, 122 were randomized. Ten subjects dropped out after randomization; three from 5 g/d testosterone arm and seven from 10 g/d testosterone arm. One subject who dropped out was randomized to rhGH placebo, four to 3 $\mu$g/kg · d rhGH and five to 5 $\mu$g/kg · d rhGH. Of the 112 participants who completed assessments at wk 16, 106 subjects were included in this analysis because serum for P3NP measurement was not available at baseline or during wk 4, 8, or 16 in six subjects.

Baseline characteristics of the 106 participants were typical of community-dwelling, older men without functional limitations (Table 1) (29). The baseline body mass index (BMI) was 26.2 kg/m$^2$, testosterone 363 ± 97 ng/dl, and IGF-I 112 ± 29 ng/dl. The BMI, testosterone, IGF-I, LBM, ASM, $VO_{2max}$, or P3NP levels did not differ among groups (Table 1).

Changes in testosterone and IGF-I levels in HORMA participants have been published (11). Briefly, treatment with testosterone and rhGH produced dose-related increments in testosterone and IGF-I concentrations. Testosterone levels increased in subjects receiving 5 g/d by 143 ± 379 ng/dl, which was lower ($P < 0.001$) than the increase of 510 ± 503 ng/dl in those receiving 10 g/day. For rhGH at 0, 3, and 5 $\mu$g/kg · d, levels of IGF-I increased by 6 ± 28 ng/dl ($P = 0.15$), 64 ± 44 ng/dl ($P < 0.001$), and 108 ± 51 ng/dl ($P < 0.001$), respectively, with a trend across rhGH dose groups ($P < 0.001$).

Changes in body composition have been published (18). Briefly, in the 106 men who were included in this analysis, LBM and ASM increased significantly from baseline to wk 16 ($P < 0.02$) within each of the six groups (18). The changes in LBM from baseline to wk 16 were higher in men receiving 10 g testosterone gel than in those receiving 5 g testosterone gel. As reported (18), there was a linear trend across increasing doses of rhGH for increases in LBM.

**TABLE 1.** Baseline characteristics of the 106 subjects who were included in this analysis

| Daily dose by study Group Variable | Testosterone 5 g | | | Testosterone 10 g | | | *P* value[a] |
|---|---|---|---|---|---|---|---|
| | GH 0 (n = 19) Group A | GH 3 μg (n = 17) Group B | GH 5 μg (n = 20) Group C | GH 0 (n = 19) Group D | GH 3 μg (n = 15) Group E | GH 5 μg (n = 16) Group F | |
| Age (yr) | 72.7 ± 5.1 | 71.4 ± 4.1 | 70.0 ± 4.1 | 69.9 ± 4.5 | 69.2 ± 2.5 | 70.8 ± 3.8 | 0.24[b] |
| Ethnicity/race | | | | | | | |
| Caucasian | 14 (74%) | 17 (100%) | 18 (90%) | 14 (74%) | 13 (87%) | 16 (100%) | 0.27 |
| Minority | 5 (26%) | 0 (0%) | 2 (10%) | 5 (26%) | 2 (13%) | 0 (0%) | |
| Hormones | | | | | | | |
| Total testosterone (ng/dl) | 385 ± 107 | 383 ± 101 | 373 ± 89 | 355 ± 97 | 372 ± 85 | 302 ± 90 | 0.13 |
| IGF-I (ng/ml) | 101 ± 23 | 108 ± 25 | 115 ± 31 | 108 ± 29 | 130 ± 31 | 112 ± 32 | 0.10[b] |
| Body composition | | | | | | | |
| Weight (kg) | 79.1 ± 10.4 | 81.6 ± 12.9 | 85.7 ± 11.2 | 85.1 ± 13.9 | 85.4 ± 14.5 | 83.2 ± 11.6 | 0.54 |
| BMI (kg/m$^2$) | 26.8 ± 3.5 | 26.1 ± 3.0 | 28.2 ± 3.2 | 28.4 ± 3.8 | 27.2 ± 3.4 | 27.3 ± 3.3 | 0.33 |
| Total LBM (kg) | 55.6 ± 5.0 | 58.7 ± 8.2 | 59.2 ± 8.1 | 58.9 ± 6.2 | 58.0 ± 6.9 | 58.3 ± 4.9 | 0.60 |
| Appendicular LBM (kg) | 24.2 ± 2.4 | 25.7 ± 3.7 | 26.0 ± 3.7 | 25.7 ± 2.8 | 25.3 ± 3.4 | 25.6 ± 2.4 | 0.56 |
| Muscle performance | | | | | | | |
| $VO_2$ peak (ml/kg · min) | 23.9 ± 6.2 | 26.4 ± 4.0 | 24.1 ± 3.5 | 24.3 ± 6.9 | 23.9 ± 3.9 | 25.3 ± 4.2 | 0.72 |
| P3NP (ng/ml) | 4.7 ± 1.2 | 4.4 ± 1.0 | 4.5 ± 0.9 | 4.7 ± 1.1 | 4.2 ± 1.0 | 4.4 ± 1.0 | 0.76 |

Data are mean ± SD. $VO_2$, $O_2$ consumption.

[a] ANOVA for continuous variables and $\chi^2$ test for discrete variables (*e.g.* ethnicity) was used.

[b] From Kruskal-Wallis test.

Baseline serum P3NP concentrations were similar in the six treatment groups (Table 2). P3NP levels increased during treatment and were significantly higher at wk 4, 8, and 16 compared with baseline. In pairwise comparisons and using Wald trend analysis, the increases in P3NP levels became greater over time (time trend $P < 0.0001$) (Table 2). P3NP concentrations were higher during wk 16 than at baseline ($P < 0.0001$) or wk 4 ($P < 0.0001$) but did not differ from those at wk 8. In men receiving testosterone alone (groups A and D), P3NP levels reached a plateau at wk 4 and did not differ between wk 4 and 8. However, in men treated with testosterone plus any dose of rhGH, wk 8 P3NP levels were significantly higher than wk 4 levels (Table 2).

Changes in P3NP from baseline to wk 4, 8, and 16 were significantly correlated with changes in LBM; the highest correlations were observed with LBM change from baseline to wk 16 (r = 0.53, $P < 0.0001$) as well as with changes in ASM from baseline to wk 16 (r = 0.40, $P < 0.0001$) (Table 3 and Fig. 1) When subjects were categorized into high responders and low responders based on whether the change in LBM from baseline to wk 16 was greater than or less than the median change (1.4 kg), the change in P3NP was significantly higher in the high re-

**TABLE 2.** Change in Serum P3NP levels (ng/ml) by treatment group and duration of treatment

| Body composition | Testosterone 5 g/day | | | Testosterone 10 g/day | | | Total (n = 106) | *P* values ANOVA (trend)[a] |
|---|---|---|---|---|---|---|---|---|
| | GH 0[a] (n = 19) Group A | GH 3 μg (n = 17) Group B | GH 5 μg (n = 20) Group C | GH 0 (n = 19) Group D | GH 3 μg (n = 15) Group E | GH 5 μg (n = 16) Group F | | |
| Baseline to wk 4 | | | | | | | | |
| Baseline | 4.70 ± 1.20 | 4.41 ± 1.05 | 4.48 ± 0.93 | 4.70 ± 1.10 | 4.21 ± 1.04 | 4.44 ± 1.03 | 4.51 ± 1.05 | 0.76 (0.44) |
| Week 4 | 6.11 ± 1.56 | 6.09 ± 1.92 | 7.00 ± 2.26 | 6.39 ± 2.10 | 5.99 ± 1.63[a] | 8.08 ± 2.73[b] | 6.61 ± 2.14 | 0.04 (0.04) |
| Change | 1.41 ± 1.84[a] | 1.68 ± 1.93 | 2.52 ± 2.04 | 1.69 ± 1.74[a] | 1.78 ± 1.80 | 3.63 ± 2.38[b] | 2.10 ± 2.05 | 0.01 (0.009) |
| *P* value[b] | 0.004[b] | 0.002 | <0.0001 | 0.0005 | 0.002 | <0.0001 | <0.0001 | |
| Baseline to wk 8 | | | | | | | | |
| Week 8 | 6.15 ± 1.36[a,c] | 7.22 ± 1.93[a] | 10.12 ± 4.60[d] | 6.45 ± 2.48[a,c] | 7.35 ± 2.35[a] | 11.51 ± 4.47[b] | 8.10 ± 3.65 | <0.0001 (0.0003) |
| Change | 1.45 ± 1.86[a,c] | 2.81 ± 1.87[a] | 5.64 ± 4.37[d] | 1.75 ± 2.09[a,c] | 3.14 ± 2.50[a] | 7.07 ± 4.22[b] | 3.60 ± 3.59 | <0.0001 (<0.0001) |
| *P* value | 0.003 | <0.0001 | <0.0001 | 0.002 | 0.0003 | <0.0001 | <0.0001 | |
| Baseline to wk 16 | | | | | | | | |
| Week 16 | 6.30 ± 1.82[a,c] | 7.46 ± 2.84[a] | 10.07 ± 4.17[d] | 6.41 ± 1.73[a,c] | 8.01 ± 2.27[a] | 11.51 ± 3.63[b] | 8.24 ± 3.42 | <0.0001 (<0.0001) |
| Change | 1.60 ± 1.90[a,c] | 3.05 ± 2.32[a] | 5.59 ± 3.81[d] | 1.71 ± 1.47[a,c] | 3.80 ± 2.50[a] | 7.06 ± 3.57[b] | 3.74 ± 3.33 | <0.0001 (<0.0001) |
| *P* value | 0.002 | <0.0001 | <0.0001 | <0.0001 | <0.0001 | <0.0001 | <0.0001 | |

Data are means ± SD. Pairs of groups with *different letters* (*e.g.* a or b) are significantly different using the Tukey pairwise comparison procedure ($P < 0.05$).

[a] One-way ANOVA across treatment groups and Wald trend analysis across treatment groups.

[b] Paired *t* test.

**TABLE 3.** Univariate relationships of change in P3NP from baseline and changes in LBM, ASM, and hormone levels

| Variable | r (Pearson) | *P* |
|---|---|---|
| Δ Total LBM[a] | | |
| Δ P3NP at wk 4[b] | 0.26 | 0.007 |
| Δ P3NP at wk 8 | 0.36 | 0.0002 |
| Δ P3NP at wk 16 | 0.53 | <0.0001 |
| Δ ASM | | |
| Δ P3NP at wk 4 | 0.17 | 0.07 |
| Δ P3NP at wk 8 | 0.25 | 0.01 |
| Δ P3NP at wk 16 | 0.40 | <0.0001 |
| Δ Testosterone levels | | |
| Δ P3NP at wk 4 | 0.26 | 0.007 |
| Δ P3NP at wk 8 | 0.22 | 0.02 |
| Δ P3NP at wk 16 | 0.29 | 0.003 |
| Δ IGF-I | | |
| Δ P3NP at wk 4 | 0.28 | 0.004 |
| Δ P3NP at wk 8 | 0.50 | <0.0001 |
| Δ P3NP at wk 16 | 0.65 | <0.0001 |

[a] Change was calculated as wk 16 minus baseline for all four predictors.

[b] Change was calculated as wk 4, 8, or 16 value minus baseline value.

sponders than in the low responders at 4 ($P < 0.004$), 8 ($P < 0.001$), and 16 (P0.0001) wk, respectively (Table 4). Similarly, men who experienced more than the median change in ASM demonstrated greater increments in P3NP levels than those who gained less than the median change (Table 4). By logistic regression, the odds of a greater change in P3NP from baseline to wk 16 were substantially higher in men in the highest quartiles of change in LBM [odds ratio 9.0, 95% confidence interval (CI) 2.6–31.8] or change in ASM from baseline (odds ratio 4.6, 95% CI 1.4–15.2) than those in the lowest quartile. Thus, changes in P3NP were strongly associated with changes in LBM and ASM.

Importantly, changes in P3NP concentrations from baseline to wk 4 (r = 0.26, $P = 007$) and wk 8 (r = 0.36, $P = 0.0002$) were significantly correlated with change in LBM from baseline to wk 16 (Fig. 1C). Similarly, changes in P3NP concentrations from baseline to wk 4 (r = 0.17, $P = 0.07$) and 8 (r = 0.25, $P = 0.01$) were predictive of changes in ASM from baseline to wk 16.

We examined the relationship of P3NP with changes in composite strength of upper- and lower-body muscle groups. In univariate regression analysis, there was a marginal correlation of 0.20 ($P = 0.056$; Spearman) for change in P3NP at wk 4 and change in composite strength at wk 16. We examined separately the relationship of change in P3NP at wk 4 with changes in composite strength in 39 men who received testosterone alone (study groups A and D); in these men, there was a significant correlation between increases in P3NP at wk 4 and improvements in strength (r = 0.36, $P = 0.041$).

The changes in P3NP concentrations from baseline to wk 4, 8, and 16 were correlated significantly with changes in testosterone concentrations (Table 3 and Fig. 2A) from baseline to wk 16. The changes in P3NP levels from baseline to wk 4, 8, and 16 were significantly higher in men who experienced greater increase in testosterone levels (greater than median) than in those who had lesser increase (less than median) (Table 4). By logistic regression, odds of greater change in P3NP were significantly higher in men in the highest quartile of change in testosterone levels than those in the lowest quartile of change in testosterone levels (OR 5.3, 3.3, and 2.3 for baseline to wk 4, 8, and 16 change in P3NP).

Changes in P3NP concentrations were also correlated significantly with changes in IGF-I concentrations (Table 3 and Fig. 2B). The changes in P3NP from baseline to wk 16 were higher in participants who had greater than the median change in IGF-I than in those who had less than the median change (Table 4). Even more strikingly, men in the highest quartile for IGF-I change had substantially greater likelihood of having greater change in P3NP than those in the lowest quartile (OR 12.9 and 150.0 for high P3NP response from baseline to wk 8 and 16, respectively).

We performed univariate and multivariate regression analyses to examine the determinants of change in P3NP levels from baseline. Baseline BMI, testosterone and IGF-I levels, LBM, and ASM were not significantly associated with either baseline P3NP levels or changes in P3NP levels. Age was inversely related to change in P3NP from baseline to wk 16 in both univariate and multivariate models, after adjusting for baseline BMI, LBM, ASM, testosterone, IGF-I, and change in testosterone and IGF-I. In multivariate analyses, change in IGF-I level from baseline emerged as the most robust predictor of change in P3NP levels, which was significantly associated with change in P3NP ($P < 0.0001$), even after adjusting for BMI, age, LBM, ASM, testosterone, and IGF-I, and changes in testosterone and changes in LBM.

The Pearson coefficients of the association of changes in P3NP (r = 0.53, $P < 0.0001$) and IGF-I (r = 0.40, $P < 0.0001$) with change in LBM were slightly better for P3NP than for IGF-I. To determine the relative ability of the two markers in predicting change in LBM and ASM, we performed stepwise regression using change in LBM or ASM from baseline to wk 16 as the dependent variable and changes in P3NP, IGF-I, age, and change in testosterone levels as independent variables; these independent variables were selected because in univariate analyses, they were correlated with the dependent variable at $P < 0.20$. In these models, change in P3NP from baseline to wk 8 or 16 was the strongest predictor of change in LBM and ASM (Table 5 and 6). Age and change in testosterone levels added

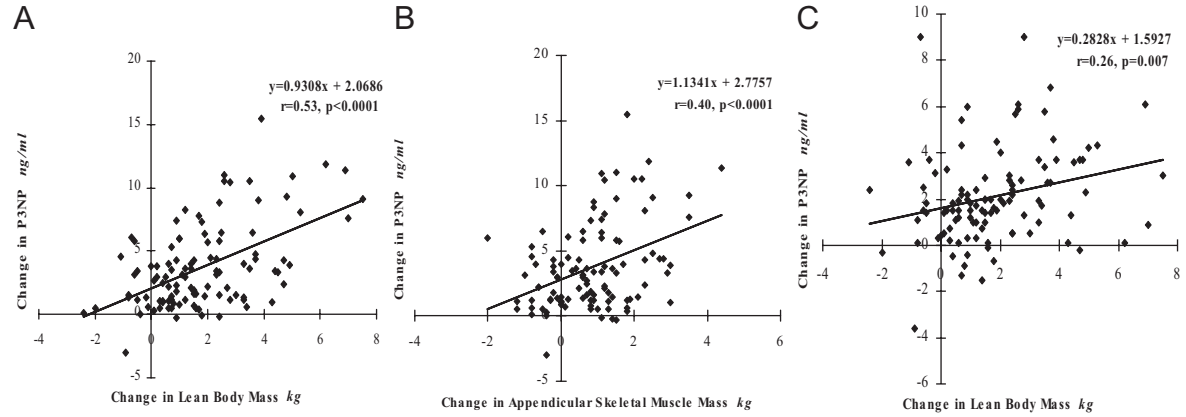


**FIG. 1.** A, Regression plot of change in total lean body mass (kilograms) from baseline to wk 16 relative to change in serum P3NP concentrations (nanograms per milliliter) from baseline to wk 16. B, Change in appendicular lean mass (kilograms) from baseline to wk 16 was plotted against change in serum P3NP concentrations (nanograms per milliliter) from baseline to wk 16. C, Change in lean body mass (kilograms) from baseline to wk 16 was plotted against change in serum P3NP concentrations (nanograms per milliliter) from baseline to wk 4 (n = 106 for all three panels).

only marginally to the predictive ability of the model and IGF-I did not add significantly to the model ($P > 0.10$).

To test for interaction between testosterone and IGF-I relative to the changes in P3NP, we conducted a two-way ANOVA; this analysis revealed a marginal interaction at wk 16 ($P = 0.06$; data not shown). In the stratified analysis at wk 16, there was a significant effect of testosterone dose in the high IGF-I group ($P = 0.01$); additionally, IGF-I/rhGH had significant effects on P3NP at both the high ($P = 0.0004$) and low testosterone dose ($P < 0.0001$).

Because changes in LBM, testosterone, and IGF-I levels are interrelated, we performed linear stepwise regression

**TABLE 4.** Change in P3NP levels (nanograms per milliliter) in high and low responders compared with changes in LBM, ASM, and hormone levels

| | Δ Total LBM (kg) | | |
|---|---|---|---|
| | Low (−2.4–1.4 kg) (n = 51) | High (1.5–7.5 kg) (n = 55) | *P* (*t* test) |
| Δ P3NP at wk 4 | 1.51 ± 1.97 | 2.65 ± 1.99 | 0.004 |
| Δ P3NP at wk 8 | 2.47 ± 2.34 | 4.65 ± 4.21 | 0.001 |
| Δ P3NP at wk 16 | 2.49 ± 2.16 | 4.90 ± 3.79 | 0.0001 |
| | Δ ASM | | |
| | Low (−2.0 to 0.8 kg) (n = 54) | High (0.9–4.4 kg) (n = 52) | *P* (*t* test) |
| Δ P3NP at wk 4 | 1.83 ± 1.96 | 2.39 ± 2.12 | 0.16 |
| Δ P3NP at wk 8 | 2.62 ± 2.25 | 4.61 ± 4.39 | 0.005 |
| Δ P3NP at wk 16 | 2.56 ± 2.02 | 4.97 ± 3.94 | 0.002 |
| | Δ Testosterone (nanograms per deciliter) | | |
| | Low (−497 to 174 ng/dl) (n = 52) | High (175–1854 ng/dl) (n = 54) | *P* (*t* test) |
| Δ P3NP at wk 4 | 1.53 ± 1.59 | 2.65 ± 2.30 | 0.004 |
| Δ P3NP at wk 8 | 2.65 ± 2.29 | 4.54 ± 4.33 | 0.005 |
| Δ P3NP at wk 16 | 2.68 ± 2.41 | 4.76 ± 3.77 | 0.001 |
| | Δ IGF-I (nanograms per milliliter) | | |
| | Low (−55 to 44 ng/ml) (n = 52) | High (48–226 ng/ml) (n = 54) | *P* (*t* test) |
| Δ P3NP at wk 4 | 1.57 ± 1.83 | 2.64 ± 2.13 | 0.007 |
| Δ P3NP at wk 8 | 2.00 ± 2.04 | 5.20 ± 4.09 | <0.0001 |
| Δ P3NP at wk 16 | 1.88 ± 1.81 | 5.59 ± 3.48 | <0.0001 |

Δ P3NP was calculated as the difference between posttreatment level (*e.g.* wk 4, 8, or 16 value) and baseline P3NP level. The subjects were categorized as low or high responders, depending on whether the change in the respective variable from baseline to wk 16 was less or more than the median change.

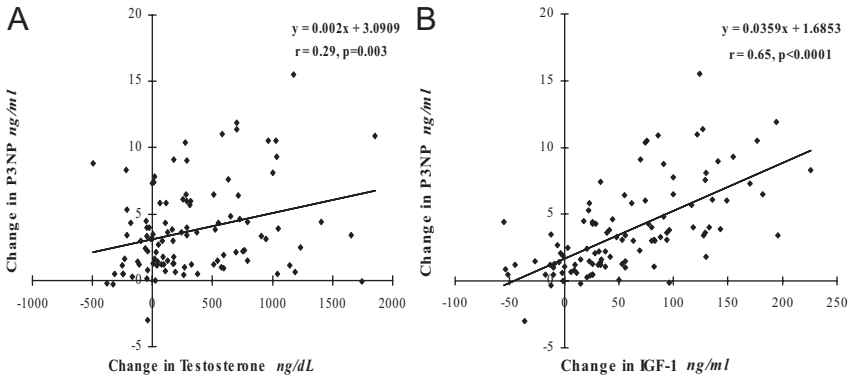


**FIG. 2.** Figure shows regression plots of the changes in serum P3NP after 16 wk relative to changes in serum testosterone (A) and serum IGF-I (B) after 16 wk of therapy.

to determine the relative contribution of each of these variables to the variance of change in P3NP levels. We included change in LBM, IGF-I, and testosterone and age in this model because each of these variables was associated with change in P3NP in univariate analyses with $P < 0.20$. In stepwise regression, change in LBM was the best predictor of change in P3NP that alone explained 28% of variance of change in P3NP level. When added after entering change in LBM to the model, change in IGF-I explained 22% of the additional variance in change in P3NP levels, and change in testosterone levels and age were not significant contributors. Similarly, when we used change in ASM as the first variable in stepwise regression model, 16% of variance was explained by this variable. Change in IGF-I explained an additional 31% of the variance, and other variables did not add to the predictive ability of the model.

**TABLE 5.** Stepwise regression of the predictors of change in LBM

| DV = Δ LBM wk 16 | Parameter estimate | Partial $r^2$* | *P* |
|---|---|---|---|
| Model 1 | | | |
| Δ P3NP wk 8 | 0.14 | 0.13 | 0.006 |
| Baseline age | −0.09 | 0.04 | 0.02 |
| Δ Testosterone wk 8 | 0.001 | 0.04 | 0.03 |
| Model 2 | | | |
| Δ P3NP wk 16 | 0.24 | 0.30 | <0.0001 |
| Δ Testosterone wk 16 | −0.07 | 0.02 | 0.08 |
| Baseline age | 0.001 | 0.05 | 0.004 |

To determine the relative contribution of variables that predict change in total lean body mass (LBM) from baseline to wk 16, we performed stepwise regression. The dependent variable (DV) was change in LBM. The independent variables that were included in the analyses were change in P3NP from baseline to wk 8 (model 1) or change in P3NP from baseline to wk 16 (model 2), change in IGF-I or testosterone levels and age because their adjusted $P < 0.2$. P3NP emerged as the strongest predictor of change in the LBM model. IGF-I was not a significant contributor to the models ($P > 0.10$) after adjusting for all previously selected variables. *, Partial $r^2$ is the additional contribution of a variable in predicting change in LBM, adjusting for prior variables in stepwise regression.

We sought to determine the magnitude of change in P3NP concentrations that was associated with each kilogram gain in LBM and 0.5 kg gain in ASM; this magnitude of change in P3NP might arguably be deemed the MCID in P3NP concentration. The bootstrapping method revealed that each kilogram change in LBM from baseline to wk 16 was associated with 0.93 ng/ml (95% CI 0.83–1.02) change in P3NP concentration from baseline to wk 16 (Fig. 3A) and 0.28 ng/ml (95% CI 0.22–0.36) change in P3NP from baseline to wk 4 (Fig. 3B). Similarly, 0.5 kg change in ASM was associated with 0.57 ng/ml (95% CI 0.57–0.57) change in P3NP from baseline to wk 16 and 0.15 ng/ml (95% CI 0.15–0.16) change from baseline to wk 4.

## Discussion

Changes in serum P3NP levels are predictive of changes in total LBM and ASM in community-dwelling older men administered testosterone with or without rhGH. Importantly, changes in P3NP levels at wk 4 were predictive of changes in LBM and ASM at wk 16. Subjects who experienced greater gains in LBM and ASM during testosterone and rhGH administration were more likely to have greater increments in P3NP levels. In stepwise regression models, change in P3NP levels from baseline was the best predictor of changes in LBM and ASM. These data indicate that increases in serum P3NP concentrations may serve as a useful early biomarker of lean

**TABLE 6.** Stepwise regression of the predictors of change in ASM

| DV = Δ ASM wk 16 | Parameter estimate | Partial $r^2$* | *P* |
|---|---|---|---|
| Model 1 | | | |
| Δ P3NP wk 8 | 0.08 | 0.06 | 0.01 |
| Model 2 | | | |
| Δ P3NP wk 16 | 0.26 | 0.28 | <0.0001 |
| Δ Testosterone wk 16 | 0.001 | 0.05 | 0.005 |

To determine the relative contribution of variables that predict change in total ASM from baseline to wk 16, we performed stepwise regression. The dependent variable (DV) was change in ASM. The independent variables that were included in the analyses were change in P3NP from baseline to wk 8 (model 1) or change in P3NP from baseline to wk 16 (model 2), change in IGF-I, or testosterone levels and age because their adjusted $P < 0.2$. P3NP emerged as the strongest predictor of change in the ASM model. IGF-I was not a significant contributor to the models ($P > 0.10$) after adjusting for all previously selected variables. *, Partial $r^2$ is the additional contribution of a variable in predicting change in LBM, adjusting for prior variables in stepwise regression.

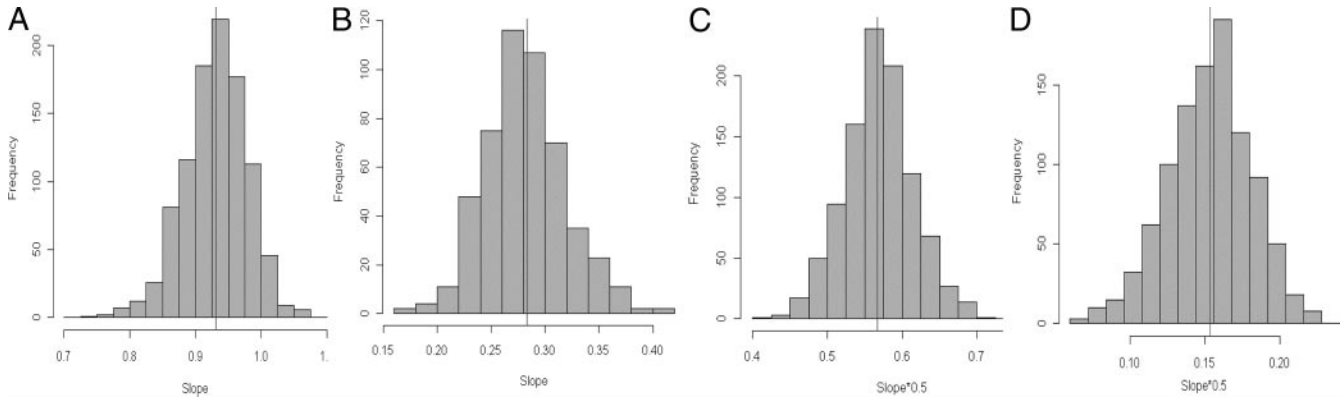


**FIG. 3.** To determine the magnitude of change in P3NP concentrations that was associated with changes in lean mass, we used the boot strapping method in which each bootstrap sample set contained 90% of the original sample randomly drawn from the original 106 samples without replacement. A and B, Histograms show the distribution of the slopes (change in P3NP for each kilogram change in total LBM from baseline to wk 16). A, Each kilogram change in LBM was associated with 0.93 ng/ml (95% CI 0.83–1.02) change in P3NP concentration from baseline to wk 16. B, Each kilogram change in LBM was associated with 0.28 ng/ml (95% CI 0.22–0.36) change in P3NP from baseline to wk 4. C and D, Histograms show the distribution of the slopes (change in P3NP for each 0.5 kg change in ASM). C, Each 0.5 kg change in ASM was associated with 0.57 ng/ml (95% CI 0.57, 0.57) change in P3NP from baseline to wk 16. D, Each 0.5 kg change in ASM was associated with 0.15 ng/ml (95% CI 0.15, 0.16) change in P3NP from baseline to wk 4.

body mass improvements during testosterone supplementation either alone or in combination with rhGH administration.

The time and expense associated with efficacy trials of promyogenic therapies are major retardants in the drug development process. Randomized trials of 6–12 months duration may be needed to demonstrate efficacy. The availability of robust early predictive biomarkers of an anabolic response would accelerate human studies of anabolic therapies. Ultimately, the efficacy of function promoting anabolic therapies will have to be evaluated in terms of improvements in physical function measures and health outcomes. However, to the extent that promyogenic molecules currently in development, such as testosterone, selective androgen receptor modulators, myostatin antagonists, and rhGH, act primarily by increasing skeletal muscle mass, biomarkers, such as P3NP, that are predictive of subsequent skeletal muscle mass gains could expedite screening of candidate compounds in humans.

In participants receiving only testosterone, increases in P3NP at wk 4 were significantly associated with improvements in composite strength of the major muscle groups in the upper and lower extremities. This suggests that testosterone-induced increases in P3NP reflect subsequent increases in functional myofibrillar proteins and not just general fibrosis or remodeling of other soft lean tissues. It is possible that P3NP increase during rhGH administration may reflect synthesis of nonmyofibrillar proteins or remodeling of other tissues, consistent with the failure of previous rhGH studies to demonstrate muscle strength gains (30–32). We recognize that some classes of function promoting therapies, such as vitamin D, may improve physical function by different mechanisms and serum markers of LBM change, such as P3NP, may not be useful biomarkers for those drugs.

Previous studies reported that P3NP levels increase rapidly after administration of rhGH and these elevations in P3NP levels persist for several days after rhGH discontinuation (10, 14). In our study, serum P3NP levels were significantly elevated above baseline at 4 wk. The men receiving testosterone alone demonstrated a plateau in P3NP levels by wk 4, but the plateau was not reached until wk 8 in those receiving testosterone plus rhGH. Because we did not have a rhGH-alone group, the differences in time course of P3NP response to testosterone and rhGH need confirmation. However, wk 8 and 16 values were not significantly different, suggesting that a plateau had been reached by 8 wk in all men.

P3NP has been explored as a marker of GH doping in athletes (6, 10, 12, 13, 15, 17) and shown to respond to exercise (11) and testosterone (10). In previous studies, administration of supraphysiological doses of rhGH has been associated with significant elevations of P3NP levels (10), which persisted for 8 wk after GH discontinuation (12). A multinational team, the GH-2000, has proposed the combined application of IGF-I and P3NP for detection of rhGH doping (13, 15, 16). P3NP levels rise after a bone fracture or soft tissue muscle injury (9, 33–35). P3NP response to rhGH administration may be affected by the age, gender, and stage of pubertal maturation, whereas ethnicity has only a small effect (6, 17, 36). Our data show that P3NP also increased after testosterone administration in men who did not receive rhGH; thus, the P3NP response is not unique to rhGH. Furthermore, our data provide the first evidence that changes in P3NP are robustly associated

with changes in LBM and ASM during testosterone administration with or without rhGH. Similar to the data reported from the GH-2000 study (12–14), we found that changes in IGF-I levels were robustly associated with changes in P3NP levels. Because rhGH administration and higher levels of testosterone increase circulating IGF-I levels (37, 38), it is possible that the effects of both anabolic agents on skeletal muscle might be mediated through IGF-I. The relative roles of testosterone and IGF-I in regulation of circulating P3NP levels should be investigated. We cannot determine from these data whether the changes in P3NP reflect changes in P3NP production or clearance.

P3NP meets many criteria for an early biomarker (39). It can be measured with precision and accuracy, it responds rapidly to anabolic interventions, early changes in P3NP predict subsequent lean mass acquisition, and there is biological plausibility linking change in P3NP and lean mass acquisition. It is possible that a minimal clinically important difference for change in lean body mass could be predicted from early changes in P3NP. In a metaanalysis, testosterone replacement of androgen-deficient men was associated with about 1.2 kg gain in lean body mass (1). Similarly, the first-generation trials of selective androgen receptor modulators have revealed 1.0–1.5 kg increase in LBM during selective androgen receptor modulator administration (40). In our study, at 16 wk, each kilogram of change in LBM was associated with 0.93 ng/ml increase in P3NP. Thus, P3NP has the characteristics of a potentially valuable biomarker of skeletal muscle mass acquisition in early-phase trials of function promoting anabolic therapies that act by increasing skeletal muscle mass.

## Acknowledgments

We thank Drs. Jagadish Ulloor and Anqi Zhang for the hormone analyses, and General Clinical Research Center staff for their assistance with the study, and Drs. David Shaywitz and Ken Baldwin for their helpful discussions.

Address all correspondence and requests for reprints to: Shalender Bhasin, M.D., Professor of Medicine and Director, Boston Claude D. Pepper Older Americans Independence Center for Function Promoting Therapies, 670 Albany Street, Second Floor, Boston Medical Center, Boston, Massachusetts 02118. E-mail: bhasin@bu.edu.

This work was supported by National Institutes of Health Grants R01AG18169 and M0I RR000043 at University of Southern California, Grant M01 RR000036 at Washington University School of Medicine, and the U.S. Department of Agriculture (USDA) ARS Cooperative Agreement 58-1950-9-001; the National Center for Research Resources General Clinical Research Center Grant M01 RR000054 at Tufts University, where any opinions, findings, conclusions, or recommendations expressed in this publication are those of the authors and do not necessarily reflect the view of the USDA; and Grants 1RO1AG22356 and 5P30AG031679 at Boston Medical Center. The study therapies were provided by Solvay Pharmaceuticals, Genentech Inc., and Tap Pharmaceutical Inc.

Disclosure Summary: F.R.S. and S.B. have received grant support from Solvay Pharmaceuticals and Merck, Inc. for research that is unrelated to the study reported in this manuscript. S.B. and K.Y. have received new grant support from the National Institutes of Health unrelated to this study. E.F.B. has received grant support from Solvay Pharmaceuticals. The P3NP assays were supported by Merck and Co. G.T.O., A.R., F.C., R.L., and J.A.T. are Merck employees. E.J.H., M.K., E.T.S., and C.C.-S. have nothing to declare.

## References

1. **Bhasin S, Calof OM, Storer TW, Lee ML, Mazer NA, Jasuja R, Montori VM, Gao W, Dalton JT** 2006 Drug insight: testosterone and selective androgen receptor modulators as anabolic therapies for chronic illness and aging. Nat Clin Pract 2:146–159
2. **Niemelä O** 1985 Radioimmunoassays for type III procollagen aminoterminal peptides in humans. Clin Chem 31:1301–1304
3. **Niemelä O, Risteli L, Parkkinen J, Risteli J** 1985 Purification and characterization of the N-terminal propeptide of human type III procollagen. Biochem J 232:145–150
4. **Prockop DJ, Kivirikko KI, Tuderman L, Guzman NA** 1979 The biosynthesis of collagen and its disorders (first of two parts). N Engl J Med 301:13–23
5. **Nguyen TV, Nelson AE, Howe CJ, Seibel MJ, Baxter RC, Handelsman DJ, Kazlauskas R, Ho KK** 2008 Within-subject variability and analytic imprecision of insulinlike growth factor axis and collagen markers: implications for clinical diagnosis and doping tests. Clin Chem 54:1268–1276
6. **Nelson AE, Howe CJ, Nguyen TV, Leung KC, Trout GJ, Seibel MJ, Baxter RC, Handelsman DJ, Kazlauskas R, Ho KK** 2006 Influence of demographic factors and sport type on GH-responsive markers in elite athletes. J Clin Endocrinol Metab 91:4424–4432
7. **Danne T, Grüters A, Schuppan D, Quantas N, Enders I, Weber B** 1989 Relationship of procollagen type III propeptide-related antigens in serum to somatic growth in healthy children and patients with growth disorders. J Pediatr 114:257–260
8. **Verde GG, Santi I, Chiodini P, Cozzi R, Dallabonzana D, Oppizzi G, Liuzzi A** 1986 Serum type III procollagen propeptide levels in acromegalic patients. J Clin Endocrinol Metab 63:1406–1410
9. **Erotokritou-Mulligan I, Bassett EE, Bartlett C, Cowan D, McHugh C, Seah R, Curtis B, Wells V, Harrison K, Sönksen PH, Holt RI** 2008 The effect of sports injury on insulin-like growth factor-I and type 3 procollagen: implications for detection of GH abuse in athletes. J Clin Endocrinol Metab 93:2760–2763
10. **Nelson AE, Meinhardt U, Hansen JL, Walker IH, Stone G, Howe CJ, Leung KC, Seibel MJ, Baxter RC, Handelsman DJ, Kazlauskas R, Ho KK** 2008 Pharmacodynamics of GH abuse biomarkers and the influence of gender and testosterone: a randomized double-blind placebo-controlled study in young recreational athletes. J Clin Endocrinol Metab 93:2213–2222
11. **Garma T, Kobayashi C, Haddad F, Adams GR, Bodell PW, Baldwin KM** 2007 Similar acute molecular responses to equivalent volumes of isometric, lengthening, or shortening mode resistance exercise. J Appl Physiol 102:135–143
12. **Longobardi S, Keay N, Ehrnborg C, Cittadini A, Rosén T, Dall R, Boroujerdi MA, Bassett EE, Healy ML, Pentecost C, Wallace JD, Powrie J, Jørgensen JO, Saccà L** 2000 GH (GH) effects on bone and collagen turnover in healthy adults and its potential as a marker of

GH abuse in sports: a double blind, placebo-controlled study. The GH-2000 Study Group. J Clin Endocrinol Metab 85:1505–1512
13. **Erotokritou-Mulligan I, Bassett EE, Kniess A, Sönksen PH, Holt RI** 2007 Validation of the GH (GH)-dependent marker method of detecting GH abuse in sport through the use of independent data sets. Growth Horm IGF Res 17:416–423
14. **Wallace JD, Cuneo RC, Lundberg PA, Rosén T, Jørgensen JO, Longobardi S, Keay N, Sacca L, Christiansen JS, Bengtsson BA, Sönksen PH** 2000 Responses of markers of bone and collagen turnover to exercise, GH (GH) administration, and GH withdrawal in trained adult males. J Clin Endocrinol Metab 85:124–133
15. **Powrie JK, Bassett EE, Rosen T, Jørgensen JO, Napoli R, Sacca L, Christiansen JS, Bengtsson BA, Sönksen PH** 2007 Detection of GH abuse in sport. Growth Horm IGF Res 17:220–226
16. **McHugh CM, Park RT, Sönksen PH, Holt RI** 2005 Challenges in detecting the abuse of GH in sport. Clin Chem 51:1587–1593
17. **Erotokritou-Mulligan I, Bassett EE, Cowan DA, Bartlett C, McHugh C, Sönksen PH, Holt RI** 2009 Influence of ethnicity on IGF-I and procollagen III peptide (P-III-P) in elite athletes and its effect on the ability to detect GH abuse. Clin Endocrinol (Oxf) 70: 161–168
18. **Sattler F, Castaneda-Sceppa C, Binder E, Schroeder E, Wang Y, Bhasin S, Kawakubo M, Stewart Y, Yarasheski K, Ulloor J, Colletti P, Roubenoff R, Azen S** 2009 Testosterone and GH improve body composition and muscle performance in older men. J Clin Endocrinol Metab 94:1991–2001
19. **Schroeder ET, Castaneda-Sceppa C, Wang Y, Binder EF, Kawakubo M, Stewart Y, Storer T, Roubenoff R, Bhasin S, Yarasheski KE, Sattler FR, Azen SP** 2007 Hormonal Regulators of Muscle and Metabolism in Aging (HORMA): design and conduct of a complex, double masked multicenter trial. Clin Trials (London, England) 4:560–571
20. **Swerdloff RS, Wang C, Cunningham G, Dobs A, Iranmanesh A, Matsumoto AM, Snyder PJ, Weber T, Longstreth J, Berman N** 2000 Long-term pharmacokinetics of transdermal testosterone gel in hypogonadal men. J Clin Endocrinol Metab 85:4500–4510
21. **Lucidi P, Lauteri M, Laureti S, Celleno R, Santoni S, Volpi E, Angeletti G, Santeusanio F, De Feo P** 1998 A dose-response study of GH (GH) replacement on whole body protein and lipid kinetics in GH-deficient adults. J Clin Endocrinol Metab 83:353–357
22. **Toogood AA, Shalet SM** 1999 GH replacement therapy in the elderly with hypothalamic-pituitary disease: a dose-finding study. J Clin Endocrinol Metab 84:131–136
23. **Amato G, Mazziotti G, Di Somma C, Lalli E, De Felice G, Conte M, Rotondi M, Pietrosante M, Lombardi G, Bellastella A, Carella C, Colao A** 2000 Recombinant GH (GH) therapy in GH-deficient adults: a long-term controlled study on daily versus thrice weekly injections. J Clin Endocrinol Metab 85:3720–3725
24. **Fleck S** 1997 Designing resistance training programs. In: Human kinetics. 2nd ed. Human Kinetics Publishers: Champaign, IL; 98–100
25. **Schroeder ET, Wang Y, Castaneda-Sceppa C, Cloutier G, Vallejo AF, Kawakubo M, Jensky NE, Coomber S, Azen SP, Sattler FR** 2007 Reliability of maximal voluntary muscle strength and power testing in older men. J Gerontol 62:543–549
26. **Singh AB, Lee ML, Sinha-Hikim I, Kushnir M, Meikle W, Rockwood A, Afework S, Bhasin S** 2006 Pharmacokinetics of a testosterone gel in healthy postmenopausal women. J Clin Endocrinol Metab 91:136–144
27. **Sir-Petermann T, Codner E, Pérez V, Echiburú B, Maliqueo M, Ladrón de Guevara A, Preisler J, Crisosto N, Sánchez F, Cassorla F, Bhasin S** 2009 Metabolic and reproductive features before and during puberty in daughters of women with polycystic ovary syndrome. J Clin Endocrinol Metab 94:1923–1930
28. **Risteli J, Niemi S, Trivedi P, Mäentausta O, Mowat AP, Risteli L** 1988 Rapid equilibrium radioimmunoassay for the amino-terminal propeptide of human type III procollagen. Clin Chem 34:715–718
29. **Deley G, Kervio G, Van Hoecke J, Verges B, Grassi B, Casillas JM** 2007 Effects of a one-year exercise training program in adults over 70 years old: a study with a control group. Aging Clin Exp Res 19:310–315
30. **Yarasheski KE** 1994 GH effects on metabolism, body composition, muscle mass, and strength. Exercise and sport sciences reviews 22: 285–312
31. **Giannoulis MG, Sonksen PH, Umpleby M, Breen L, Pentecost C, Whyte M, McMillan CV, Bradley C, Martin FC** 2006 The effects of GH and/or testosterone in healthy elderly men: a randomized controlled trial. J Clin Endocrinol Metab 91:477–484
32. **Blackman MR, Sorkin JD, Münzer T, Bellantoni MF, Busby-Whitehead J, Stevens TE, Jayme J, O'Connor KG, Christmas C, Tobin JD, Stewart KJ, Cottrell E, St. Clair C, Pabst KM, Harman SM** 2002 GH and sex steroid administration in healthy aged women and men: a randomized controlled trial. JAMA 288:2282–2292
33. **Haukipuro K, Risteli L, Kairaluoma MI, Risteli J** 1990 Aminoterminal propeptide of type III procollagen in serum during wound healing in human beings. Surgery 107:381–388
34. **Kurdy NM, Bowles S, Marsh DR, Davies A, France M** 1998 Serology of collagen types I and III in normal healing of tibial shaft fractures. J Orthop Trauma 12:122–126
35. **Veitch SW, Findlay SC, Hamer AJ, Blumsohn A, Eastell R, Ingle BM** 2006 Changes in bone mass and bone turnover following tibial shaft fracture. Osteoporos Int 17:364–372
36. **Giannoulis MG, Boroujerdi MA, Powrie J, Dall R, Napoli R, Ehrnborg C, Pentecost C, Cittadini A, Jørgensen JO, Sonksen PH** 2005 Gender differences in GH response to exercise before and after rhGH administration and the effect of rhGH on the hormone profile of fit normal adults. Clin Endocrinol (Oxf) 62:315–322
37. **Hobbs CJ, Plymate SR, Rosen CJ, Adler RA** 1993 Testosterone administration increases insulin-like growth factor-I levels in normal men. J Clin Endocrinol Metab 77:776–779
38. **Bhasin S, Woodhouse L, Casaburi R, Singh AB, Bhasin D, Berman N, Chen X, Yarasheski KE, Magliano L, Dzekov C, Dzekov J, Bross R, Phillips J, Sinha-Hikim I, Shen R, Storer TW** 2001 Testosterone dose-response relationships in healthy young men. Am J Physiol 281:E1172–E1181
39. **Baker 3rd GT, Sprott RL** 1988 Biomarkers of aging. Exp Gerontol 23:223–239
40. **Bhasin S, Jasuja R** 2009 Selective androgen receptor modulators as function promoting therapies. Curr Opin Clin Nutr Metab Care 12:232–240