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Evidence for a decrease in cardiovascular risk factors following recombinant growth hormone administration in abstinent anabolic-androgenic steroid users

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Abstract

Objectives: To determine whether six days recombinant human growth hormone (rhGH) in an abstinent anabolic-androgenic steroid (AAS) group had any cardiovascular and biochemical effects compared with a control group.
Methods: Male subjects ($n = 48$) were randomly divided, using a single blind procedure into two groups: (1) control group (C) $n = 24$, mean ± SD, age 32 ± 11 years; height 1.8 ± 0.06 m; (2) rhGH using group (0.058 IU kg^{-1} day^{-1}) (GH) $n = 24$, mean ± SD, age 32 ± 9 years; height 1.8 ± 0.07 m. Physiological responses, anthropometry, arterial pulse wave velocity (APWV), blood pressure (BP), heart rate (HR), peak oxygen uptake (VO_2 peak) and biochemical indices were investigated.
Results: Body mass index, fat-free mass index and VO_2 peak significantly increased while body fat significantly decreased within GH (all $P < 0.017$). Insulin like growth factor-I significantly increased within GH ($P < 0.017$) and compared with C ($P < 0.05$). Serum sodium significantly increased ($P < 0.017$) and serum homocysteine, high sensitivity C-reactive protein, thyroid stimulating hormone and tetra-iodothyronine (T_4), significantly decreased within GH (all $P < 0.017$). T_4 significantly decreased compared with C ($P < 0.05$).

Arterial pulse wave velocity, peak and recovery systolic and diastolic BP, significantly decreased compared with C ($P < 0.05$). Resting HR and rate pressure product (RPP) significantly increased compared with C ($P < 0.05$).
Conclusion: The findings of this study suggest that short term use of rhGH may have beneficial effects on endothelial function and specific inflammatory markers of cardiovascular disease in abstinent AAS users, but may have an adverse effect on the cardiovascular system, as evidenced by the increase in resting RPP.

Keywords: Arterial pulse wave velocity; Endothelial function; Inflammation; Growth hormone treatment

1. Introduction

Increased stiffness of large elastic arteries leads to elevated systolic blood pressure (SBP) and hence, left ventricular hypertrophy (LVH) [1]. Arterial stiffness is associated with atherosclerosis, hyperlipidaemia [2], coronary heart disease (CHD) [3], stroke [4] and is present

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in healthy young subjects with a family history of myocardial infarction [5] or diabetes mellitus [6]. In hypertensive subjects without a history of overt cardiovascular disease (CVD), arterial pulse wave velocity (APWV, a surrogate measure of stiffness and therefore CHD) independently predicts the occurrence of CV events [7]. Arterial endothelial dysfunction (a cause of increased stiffness) is reversed by recombinant human growth hormone (rhGH) therapy, in growth hormone deficiency (GHD) [8]. RhGH also decreases total cholesterol (TC) and increases high density lipoprotein cholesterol (HDL-C), which favourably influences the risk for atherogenesis [9].

There is a direct correlation between CHD and diabetes and an elevated C-reactive protein (CRP) (an acute-phase protein, secreted by hepatocytes in response to in vivo inflammatory events) [10].

Higher levels of physical activity are consistently associated with lower CRP levels [11]. Both CRP [12] and homocysteine (HCY, a product of methionine metabolism) [13] are directly linked with arterial endothelial dysfunction and increased APWV. In combination, they are significantly related to a history of CVD [14]. Aerobic exercise training increases arterial compliance and reduces systolic blood pressure [15], whereas whole body arterial compliance is lowered in strength-trained individuals [16].

The purpose of this study was to examine the effects of short-term rhGH administration on body composition, and specific CV risk markers in abstinent anabolic–androgenic steroid (AAS) using strength trained athletes and to compare these subjects with age-matched, abstinent AAS using, apparently healthy, strength trained controls.

2. Methods

2.1. Subjects

Approval for the study was obtained from the University ethics committee. Subjects read the details of experimental protocol. Subjects provided written consent prior to data collection. Subjects abstained from taking AAS for a period of 12 weeks, prior to the current investigation. Subjects in both groups followed synchronised training times and intensity, which did not differ. In order to avoid confounding effects on liver and muscle enzymes, they abstained from physical activity for a minimum of 24 h before each testing day. Prior to the start of the investigation, a drug screen was performed on each subject to exclude potential confounding of results by surreptitious use of AAS or other agents. Urinalysis was performed at a world anti-doping agency (WADA) accredited laboratory.

2.2. Study design

Male subjects ($n = 48$) were randomly assigned, using a single blind procedure, into two groups:

(1) control group (C) $n = 24$, mean ± SD, age 32 ± 11 years; height 1.8 ± 0.06 m;
(2) rhGH using group (GH) $n = 24$, mean ± SD, age 32 ± 9 years; height 1.8 ± 0.07 m.

Physiological tests were performed in the same order for both the experimental group and the control group. Subjects were familiarised with testing procedures. Subjects were examined daily over a period of six weeks between the hours of 09:00 and 11:00 and were anonymous to each other. The dosage of rhGH used was 0.058 IU kg^{-1} day^{-1} (0.019 mg kg^{-1} day^{-1}) and an administration diary was recorded. The rhGH certificate of analysis was provided by GeneScience Pharmacuticals Co., Ltd. This experimental design complied with the review of multiple studies by Hjrobartsson and Gotzsche, without the administration of placebo for ethical reasons, when measuring objective outcomes [17].

Subjects were examined prior to the commencement of rhGH administration (day 1), one day after six days administration (day 7), and eight days after cessation (day 14). Dietary intake was strictly monitored, using a fourteen day dietary recall (Nutri-check, Heath Options Ltd, Eastbourne, UK).

2.3. Blood sampling

Phlebotomy was conducted in the fasted state, following 30 min rest in the supine position [18] using the standard venepuncture method (Becton Dickinson, Rutherford, NJ, USA between the hours of 09:00 and 09:30 accounting for diurnal biological variation of male sex hormones [19]. Serum analytes were measured using standard methods.

Serum tetra-iodothyronine (T_4), thyroid stimulating hormone (TSH), cortisol, luteinising hormone (LH), follicle stimulating hormone (FSH), testosterone (T) and prolactin (PRL) were measured with chemiluminescent immunoassay on an Advia Centaur (Bayer Diagnostics, Newbury, UK). Serum sodium, glucose, urea, total cholesterol (TC) and triglycerides (TG) were measured by dry-slide technology on an Ortho Vitros 950 analyzer (Ortho Clinical Diagnostics, High Wycombe, Bucks UK). Serum HDL-C was analysed on an ILab 600 using a homogeneous direct method in which reaction with non-HDL cholesterol is prevented by addition of anti-human lipoprotein antibody (Instrumentation Laboratory, Warrington, UK). Serum low density lipoprotein cholesterol (LDL-C) was derived from the Friedewald equation [20].

High sensitivity C-reactive protein (HsCRP) was analysed using a latex-enhanced immunoturbimetric assay (Randox Laboratories, Crumlin, Northern Ireland). HCY was measured from plasma blood by fluorescence polarization immunoassay (FPIA) using the IMX® system analyser IMX® reagents (ABBOTT Laboratories, UK). IGF-I was analysed using the standard Nichols Institute Diagnostics IGF-I Immunoradiometric Assay (IRMA), which employs two region-restricted affinity purified polyclonal antibodies (Nichols Institute Diagnostics, San Ciemente, CA 92673 USA) calibrated against the world health authority 1st IRP IGF-I 87/518.

Recombinant and pituitary GH were analysed using two immunofluorometric assays, one measuring 22 kDa hGH and the other total hGH (22 and 20 kDa) [21].

2.4. Body composition assessment

Body mass (BM, kg) was measured using a calibrated balanced weighing scales (Seca, Cranlea Ltd, UK) and stature was measured using a stadiometer (Seca, Cranlea Ltd, UK). Body mass index (BMI, $kg\,m^{-2}$) was calculated by dividing the subject's weight in kilograms (kg) by the square of the subject's stature in meters. Body density was determined using hydrostatic weighing procedures previously described by [22]. Following a familiarisation trial, underwater weight was determined five times. The mean of five trials was used as the criterion value. Gastrointestinal volume was assumed to be 0.1 litres (L) and residual lung volume (RLV) was estimated to be 24% of forced vital capacity and ranged from 0.9–1.4 L, which was within normal limits [23]. Body fat was estimated from body density, using the equation of Siri [24].

Fat free mass (FFM, kg) was calculated by subtracting fat mass from total body mass (TBM). Fat free mass index (FFMI, $kg\,m^{-2}$) was calculated by dividing the subject's FFM in kg by the square of the subject's stature in meters.

2.5. Arterial pulse wave velocity

Arterial pulse wave velocity was measured simultaneously and non-invasively in the supine subject's arm and leg by oscillometry (time resolution ±2 ms; QVL SciMed (Bristol, UK). The right arm and right leg were used for all the studies. All measurements were made by the same operator. Non-occlusive cuffs were placed over the brachial artery in the antero-superior position of the right upper arm and over the radial artery in the antero-inferior aspect of the right lower arm at the wrist and over the femoral artery at the position of the antero-superior aspect of the upper right thigh and over the anterior tibial artery at the ankle, prior to it becoming the dorsalis pedis artery. The cuffs were connected by "compliant" tubing to pressure transducers and inflated to 65–70 mmHg for measurement of pulse waves. Pulse pressure waveforms caused by the volume displacement of the travelling pulse wave were obtained from each of the four cuffs. A computer program characterized the waveform with respect to time at 30, 40, and 50% of peak pressure along its ascending limb and measured the transit time of normal pulse waves between the pairs of upper limb and of lower limb cuffs. It was designed to discard ectopic beats and abnormal waveforms. Transit times between the proximal and distal sites of the upper and lower limb pairs of cuffs were measured as the average of the time delays between each of the time points in each pulse and are given as the average of the transit times in 10 consecutive beats. PWV was derived as the distance between the proximal edges of each pair of cuffs divided by the transit time in $m\,s^{-1}$. Each recording of PWV took 30 s. Throughout each study PWV was measured for the first 12 min (12 instructions). Each instruction consists of the artery being in a state of vascular occlusion, at a pressure of 65–70 mmHg, for 30 s. PWV was measured for the following 30 s, in a state of non-occlusion. After 12 instructions, there was distal vascular occlusion for 5 min at a pressure of 65–70 mmHg (instruction 13). Following the 5 min occlusion, PWV was repeatedly measured for a further 12 instructions. Reproducibility of supine PWV was assessed by within-subject coefficients of variation over 30 min of consecutive measurements and of measurements repeated one month apart. All subjects attended in the fasted state and at the same time on the morning of each study, having avoided caffeine-containing beverages for 12 h. All interventions and measurements were preceded by a preliminary period of >20 min supine rest in a quiet, temperature-controlled room at 22 °C.

2.6. Electrocardiography

A 12 lead electrocardiogram (ECG) was performed on all subjects in accordance with the position statement outlined by the American Heart Association [25]. Resting (rest), peak and 8 min post-exercise, recovery (rec) heart rates (HR) were recorded. Subjects underwent a functional diagnostic exercise test, on a motorised treadmill (Powerjog GXC200, Birmingham, UK), using the Bruce protocol [26]. The test was symptom limited and terminated at volitional exhaustion, or on achieving peak oxygen uptake (VO_2 peak) (obtained using an online computerised gas analysis system CPX/D [Medgraphics, Beaver Medical Ltd, UK]), indicated by respiratory exchange ratio (RER) >1.15.

2.7. Calculations and statistical analyses

Data were analysed using a computerised statistical package (SPSS 14.0 for Windows, Surrey, England)

using parametric statistics. Significance was set at the $P < 0.05$ level. Data are presented as means ± standard deviation (SD). The power of the test was calculated at 95%. Confirmation that all dependent variables were normally distributed was assessed via repeated Kolmogorov-Smirnov tests. Changes in selected dependent variables as a function of time and condition were assessed using a two way repeated measures analysis of variance (ANOVA). Following simple main and interaction effects, Bonferroni-corrected paired samples *t*-tests were applied to make *posteriori* comparisons of the effect of time at each level of the condition factor. The rate pressure product (RPP) was calculated as heart rate multiplied by systolic SBP.

3. Results

Three subjects in the treatment group reported more frequent headaches, which responded to simple analgesia. One subjects reported the symptoms of extrasystoles, but there were no adverse ECG changes. There were no changes within the control group. No xenobiotic AAS were detected in urine, complying with the inclusion criterion. Demographic characteristics of the subjects are presented in Table 1. Body mass index (BMI, kg m^{-2}) and fat free Mass Index (FFMI) significantly increased within the GH group ($P < 0.017$) and significantly decreased eight days following cessation of rhGH administration ($P < 0.017$). Body fat significantly decreased within the GH group ($P < 0.017$) and remained significantly decreased eight days following cessation of rhGH administration ($P < 0.017$). In the GH group the VO_2 peak significantly increased on rhGH administration and on rhGH cessation ($P < 0.017$), Table 1.

Values for Arterial Pulse Wave Velocity (APWV, m s^{-1}) are presented in Table 2. In the GH group the lower limb pre-occlusion velocity was significantly decreased on rhGH administration and on rhGH cessation compared

Table 1
Subject demographics for control group v growth hormone (GH [Treatment]) group

Variables	Control group			Treatment group		
Day	1	7	14	(PRE-GH) 1	(on-GH) 7	(POST-GH) 14
BM	89.8 ± 12.7	89.6 ± 12.6	89.5 ± 12.7	86.1 ± 12.0	86.7 ± 12.1*	85.5 ± 11.8*,¥
BMI	28.0 ± 3.1	27.9 ± 3.1	27.9 ± 3.1	27.5 ± 3.0	27.7 ± 3.1*	27.3 ± 3.0*,¥
Body fat	21.9 ± 3.8	21.7 ± 3.8	21.6 ± 4.0	20.0 ± 6.0	19.0 ± 6.0*	19.1 ± 5.8*
FFMI	21.8 ± 2.1	21.8 ± 2.1	21.8 ± 2.1	21.9 ± 1.9	22.3 ± 1.9*	22.0 ± 1.9¥
RT	12.2 ± 3.6	12.2 ± 3.6	12.2 ± 3.6	12.2 ± 3.6	12.2 ± 3.6	12.2 ± 3.6
WT	4.4 ± 1.1	4.3 ± 1.2	4.5 ± 1.1	4.7 ± 1.1	4.6 ± 1.3	4.5 ± 1.2
TT	46 ± 12	47 ± 15	45 ± 11	47 ± 9	45 ± 13	46 ± 12
Energy intake	18050 ± 4100	18100 ± 2020	18175 ± 3100	17900 ± 3020	18450 ± 3900	18100 ± 2020
Protein intake	205 ± 60	195 ± 55	213 ± 45	207 ± 35	217 ± 65	210 ± 50
VO_2 peak	44.8 ± 7.9	45.4 ± 8.3	44.6 ± 8.3	41.8 ± 9.8	45.4 ± 9.9*	45.1 ± 8.2*
RPE	19.2 ± 0.7	19.0 ± 0.8	19.2 ± 0.7	19.2 ± 0.7	18.9 ± 0.6	18.8 ± 0.6
RER	1.15 ± 0.09	1.15 ± 0.08	1.16 ± 0.12	1.17 ± 0.16	1.1 ± 0.09	1.14 ± 0.13

Figures are presented as means ± standard deviations (SD).
BM, body mass (kg); BMI, body mass index (kg m^{-2}); Body fat (%); FFMI, fat free mass index (kg m^{-2}); RT, resistance training (years); WT, weight training (sessions); TT, training time (minutes); Energy intake (KJ/day); Protein intake (g/day); RPE, rate of perceived exertion; VO_2 peak, maximal oxygen uptake (ml kg^{-1} min^{-1}); RER, respiratory exchange ratio.
* $P < 0.017$, significantly different to PRE-GH.
¥ $P < 0.017$, significantly different to on-GH.

Table 2
Arterial pulse wave velocity responses for control group v growth hormone (GH [Treatment]) group

Variables	Control group			Treatment group		
Day	1	7	14	(PRE-GH) 1	(on-GH) 7	(POST-GH) 14
UL pre-occ velocity	8.76 ± 1.80	8.88 ± 1.69	8.91 ± 1.90	9.21 ± 1.93	8.69 ± 1.17	8.49 ± 1.08
UL post-occ velocity	8.31 ± 1.76	8.67 ± 1.54	8.63 ± 1.69	8.96 ± 1.80	8.65 ± 1.41	8.27 ± 0.94
LL pre-occ velocity	9.64 ± 1.62	9.79 ± 1.50	9.75 ± 1.54	9.97 ± 1.38	9.18 ± 1.60*	9.26 ± 1.52*
LL post-occ velocity	9.28 ± 1.45	9.48 ± 1.26	9.37 ± 1.26	9.84 ± 1.84	9.27 ± 1.33	9.35 ± 1.49

Figures are presented as means ± standard deviations (SD).
UL pre-occ velocity, upper limb pre-occlusion velocity (m s^{-1}); UL post-occ velocity, upper limb post-occlusion velocity; LL pre-occ velocity, lower limb pre-occlusion velocity; LL post-occ velocity, lower limb post-occlusion velocity.
* $P < 0.017$, significantly different to PRE-GH.

Table 3
Heart rate and blood pressure responses, for control v growth hormone (GH [Treatment]) group

Variables	Control group			Treatment group		
Day	1	7	14	(PRE-GH) 1	(on-GH) 7	(POST-GH) 14
HR-rest	66 ± 16	67 ± 16	67 ± 14	72 ± 14	78 ± 11[†]	75 ± 18
HR-peak	185 ± 12	185 ± 13	183 ± 12	185 ± 7	185 ± 8	185 ± 7
HR-rec	103 ± 19	103 ± 19	104 ± 19	100 ± 8	100 ± 7	99 ± 9
SBP-rest	125 ± 12	124 ± 12	125 ± 11	126 ± 10	125 ± 12	122 ± 9
SBP-peak	199 ± 20	201 ± 18	198 ± 18	192 ± 21	190 ± 16[†]	188 ± 28
SBP-rec	129 ± 17	127 ± 18	126 ± 16	132 ± 10	130 ± 12	121 ± 9[¥,*]
RPP-rest	83 ± 22	84 ± 24	85 ± 22	90 ± 18	97 ± 14[†]	93 ± 28
RPP-peak	367 ± 42	367 ± 40	362 ± 41	355 ± 41	351 ± 35	348 ± 52
RPP-rec	133 ± 26	131 ± 28	131 ± 27	132 ± 17	129 ± 14	119 ± 16[*]

Figures are presented as means ± standard deviations (SD).
Heart rate (HR; bpm), systolic blood pressure (SBP; mm Hg), diastolic blood pressure (DBP), and rate pressure product (RPP; bpm mmHgX10−2)
-rest, resting; -peak, peak; -rec = 8 min recovery.
[*] $P < 0.017$ significantly different to PRE-GH.
[¥] $P < 0.017$ significantly different to on-GH.
[†] $P < 0.05$ significantly different to C group.

with pre-administration (both $P < 0.017$). Electrocardiography was unremarkable in all subjects, demonstrating no adverse effect of rhGH on electrical activity.

Results of the effects of rhGH on the cardiovascular responses: heart rate (HR), systolic blood pressure (SBP), diastolic blood pressure (DBP), and rate pressure product (RPP) responses are shown in Table 3. HR-rest significantly increased compared with the C group ($P < 0.05$).

SBP-peak significantly decreased compared with the C group ($P < 0.05$). SBP-rec significantly decreased within the GH group eight days following cessation of rhGH administration ($P < 0.017$). RPP-rest significantly increased compared with the C group ($P < 0.05$).

Table 4
Serum analytes responses for control group v growth hormone (GH) group

Variables	Control group			Treatment group			Reference range
Day	1	7	14	(PRE-GH) 1	(on-GH) 7	(POST-GH) 14	
Glucose (mmol/L)	4.7 ± 0.7	4.7 ± 0.7	4.7 ± 0.7	4.9 ± 0.6	5.0 ± 0.6	4.9 ± 0.5	(3.0–10.5)
Sodium (mmol/L)	139.6 ± 8.4	141.5 ± 3.1	140.5 ± 5.8	140.6 ± 2.7	142 ± 2.4[*]	142 ± 2.4[*]	(136–145)
Urea (mmol/L)	6.4 ± 2.3	6.3 ± 1.9	6.6 ± 2.2	5.6 ± 1.3	5.8 ± 1.6	7.1 ± 2.8[¥]	(2.3–6.9)
Creatinine (mmol/L)	95.9 ± 17	92.3 ± 13	95.6 ± 15	103.3 ± 25	92.7 ± 13[*]	105.3 ± 24[¥]	(50–100)
Total protein (mmol/L)	76.5 ± 10	78.6 ± 7	76.4 ± 8	75.7 ± 5	73.1 ± 5[*]	75.3 ± 5	(58–80)
Albumen (mmol/L)	44.1 ± 6	45.8 ± 4	45 ± 6	44.4 ± 4	42.5 ± 4[*]	43.9 ± 5	(38–50)
TC (mmol/L)	4.4 ± 1.0	4.6 ± 0.9	4.5 ± 1.1	4.7 ± 0.9	4.4 ± 0.7[*]	4.7 ± 1.0	(3–7)
TG (mmol/L)	1.0 ± 0.4	1.1 ± 0.5	1.1 ± 0.4	1.2 ± 0.5	1.1 ± 0.6	1.4 ± 0.9	(0.6–2.10)
HDL (mmol/L)	1.2 ± 0.4	1.3 ± 0.3	1.2 ± 0.3	1.2 ± 0.3	1.2 ± 0.2	1.2 ± 0.3	(0.8–1.9)
LDL (mmol/L)	2.7 ± 0.8	2.8 ± 0.8	2.8 ± 1.0	2.9 ± 0.9	2.7 ± 0.7	2.9 ± 0.9	(<3)
hsCRP (mg/L)	1.35 ± 1.9	1.38 ± 2.1	1.44 ± 2.1	1.77 ± 2.1	1.29 ± 1.6[*]	1.7 ± 2.8	(<3)
HCY (μmol/L)	12.5 ± 4.2	13.3 ± 4.7	13.1 ± 4.1	13.2 ± 4.0	11.7 ± 3.1[*]	13.1 ± 4.3	(<12)
T_4 (pmol/L)	15.6 ± 1.9	15.3 ± 1.8	15.9 ± 1.7	15.3 ± 2.0	14.2 ± 1.6[*,†]	15.0 ± 1.5	(9.6–26.5)
TSH (mU/L)	1.84 ± 0.8	1.92 ± 0.7	1.84 ± 0.7	2.3 ± 1.1	1.5 ± 0.8[*]	2.2 ± 1.0	(0.6–4.8)
LH (IU/L)	4.18 ± 2.1	3.8 ± 1.9	3.7 ± 2.0	3.6 ± 2.3	3.1 ± 2.4	3.4 ± 2.1	(1.0–8.0)
FSH (IU/L)	3.9 ± 2.1	4.0 ± 2.2	3.9 ± 2.2	3.4 ± 2.0	3.0 ± 1.8	3.6 ± 2.1	(1.0–9.0)
T (nmol/L)	17.5 ± 5.2	17.5 ± 5.6	17.4 ± 5.4	16.2 ± 6.0	15.3 ± 5.7	14.5 ± 5.0	(10–35)
PRL (IU/L)	175 ± 102	142 ± 55	159 ± 108	205 ± 110	199 ± 97[†]	169 ± 101	(120–300)
Cortisol (nmol/L)	389 ± 162	378 ± 125	404 ± 146	402 ± 133	381 ± 138	389 ± 149	(220–720)
PSA (ng/mL)	0.6 ± 0.2	0.6 ± 0.2	0.5 ± 0.2	0.6 ± 0.4	0.6 ± 0.4	0.6 ± 0.3	(<5.0)
IGF-1(ng/ml)	179 ± 47	169 ± 50	175 ± 53	159 ± 54	323 ± 93[*,†]	175 ± 61[¥]	

Figures are presented as means ± standard deviations (SD).
TC, total cholesterol; TG, triglycerides; HDL, high density lipoprotein; LDL, low density lipoprotein; hsCRP, hsC-reactive protein; HCY, homocysteine; T_4, thyroxine; TSH, thyroid stimulating hormone; LH, luteinising hormone; FSH, follicle stimulating hormone; T, testosterone; PRL, prolactin; PSA, prostate specific antigen; IGF-I, insulin like growth Factor-I.
[*] $P < 0.017$, significantly different to PRE-GH.
[¥] $P < 0.017$, significantly different to on-GH.
[†] $P < 0.05$, significantly different to C group.

RPP-rec significantly decreased within the GH group, eight days following cessation of rhGH administration compared with pre-administration of rhGH ($P < 0.017$). Diastolic BP (DBP) significantly decreased within the GH group, eight days following cessation of rhGH administration compared with pre-administration of rhGH ($P < 0.017$).

Results of the effects of the drug on the serum analytes are shown in Table 4. Serum sodium significantly increased within the GH group ($P < 0.017$) on administration of rhGH and urea and creatinine were significantly increased, on-cessation of rhGH ($P < 0.017$). Creatinine, total protein, albumin, T_4, TSH, hsCRP, HCY and TC all significantly decreased within the GH group (all $P < 0.017$) on rhGH administration. PRL significantly increased and T_4 was significantly decreased on administration of rhGH when compared with the C group ($P < 0.05$). IGF-I significantly increased within the GH group ($P < 0.017$) on administration of rhGH and compared with the C group ($P < 0.017$). Individual responses for IGF-I are presented in Fig. 1.

4. Discussion

4.1. Effects on anthropometry

The administration of a supraphysiological dose of rhGH 0.058 IU kg^{-1} day^{-1} for six days, in abstinent AAS users significantly altered anthropometry within the treatment group, but not compared with controls. This may have been as a consequence of the dosage used and the time period of administration. It significantly decreased body fat and significantly increased IGF-I and FFMI consistent with previous research [27].

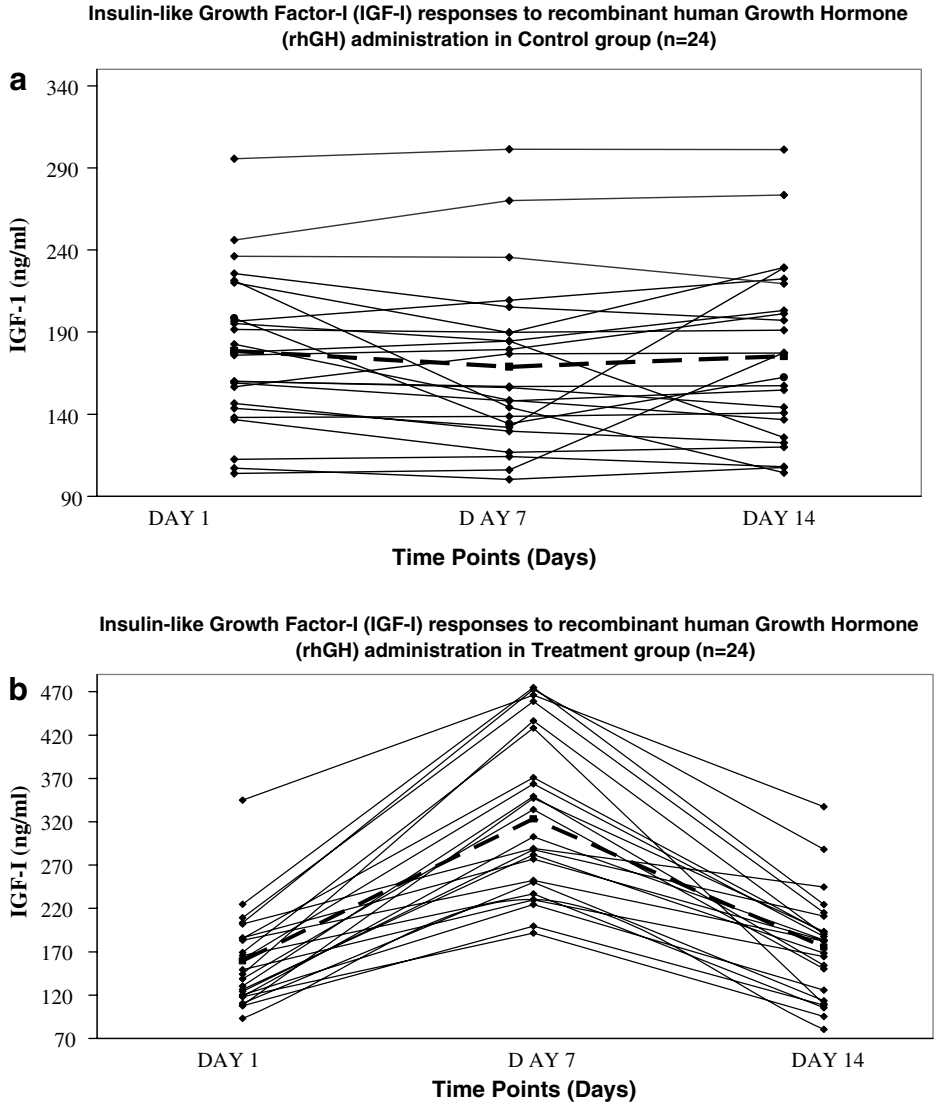

Fig. 1. Comparison between control group and treatment group for individual subject responses for insulin-like growth factor (IGF-I).

Protein and kilocaloric intakes were constant, which could have biased the responses.

4.2. Effects on APWV

There were no differences between the groups. The significant decrease in the lower limb pre-occlusion velocity within the GH group is comparable with the effects of rhGH on endothelial dysfunction in GHD [8]. Polymorphic variation in the fibrillin-1 receptor [28], angiotensin II type-1 receptor and endothelin receptor genes are genetic influences considered to be related to stiffness [29]. The angiotensin-converting enzyme (ACE) I/D polymorphism has also been associated with stiffness [30]. Reduced nitric oxide (NO) production and bioavailability and increased sympathetic activity and reduced conversion of thyroxine to triiodothyronine in the GHD state can contribute to changes in microcirculation, which can be combated by replacement [31]. The known effects of rhGH on collagen metabolism may also in part contribute to the significant lowering of APWV in this present study [32] since the sum of procollagen type I carboxy-terminal propeptide and carboxy-terminal telopeptide is significantly greater in untreated essential hypertensives [33].

4.3. Effects on HR, BP and RPP

The significantly elevated HR-rest and RPP-rest and significantly decreased SBP-peak, compared with the control group is also prevalent in acromegaly and may be dosage related. This may predispose an individual to long term endothelial dysfunction and could be accounted for by an inverse correlation of NO levels with GH and IGF-I [34]. GH administration in rats, for 2 weeks corroborated the current research, impairing baroreceptor function, via activation of the NO-system, elevating HR and lowering BP [35].

4.4. Effects on serum CV analytes

There was a significant elevation of urea one week post-cessation of rhGH. An elevation in serum uric acid has been recently associated with increased risk for future hypertension and as an independent risk factor for poor CVD prognosis [36]. The mechanisms are thought to be the direct action of uric acid on smooth muscle and vascular endothelium. The significant decrease in creatinine, total protein and albumin within the GH group, on rhGH administration suggested these may have been used as substrates for anabolism. The significant urea elevation one week post-cessation suggested that the group may have reverted to a catabolic phase of protein metabolism as a rebound effect of rhGH cessation, despite analytes being within the reference ranges. The significant reduction of TC, hsCRP and body fat in the present study supports the effects of replacement therapy in GHD [37] and is possibly dose related [38]. Higher levels of CRP in a population-based cardiovascular risk study have been identified [39]. Physical activity, which stimulates GH production, is consistently associated with significant lowering of CRP levels [11].

RhGH administration has also lowered HCY in GHD, without a correlation in CRP or interleukin (IL)-6 [40], unlike this study where HCY and CRP have both significantly decreased. IL-6 is a regulator of CRP and has a key role in initiation of inflammation. Hyperhomocysteinaemic patients have elevated serum levels of CRP and IL-6 without accompanying hypercholesterolaemia or CVD [41]. The mechanism by which hyperhomocysteinaemia and elevated CRP are related to vascular disease are unclear. IL-6 may be involved, increasing the release of monocyte chemoattractant protein-1 from peripheral mononuclear cells, which enhances the effects in cells from patients with hyperhomocysteinaemia. This did not concur with a recent five year interventive lowering of HCY by B6 and B12 [42], where no improvement on CV mortality was demonstrated. The significant lowering of both HCY and TC is a unique finding in an unusual cohort of previous AAS using individuals. It is possible that the abuse of AAS sensitises these individuals to microvascular insult, which predisposes them to CV morbidity. HCY impairs vascular endothelial function through significant reduction of nitric oxide production, possibly potentiating oxidative stress and atherogenic development [43]. HCY has been shown to increase ROS, 1.85-fold [44]. Expression of the mitochondrial biogenesis factors, nuclear respiratory factor-1 and mitochondrial transcription factor A, were significantly elevated in HCY-treated cells. These changes were accompanied by an increase in mitochondrial mass and higher mRNA and protein expression of the subunit III of cytochrome C oxidase. These effects were significantly prevented by pre-treatment with the antioxidants, catechin and trolox, which modulated the adverse vascular effects of HCY [44]. AAS, particularly oral ingestion of 17-α-alkylated AAS, have been shown to produce a marked dyslipidaemia [45], to increase platelet aggregability [46] and to adversely alter endothelial function [47]. The administration of rhGH would appear to counteract these potentially adverse effects.

5. Conclusion

GHD states are associated with a variety of CV pathologies, which appear to be ameliorated by replacement therapy. The findings of this study suggest that short term use of rhGH in individuals, who may have been compromised by previous administration of AAS, appeared to have beneficial effects on endothelial

function as evidenced by significant lowering of APWV and specific inflammatory markers of cardiovascular disease, as evidenced by significant lowering of HCY and CRP. However, further research is required in healthy individuals to determine the extent of any rhGH benefit and elucidate any adverse cardiovascular effects as evidenced by the significant increase in resting RPP.

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