Short-term rhGH increases PIIINP, a biomarker of endothelial dysfunction

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Abstract— Objectives: In arterial hypertension, amino-terminal propeptide of type III procollagen (PIIINP) is elevated in arterial aneurysm tissue and associated with a poor prognosis following acute myocardial infarction (MI). Recombinant human growth hormone (rhGH) administration attenuates endothelial dysfunction but increases PIIINP. This study was conducted to establish if short-term rhGH administration affects PIIINP, endothelial function and selected cardiovascular disease (CVD) risk factors, in healthy males.

Design: Method: Male subjects (n=48) were randomly assigned into two groups:

(1): control group (C) n=24, mean ± SD, age 32 ± 11 years; height 1.8 ± 0.06 metres;

(2): rhGH administration group (rhGH) n=24, mean ± SD, age 32 ± 9 years; height 1.8 ± 0.07 metres.

Blood pressure (BP), heart rate (HR), arterial pulse wave velocity (APWV), and biochemical indices were investigated.

Results: PIIINP (0.28±0.1 vs. 0.42±0.2, U/ml); Insulin like growth factor-I (159±54 vs. 323±93, ng.mL^{-1}); resting HR (72±14 vs. 78±11, b.p.m.) and rate pressure product (RPP) (90±18 vs. 97±14, bpm x mm.Hg x 10^{-2}) all significantly increased (P<0.05). Total cholesterol (4.7±0.9 vs. 4.4±0.7, mmol.L^{-1}); high sensitivity C-reactive protein (1.77±2.1 vs. 1.29±1.6, mg.L^{-1}); serum homocysteine (13.2±4.0 vs. 11.7±3.1, μmol.L^{-1}) and APWV (9.97±1.38 vs. 9.18±1.6, m.s^{-1}) all significantly decreased (P<0.05).

Conclusion: Paradoxically, there was an improvement in CVD inflammatory markers and APWV; but PIIINP and resting RPP increased.

Elevated PIIINP may have a confounding adverse effect on the endothelium, but may also provide clinical prognostic information in monitoring arterial hypertension, left ventricular function in the sub-acute phase following MI and endothelial function in aortic aneurysms.

Keywords— APWV; BP; hsCRP; Homocysteine; PIIINP; RPP.

I.   INTRODUCTION

Serum amino-terminal propeptide of type III procollagen (PIIINP) is an extension peptide of procollagen type III and reflect its synthesis. It is cleaved off procollagen type III during the biosynthesis of type III collagen, and is a circulating biochemical marker of collagen metabolism which characterizes the early stages of repair and inflammation [1]. PIIINP is associated with the repair process involving collagen deposition which influences endothelial remodeling after myocardial infarction (MI) and increasing levels from ~3 to ≥ 5 µg/L are believed to reflect enhanced collagen turnover, including synthesis and deposition as well as alteration in degradation and elimination [1]. Elevated levels appear to be associated with a poor prognosis of patients following acute non-thrombolysed MI [2]. An elevation of PIIINP greater than 5.0 µg/L has been identified as an independent predictor of cardiac death or complicating left ventricular heart failure by vascular remodeling [3]. In dilated cardiomyopathy, with restrictive mitral filling pattern and diastolic dysfunction patients with an elevated PIIINP had a worse prognosis [4]. Elevated circulating levels of PIIINP were also associated with a decreased six minute walk distance and an increased resting heart rate (HR) in pulmonary arterial hypertension (PAH) [5].

PIIINP appears to be an important biomarker which may evaluate left ventricular end-diastolic pressure (LVEDP) and may predict cardiac mortality in acute coronary syndrome (ACS) [6].

Collagen is an important protein of the extracellular matrix (ECM) that determines the physiological properties of arteries, especially the aortic wall and the development of abdominal aortic aneurysms (AAA) [7]. PIIINP is increased in AAA tissue compared with normal aortic walls affected by occlusive atherosclerotic disease [7]. A significant difference between plasma PIIINP levels exists in patients with AAAs compared with controls and the turnover of type III collagen increases with the

enlargement of the aneurysm diameter [8]. Degradation of type III collagen in the aneurysmal wall may have an important impact on AAA rupture and the increased levels of PIIINP may also be able to predict AAA prognosis [9]. Collagen production has been identified as being increased in excess growth hormone (GH) production (acromegaly) [10]. Serum PIIINP which is increased in this condition may be used as a marker for monitoring the extent of the disease process [10]. Untreated long standing acromegaly is characterised by a high HR and arterial hypertension (the hyperkinetic syndrome) and concentric biventricular hypertrophy, diastolic dysfunction, rhythm disturbances and valve dysfunction which can all exacerbate acromegalic cardiomyopathy [11].

Systolic function and cardiac performance is markedly decreased in growth hormone deficiency (GHD) but is improved following recombinant human growth hormone (rhGH) replacement [12]. Endothelial dysfunction (ED) determined by flow-mediated endothelium-dependent dilation (EDD) of the brachial artery and measured by B mode ultrasound is prevalent in GH-deficient adults, which is reversed by rhGH replacement for the period of treatment of 18 months [13]. RhGH decreases aortic systolic and diastolic pressure which may reduce cardiovascular disease (CVD) in GHD [14]. RhGH improves vascular ED in patients with chronic heart failure who are not deficient in endogenous growth hormone [15]. Short-term rhGH, four weeks (0.1 and 0.2 $IU.kg^{-1}.day^{-1}$) and two weeks (0.1 $IU.kg^{-1}.day^{-1}$) respectively, has been shown to significantly elevate PIIINP in healthy individuals by almost three times baseline levels [16, 17].

The purpose of this original study was to examine the effects of one week's supraphysiological rhGH administration on PIIINP and specific CVD parameters in an apparently healthy group of strength training males. The research team hypothesised that PIIINP would increase and that CVD parameters would decrease, following rhGH administration.

II.   METHODS

Subjects. Approval for the study was obtained from the University of South Wales ethics committee, in accordance with the declaration of Helsinki. Ethical registration committee number: HRE916. Prior to data collection, subjects were informed of experimental protocol, signed informed consent, and were instructed that they could withdraw from the study at any time. In addition, a drug screen was performed on each subject to exclude the use of xenobiotic substances. Urinalysis was performed at a world anti-doping agency (WADA) accredited laboratory. All subjects were recreational strength trainers and recreationally active. Subjects in both groups were former testosterone users and followed synchronised training

times and intensity, which did not differ. This was verified by detailed training diaries. In order to avoid confounding effects on biochemical indices, subjects abstained from physical activity for 24 hours before each testing day.

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 metres;
2): rhGH administration group (*rhGH*) *n*=24, mean ± SD, age 32 ± 9 years; height 1.8 ± 0.07 metres.

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, to avoid confounding diurnal effects and were anonymous to each other. A dosage of 0.019 $mg.kg^{-1}.day^{-1}$ rhGH was used and was considered an acceptable dosage that would provide a physiological effect, with minimal side effects, in apparently healthy subjects. An administration diary was recorded. Subjects were examined prior to the commencement of rhGH administration, day 1, day 7, and after cessation, day 14. Dietary intake was strictly monitored, using a fourteen day dietary recall (Nutri-check, Heath Options Ltd, Eastbourne, UK).

Blood sampling. Phlebotomy was conducted in the fasted state, following 30 minutes 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 and analysed in duplicate. Serum 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). The inter-assay CVs were: Total cholesterol: 1.95%; Triglycerides: 3.6%. The intra-assay CVs were: Total cholesterol: 3.8%; Triglycerides: 0.9%.

Serum high density lipoprotein cholesterol (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). The inter-assay CV was: 2.8%. The intra-assay CV was: 1.2%. Serum low density lipoprotein cholesterol (LDL-C) was derived from the Friedewald equation.

High sensitivity C-reactive protein (hsCRP) was analysed using a latex-enhanced immunoturbimetric assay (Randox Laboratories, Crumlin, Northern Ireland). The inter-assay was: 4.95%. The intra-assay CV was: 8%.

HCY was measured from plasma blood by fluorescence polarization immunoassay (FPIA) using the IMX® system

analyser IMX® reagents (ABBOTT Laboratories, UK). The inter-assay was: 6.1%. The intra-assay CV was: 4.4%.

Testosterone (T, $nmol.L^{-1}$) was analysed with the chemiluminescent immunoassay on an Advia Centaur analyser (Bayer Diagnostics, Newbury, UK). The inter-assay CV was: 12.0%. The intra-assay CV was: 7%.

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 U.S.A.) calibrated against the world health authority 1st IRP IGF-I 87/518.

The inter-assay CV was: 4.5%. The intra-assay CV was: 10.0%, 6.3% and 5.7% at serum concentrations of 61.5, 340.8 and 776.9 $ng.ml^{-1}$ respectively.

Recombinant and pituitary GH were analysed using two immunofluorometric assays, one measuring 22 kiloDalton hGH and the other total hGH (22 and 20 kiloDalton) [20]. The inter-assay CVs were: 10.0, 4.0, and 5.4% at 1.7, 12.1 and 22.2 $ng.ml^{-1}$, respectively. The intra-assay CV was 5%.

Serum amino-terminal propeptide of type III procollagen (PIIINP) was measured by a two-stage sandwich RIA (CIS Biointernational; Oris Industries, Gif-Sur-Yvette Cedex, France). The reported intra-assay variability at 0.8, 1.5 and 4.0 U/ml is 2.9, 2.9 and 4.0% respectively. The inter-assay variability at 0.25, 1.5 and 5.6 U/ml is 11.3, 7.8 and 9.3% respectively.

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). All measurements were taken barefoot in briefs. 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 [21]. 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 L-1.4 L, which was within normal limits [22]. Body fat was estimated from body density, using the equation of Siri [23].

Arterial Pulse Wave Velocity (APWV): 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. 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 seconds. Throughout each study PWV was measured for

the first 12 minutes (12 instructions). Each instruction consists of the artery being in a state of vascular occlusion, at a pressure of 65-70 mmHg, for 30 seconds. PWV was measured for the following 30 seconds, in a state of non-occlusion. After 12 instructions, there was distal vascular occlusion for 5 minutes at a pressure of 65-70 mmHg (instruction 13). Following the five minute 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 minutes supine rest in a quiet, temperature-controlled room at 22°C [24].

Electrocardiography: A resting 12 lead electrocardiogram (ECG) was performed on all subjects in accordance with the position statement outlined by the American Heart Association [25].

Blood Pressure (BP) Measurement: Blood pressure (BP) was measured in accordance with the American Heart Association Council on High Blood Pressure Research (2005) [26]. BP measurements were obtained by a single physician at the beginning of the physical examination, using a calibrated mercury column sphygmomanometer (Yamasu, Kenzmedico Co., Ltd., Japan). BP was measured from the bared arm, with the subject seated on a chair, with a back support, for five minutes with both feet on the floor and legs uncrossed. The arm was supported at heart level and the appropriate sized cuff was used, ensuring 80% of the circumference of the subject's arm was encircled. The Korotkoff sounds were measured from the brachial artery in the antecubital fossa, using the diaphragm of the stethoscope (Littmann, 3M, Loughborough, England, UK). BP was ascertained from both arms and if the difference was less than 10 mmHg the reading was taken from the right arm. All readings that were finally used were measured from the right arm, since there were no differences between the right and left arm. Two auscultatory measurements in each position were made at an interval of one minute and an average of those readings was used to represent the patient's BP. If these readings did not agree to within 5 mm.Hg for SBP and DBP a further two readings were made and the average of those multiple readings was used.

Calculations and Statistical analyses.

From published research, the sample size of *n*=24, was estimated using the nomogram method described by Altman (1982) [27]. The proposed main variable for the effect of rhGH was considered to be IGF-I and the lowest dose of rhGH required to significantly increase IGF-I, in

healthy young men and women, has been estimated to be 0.0025 mg.kg $^{-1}$.day^{-1} [28]. From the critical difference equation, described by Fraser and Fogarty, (1989) [29], Healy *et al.*, (2003) [30] estimated an increase in IGF-I of 24% was required to demonstrate a physiological effect of IGF-I on metabolism, from rhGH administration. Velloso et al., (2013) [17] monitored a dosage of 0.033 mg.kg $^{-1}$.day^{-1}/0.1 IU.kg $^{-1}$.day^{-1}, which significantly increased PIIINP (*P*<0.05). A dosage of 0.019 mg.kg $^{-1}$.day^{-1}/0.058 IU.kg $^{-1}$.day^{-1} was assumed to be an acceptable dosage that would provide a physiological effect, with minimal side effects, in a cohort of apparently healthy subjects.

Data were analysed using a computerised statistical package (PASW 22.0 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.

III.   RESULTS

The urinary concentrations of testosterone were normal and the serum gonadotrophins and testosterone were within the reference range for eugonadal men (male serum testosterone: 10-35 nmol/L, table 4). Electrocardiography was unremarkable in all subjects, demonstrating no adverse effect of rhGH on cardiac electrical or rhythmical activity.

There were no changes within the control group. Demographic characteristics of the subjects are presented in table 1. Body mass index (BMI, kg.m^{-2}) significantly increased within the rhGH administration group (*P*<0.017). Body fat significantly decreased within the rhGH administration group (*P*<0.017).

Values for APWV (m.s^{-1}) are presented in table 2. The lower limb pre-occlusion velocity was significantly decreased within the rhGH administration group (*P*<0.017).

Results of the effects of rhGH on the cardiovascular responses: heart rate (HR), systolic blood pressure (SBP), and rate pressure product (RPP) responses are shown in table 2. HR and RPP significantly increased on rhGH administration compared with the control group (*P*<0.05).

Results of the effects of the drug on the serum analytes are shown in table 3. Serum Sodium, IGF-I and PIIINP significantly increased within the rhGH administration group (*P*<0.017) and IGF-I and PIIINP also significantly increased compared with controls (*P*<0.05). Serum hsCRP, HCY, and TC, all significantly decreased within the rhGH administration group (*P*<0.017).

IV.   DISCUSSION

In this study the administration of rhGH at a dose of 0.019 mg.kg^{-1}.day^{-1} for six days, in apparently healthy individuals equated to a mean value of 1.6 mg per day, which was considered a supraphysiological dose. In healthy males and females, the GH level is normally undetectable (<0.2 µg.L^{-1}) throughout most of the day. There are approximately 10 intermittent pulses of growth hormone per 24 hours, most often at night, when the level can be as high as 30 µg.L^{-1}. This equates to an average GH secretion of circa 0.67 mg and 0.83 mg in males and females respectively, per 24 hours [31].

The effect of rhGH in elevating PIIINP and IGF-I, corroborated similar research of short-term (four and two weeks) rhGH administration [16, 17]. PIIINP concentration is currently considered an independent predictor of left ventricular end-diastolic pressure (LVEDP) and correlates with cardiac mortality and revascularization, providing an additional means of evaluating and managing patients with acute coronary syndrome [6]. Arterial PWV is a valid and non-invasive measure of arterial stiffness that may be more directly related to damage of the target organs relative to brachial blood pressure as it is less altered by heart rate, wave reflection, and antihypertensive or lipid-lowering drugs [24]. It has only been suggested as a CVD correlational independent risk marker with PIIINP in recent years [5].

The mechanism by which rhGH exerts these effects is by the stimulation of collagen metabolism which is an important protein of the extracellular matrix (ECM) which determines the physiological properties and remodeling of the arterial endothelium in aortic aneurysms [7]. PIIINP also has an effect on myocardial fibroproliferation following myocardial infarction [3]. It is suggested that this increases arterial and myocardial stiffness, thereby causing systolic and diastolic blood pressure dysfunction resulting in BP elevation [4]. Cardiac echo-reflectivity, which is a reflection of heart collagen content, is increased in patients with active acromegaly and correlates with elevated PIIINP concentrations. After treatment of acromegaly, and reduction in GH production, both parameters revert to normal [32].

Aortic APWV is also considered an important independent risk factor of CVD and an elevation can predict the

occurrence of cardiovascular events independently of classic risk factors, other than age and blood pressure [33]. The elasticity of the proximal large arteries is the result of the high elastin to collagen ratio in their walls, which progressively declines toward the periphery. Endothelium-derived nitric oxide (NO) diffuses toward the underlying vascular smooth muscle, producing relaxation, modulating arterial smooth muscle tone and distensibility [34]. By improving arterial elasticity, endothelium-derived NO reduces the arterial wave reflection and reduces left ventricular work and the pulse pressure within the aorta [34].

However, in acromegaly where GH and IGF-I levels are excessively elevated for prolonged periods of time there is increased oxidative stress coupled with diminished antioxidant capacity and the endothelial dysfunction occurs as a consequence of NO levels [35]. Both hydrogen sulfide (H_2S) and nitric oxide (NO) are gaseous transmitters, which play a critical role in regulating vascular tone. An H_2S and NO conjugated donor can time-dependently and dose-dependently relax sustained contractions induced by phenylephrine in rat aortic rings, suggesting a possible interactive role between H_2S and NO, in vasorelaxation, produced by fluctuating GH and IGF-I levels [36]. Also H_2S induces relaxation of isolated rings of human mesenteric arteries, independently of NO. Endothelium-dependent related mechanisms with the stimulation of ATP-sensitive potassium channels represents important cellular mechanisms for H_2S effect on human mesenteric arteries [37].

In this study, subjects were exposed to the shortest duration supraphysiological dosage of rhGH possible, which was considered to significantly increase PIIINP. This also caused a reduction in the lower limb pre-occlusion APWV. It is unknown whether the increase in PIIINP caused an increased turnover of the ECM in the arterial wall. The reasons for the decrease in APWV might be as a result of the increased IGF-I to a serum concentration where it increased NO [35]. IGF-I is believed to have a vascular protective role because it stimulates NO production from endothelial and vascular smooth muscle cells. The time frame and concentration levels of serum GH and IGF-I, from commencement of excess production, in acromegaly, to pathological symptomatology is still unknown. The research conducted by the GH-2000 and GH-2004 teams did not identify any serious adverse physiological effects despite the administration of double and quadruple the dose (0.033 and 0.067 mg.kg^{-1}.day^{-1} [38, 16]) for four times longer (one month) than dosages used in the present study (0.019 mg.kg^{-1}.day^{-1}). However, the researchers were attempting to identify a test for doping rather than assess CVD risk factors. The significantly decreased lower limb pre-occlusion APW velocity in the rhGH group is comparable with the effects of rhGH on endothelial dysfunction (ED) in growth hormone deficiency (GHD) [14] and in heart failure [15].

The most common short term effects following rhGH administration are from sodium and water retention and weight gain, from dependent oedema, which can frequently occur within days [39]. It does this in GHD [39] and in healthy subjects by activation of the renin-angiotensin system, increasing aldosterone secretion, by inhibiting atrial natriuretic peptide secretion and by a direct action on renal tubules [40]. In the present study, the sodium level was significantly increased, but remained within the normal reference range, but this may have accounted for the elevation in resting HR and RPP, which occurs in acromegaly.

Sustained increased plasma levels of IGF-I may predispose an individual to long term ED and may be accounted for by an inverse correlation of NO levels with GH and IGF-I excess [41]. In the present study the effect on HR and RPP which occurred, could be explained by the effects of IGF-I on NO production, impairing baroreceptor function, via activation of the NO-system, elevating HR without affecting BP [42], corroborating similar short-term rhGH administration [39].

The research group had previously identified a significant reduction of independent cardiovascular risk factors, following rhGH administration, without an identification of changes in PIIINP [43]. In coronary artery disease, hsCRP has an important function in initiation of inflammation. It is also a better predictor of mortality than fibrinogen and offers prognostic information beyond that provided by the conventional cardiovascular risk factors [44]. Also acute inflammation, determined by elevated hsCRP, significantly increases arterial stiffness (AS) in older adults [45]. These increases can be reduced in a high cardiorespiratory fitness group, compared with a low cardiorespiratory fitness group [45].

Raised levels of HCY and CRP, which are independent risk factors for atherosclerosis, are thought to elevate blood pressure and cause vascular endothelial dysfunction by reducing NO production in endothelial cells, potentiating oxidative stress, which may result in vasoconstriction and endothelin 1 increase [46].

This significant lowering of both HCY and hsCRP is a unique finding in an unusual cohort of former testosterone using individuals. It is believed that the use of supraphysiological dosages of testosterone sensitises these individuals to microvascular insult, which predisposes them to impaired vascular reactivity [47]. An elevation of HCY had previously been established by the authors' research group in users of testosterone, which might account for the enhancement of oxidative stress and represent a mechanism leading to the destruction of

vascular cells [48]. In this study, the former users of testosterone had an elevation of homocysteine, which may have sensitised the arterial endothelium, initiated atherogenesis and increased AS by modification of DNA methylation [49]. The underlying epigenetic mechanism, which is an alteration of the phenotype without a change of DNA sequence, may have contributed to altered gene expression causing vascular damage [50]. However, following a twelve week washout the baseline values of HCY were within normal limits and the same as controls. By significantly lowering HCY, the administration of short-term rhGH would appear to have exerted a beneficial effect on any adverse effect that sustained elevated HCY might cause.

The vascular protective role of IGF-I has been suggested because of its ability to stimulate NO production from endothelial and vascular smooth muscle cells [51]. IGF-I may also play a role in aging, atherosclerosis and cerebrovascular disease. Just as an excess of GH and IGF-I can cause unfavourable cardiovascular effects [11, 32] so too in cross sectional studies, low GH and IGF-I levels have been associated with unfavourable CVD risk factors, such as abnormal lipoprotein levels and hypertension [51]. In prospective studies, lower IGF-I levels predict future development of ischaemic heart disease [51].

Conclusion. The aim of the present study was to evaluate measurements of PIIINP, following short-term rhGH administration, as a diagnostic tool in relation to endothelial function. Despite the increase in PIIINP there was an improvement in APWV and CVD risk factors consistent with recent research [52]. We postulate that, over a longer period of time, an elevated PIIINP may ultimately have an adverse effect on endothelial structure and function and predict a future breakdown of the endothelium. We believe that such findings preclude the use of rhGH as either an adjunctive therapy in gender hormone replacement therapy (andropause and menopause) or as a solitary treatment of the somatopause.

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Demographic characteristics of the subjects are presented in table 1.

Table.1: Subject demographics.

Variables	**Control Group (*C*)**			**Administration Group**		
				(PRE-rhGH)	*(on-rhGH)*	*(POST-rhGH)*
Day	1	7	14	1	7	14
BM (kg)	89.8 ± 12.7	89.6 ± 12.6	89.5 ± 12.7	86.1 ± 12	86.7±12.1	85.5±11.8**
BMI (kg.m^{-2})	28 ± 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*
RT (years.)	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 (no.week	4.4 ± 1.1	4.4 ± 1.1	4.4 ± 1.1	4.4 ± 1.1	4.4 ± 1.1	4.4 ± 1.1
TT (mins.)	47 ± 15	47 ± 15	47 ± 15	47 ±15	47 ± 15	47 ± 15
Energy Intake (KJ.day^{-1})	18050±4100	18100±2020	18175±3100	17900±3020	18450±3900	18100±2020
Protein Intake (g.day^{-1})	205 ± 60	195 ± 55	213 ± 45	207 ± 35	217 ± 65	210 ± 50

Training history for control (**C**) group v growth hormone (**rhGH**) group.

Results are presented as means ± Standard Deviations (SD)

BM = Body mass; **BMI** = Body mass index; **FFMI** = Fat free mass index; **RT** = Resistance training; **WT** = Weight training; **TT** = Training time.

* = $P<0.017$ = significantly different to **PRE- rhGH**; ** = $P<0.017$ = significantly different to **on-rhGH**

Values for Cardiovascular responses are presented in table 2.

Table.2: Arterial Pulse Wave Velocity & Heart Rate and Blood Pressure Responses.

Variables	Control Group (*C*)			Administration Group		
				(PRE-rhGH)	*(on-rhGH)*	*(POST-rhGH)*
Day	1	7	14	1	7	14
UL-PRE-OCC-V (m.s⁻¹)	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-OC-V (m.s⁻¹)	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-V (m.s⁻¹)	9.64 ± 1.62	9.79 ± 1.50	9.75 ± 1.54	9.97 ± 1.38	$9.18 \pm 1.60*$	$9.26 \pm 1.52*$
LL- POST-OC-V (m.s⁻¹)	9.28 ± 1.45	9.48 ± 1.26	9.37 ± 1.26	9.84 ± 1.84	9.27 ± 1.33	9.35 ± 1.49
HR–rest (bpm)	66 ± 16	67 ± 16	67 ± 14	72 ± 14	$78 \pm 11†$	75 ± 18
SBP–rest (mm.Hg)	125 ± 12	124 ± 12	125 ± 11	126 ± 10	125 ± 12	122 ± 9

APWV (m.s⁻¹) responses for control (**C**) group v growth hormone (**rhGH**) group.

Figures are presented as means ± Standard Deviations (SD).

UL-PRE-OCC-V = Upper Limb Pre-occlusion Velocity;

UL-POST-OC-V = Upper Limb Post-occlusion Velocity;

LL-PRE-OCC-V = Lower Limb Pre-occlusion Velocity;

LL-POST-OC-V = Lower Limb Post -occlusion Velocity.

Heart rate (**HR**, bpm), Systolic Blood Pressure (**SBP**, mm.Hg),

Rate Pressure Product (**RPP**, bpm. x mm.Hg x 10⁻²) responses, for control (**C**) group v growth hormone (**rhGH**) group.

Figures are presented as means ± Standard Deviations (SD).

–rest = Resting.

† = $P<0.05$ = significantly different to **C**

* = $P<0.017$ = significantly different to **PRE-rhGH**

Serum analytes associated with cardiovascular disease (CVD) are presented in table 3.

Table.3: Serum analytes associated with cardiovascular disease.

Variables	Control Group (*C*)			Administration Group		
				(PRE-rhGH)	*(on-rhGH)*	*(POST-rhGH)*
Day	**1**	**7**	**14**	**1**	**7**	**14**
T (nmol.L⁻¹)	17.5 ± 5.2	17.5 ± 5.6	17.4 ± 5.4	16.2 ± 6.0	15.3 ± 5.7	14.5 ± 5.0
Na⁺ (mmol.L⁻¹)	139.6 ± 8.4	141.5 ± 3.1	140.5 ± 5.8	140.6 ± 2.7	$142 \pm 2.4*$	$142 \pm 2.4*$
hsCRP(mg.L⁻¹)	1.35 ± 1.9	1.38 ± 2.1	1.44 ± 2.1	1.77 ± 2.1	$1.29 \pm 1.6*$	1.7 ± 2.8
HCY (μmol.L⁻¹)	12.5 ± 4.2	13.3 ± 4.7	13.1 ± 4.1	13.2 ± 4.0	$11.7 \pm 3.1*$	13.1 ± 4.3
TG (mmol.L⁻¹)	1.0 ± 0.4	1.1 ± 0.5	1.1 ± 0.4	1.2 ± 0.5	1.1 ± 0.6	1.4 ± 0.9

HDL (mmol.L^{-1})	1.2 ± 0.4	1.3 ± 0.3	1.2 ± 0.3	1.2 ± 0.3	1.2 ± 0.2	1.2 ± 0.3
LDL (mmol.L^{-1})	2.7 ± 0.8	2.8 ± 0.8	2.8 ± 1.0	2.9 ± 0.9	2.7 ± 0.7	2.9 ± 0.9
TC (mmol.L^{-1})	4.4 ± 1.0	4.6 ± 0.9	4.5±1.1	4.7±0.9	4.4 ± 0.7*	4.7 ± 1.0
IGF-I (ng.mL^{-1})	179 ± 47	169 ± 50	175 ± 53	159 ± 54	323±93*†¥	175 ± 61
PIIINP (U/ml)	0.32 ± 0.1	0.30 ± 0.1	0.32 ± 0.1	0.28 ± 0.1	0.42 ± 0.2*†¥	0.35 ± 0.1*

Serum analytes responses for control (*C*) group v growth hormone (*GH*) group.

Figures are presented as means ± Standard Deviations (SD)

T = Testosterone; **hsCRP =** high sensitivity C-reactive Protein; **HCY** = Homocysteine; **TG** = Triglycerides;

HDL = High Density Lipoprotein; **LDL =** Low Density Lipoprotein; **TC =** Total Cholesterol;

IGF-I = Insulin-like growth factor-I; **PIIINP =** Amino-terminal propeptide of type III procollagen.

* = *P*<0.017 = significantly different to *PRE-rhGH*;

† = *P*<0.017 = significantly different to *POST-rhGH*;

¥ = *P*<0.05 = significantly different to *C*.