Brazilian adult individuals with untreated isolated GH deficiency do not have accelerated subclinical atherosclerosis

Ursula M M Costa1, Carla R P Oliveira2, Roberto Salvatori3, José A S Barreto-Filho1, Viviane C Campos2, Francielle T Oliveira2, Ivina E S Rocha2, Joselina L M Oliveira1, Wersley A Silva1 and Manuel H Aguiar-Oliveira2

1Division of Cardiology, Federal University of Sergipe, Aracaju, SE 49060-100, Brazil
2Division of Endocrinology, Federal University of Sergipe, Aracaju, SE 49060-100, Brazil
3Division of Endocrinology, Diabetes and Metabolism, The Johns Hopkins University School of Medicine, 1830 East Monument Street Suite #333, Baltimore, Maryland 21287, USA

Correspondence should be addressed to R Salvatori
Email salvator@jhmi.edu

Abstract

GH and its principal mediator IGF1 have important effects on metabolic and cardiovascular (CV) status. While acquired GH deficiency (GHD) is often associated with increased CV risk, the consequences of congenital GHD are not known. We have described a large group of patients with isolated GHD (IGHD) due to a homozygous mutation (c.57C>G>A) in the GH releasing hormone receptor gene, and shown that adult GH-naïve individuals have no evidence of clinically evident premature atherosclerosis. To test whether subclinical atherosclerosis is anticipated in untreated IGHD, we performed a cross-sectional study of 25 IGHD and 27 adult controls matched for age and gender. A comprehensive clinical and biochemical panel and coronary artery calcium scores were evaluated by multi-detector tomography. Height, weight, IGF1, homeostasis model assessment of insulin resistance, creatinine and creatine kinase were lower in the IGHD group. Median and interquartile range of calcium scores distribution was similar in the two groups: IGHD 0(0) and control 0(4.9). The vast majority of the calcium scores (20 of 25 IGHD (80%) and 18 of 27 controls (66.6%)) were equal to zero (difference not significant). There was no difference in the calcium scores classification. None of IGHD subjects had minimal calcification, which were present in four controls. Three IGHD and four controls had mild calcification. There were two IGHD individuals with moderate calcification and one control with severe calcification. Our study provides evidence that subjects with congenital isolated lifetime and untreated severe IGHD do not have accelerated subclinical coronary atherosclerosis.

Key Words
- coronary atherosclerosis
- isolated growth hormone deficiency
- calcium score
- multi-detector CT

Introduction

Growth hormone (GH) and its principal mediator insulin-like growth factor 1 (IGF1) have important effects not only on the acquisition of normal body size, but also on metabolic and cardiovascular (CV) status (1). GH and IGF1 have synergistic anabolic effect on muscle mass, but antagonist effects on insulin action (GH-reducing and IGF1 increasing insulin sensitivity) and lipolysis (GH increasing and IGF1 reducing it) (2). Adult onset GH deficiency (GHD)
has been described as model of metabolic syndrome, with visceral obesity, insulin resistance, endothelial dysfunction, increased sympathetic activity, and a pro-inflammatory profile (3, 4, 5). Although GH replacement therapy has been shown to reduce the risk profile (6), it is still unclear if this is due to GHD per se, or to confounding factors often found in acquired GHD (other pituitary hormone deficiencies, inadequate replacements and pituitary surgery or radiotherapy) (7, 8).

A model of isolated GHD (IGHD) would be preferable to study the relationship between the GH–IGF1 axis and CV risk. In Itabaianinha county, in the northeastern Brazilian state of Sergipe, we have described a large group of subjects with familial IGHD due to a homozygous (c.57 + 1G > A) mutation in the GHRH receptor gene (GHRHR) (9). The untreated IGHD adults have proportionate dwarfism, and otherwise normal pituitary function (10). They exhibit a balance between adverse and beneficial CV risk factors. The adverse factors are increased systolic blood pressure (BP), higher fat mass percentage, increased total and LDL-cholesterol and C-reactive protein (11), visceral obesity and elevated cortisol to cortisone ratio (12). The protective factors include normal serum leptin, increased adiponectin (13) and increased insulin sensitivity (14). The adult IGHD individuals do not present premature clinical carotid (15) or aortic atherosclerosis (16) nor coronary ischemia assessed by stress echocardiography (15, 17), and have similar longevity as non-affected siblings (18). Because coronary disease presents with a long latency period, it is important to know if subclinical atherosclerosis exists in these IGHD subjects. To answer this, it is necessary to assess asymptomatic individuals (19, 20, 21). Measurement of coronary artery calcium by multi-detector tomography has been shown to predict the risk of clinical coronary disease (22, 23, 24, 25). This study tested whether subclinical atherosclerosis is accelerated in these IGHD asymptomatic individuals by comparing calcium scores in IGHD and normal controls.

Subjects and methods

Subjects

In a cross-sectional study, asymptomatic IGHD and age- and sex-matched control subjects (controls) were recruited by advertising in the local Dwarfs Association building and by word of mouth among the inhabitants of Itabaianinha. Inclusion criterion for IGHD was genotype-proven homozygosity for the c.57 + 1G > A GHRHR mutation, whereas controls were normal statured individuals proven to be homozygous for the wt GHRHR allele. Exclusion criteria were: previous GH treatment, age under 18 years, CV symptoms or any evidence of active CV diseases. Twenty-five (13 females) IGHD and 27 (15 females) volunteered and were enrolled. None of these IGHD individual have participated in a previous 6-month GH depot trial (17). The Federal University of Sergipe Institutional Review Board approved these studies and all subjects gave written informed consent.

Interview and physical examination

The subjects were submitted to a detailed interview including risk factors for CV disease, hypertension, smoking, dyslipidemia, familiar CV history, co-morbidities and treatments, and to a physical examination, with measurement of body weight and height. BP was obtained by the average of three measurements obtained in the left arm after 10 min of rest in the sitting position by one physicians (UMM C) using a mercury sphygmomanometer with a cuff appropriate for the size of the arm. The Framingham risk score was estimated (26).

Laboratory assessment

Blood was collected after an overnight fast. Total cholesterol, triglycerides, glucose, insulin, urea, creatinine and creatinine kinase were measured by standard techniques. The increase in total cholesterol in this IGHD cohort is due to an increase in LDL cholesterol (11, 13, 15). IGF1 was measured by a solid-phase, enzyme-labeled chemiluminescent immunometric assay (IMMULITE 2000, Siemens Healthcare Diagnostics Products Ltd, Malvern, PA, USA), with intra- and inter-assay variabilities of 3.1 and 6.1% respectively. Insulin was measured by a solid-phase, enzyme-labeled chemiluminescent immunometric assay (IMMULITE 2000 Siemens Healthcare Diagnostics Products Ltd), with intra- and inter-assay variabilities of 4.2 and 5.1% respectively. Insulin resistance was estimated using the homeostasis model assessment of insulin resistance (HOMAIR) with the formula: fasting serum insulin (\( \mu U/ml \)) x fasting plasma glucose (mmol/l)/22.5. All the tests were carried out in the Laboratory of University Hospital of the Federal University of Sergipe, in Aracaju, Sergipe.

Coronary tomography for calcium score

Calcium score was calculated with the use of a 64-slice multi-detector tomography scanner (Siemens Somatom Definition AS) dedicated to ECG-synchronized cardiac...
studies, for a non-contrast ECG-triggered acquisition. Syngovia software (Siemens Healthcare Global) was employed to acquire the Agatston score. All computed tomographies were analyzed by a single reader. The classification of calcium score followed the American College of Cardiology/American Heart Association Task Force criteria. Calcium score >300 = severe calcification, 101–300 = moderate calcification, 11–100 = mild calcification, 1–10 = minimal calcification and 0 = absence of calcification (25).

**Statistical analysis**

Data are expressed as mean (s.d.), except for IGF1, insulin, HOMAIR and calcium scores expressed as median (interquartile range). Student’s t-test was used for variables with normal distribution, and Mann–Whitney U-test for variables without Gaussian distribution (IGF1, insulin, HOMAIR and calcium scores). Fisher’s exact test was used to analyze classification of CAC sores and percentage of previous smoking, high BP and diabetes. Statistical analysis was performed using the statistical software SPSS 19.0 version. P values under 0.05 were considered significant.

**Results**

Table 1 shows the clinical and biochemical features of the two groups. Height, weight, IGF1, insulin, HOMAIR, creatinine, creatininekinase and were lower in IGHD group.

| Table 1 | Clinical and biochemical features of IGHD and control subjects. |
|---------|------------------------------------------------------------------|
| Age (years) | 50.1 (15.9) | 51.1 (14.0) | 0.799 |
| Sex (F/M) | 13/12 | 15/12 | 2.764 |
| Smoking | 1 | 2 | 0.0001 |
| Weight (kg) | 39.3 (8.7) | 71.8 (14.4) | < 0.0001 |
| Diabetes history | 4 | 2 | 0.411 |
| Dislipidemia history | 11 | 7 | 0.0001 |
| Arterial hypertension | 8 | 16 | 0.058 |
| Height (m) | 1.2 (0.18) | 1.6 (0.1) | < 0.0001 |
| BMI (kg/m²) | 25.5 (5.7) | 26.7 (4.2) | 0.402 |
| Systolic BP (mmHg) | 122.1 (18.9) | 124.4 (15.0) | 0.622 |
| Diastolic BP (mmHg) | 78.8 (9.3) | 80.4 (6.5) | 0.486 |
| IGF1 (ng/ml) | 1.9 (0) | 132 (55) | < 0.0001 |
| Glucose (mg/dl) | 105.5 (22.7) | 105.9 (70.5) | 0.975 |
| Insulin (mU/ml) | 5.35 (3.8) | 17.3 (10.9) | < 0.0001 |
| Homa IR | 1.4 (1.0) | 4.3 (3.1) | 0.012 |
| Cholesterol (mg/dl) | 227.0 (65.1) | 210.7 (28.2) | 0.257 |
| Tryglicerides (mg/dl) | 142.0 (95.5) | 135.3 (51.3) | 0.762 |
| Urea (mg/dl) | 38.6 (9.5) | 34.7 (9.0) | 0.143 |
| Creatinine (mg/dl) | 0.7 (0.1) | 0.9 (0.2) | < 0.0001 |
| Creatininekinase (IU/l) | 82.0 (35.1) | 152.8 (141.0) | 0.017 |
| Framingham score | 5.7 (6.4) | 4.9 (4.5) | 0.596 |

Table 2 shows the calcium scores. The distribution expressed as median and interquartile range was similar in the two groups: IGHD 0(0) and control 0(4.9). The vast majority of the CAC scores (20 out 25 IGHD (80%) and 18 out 27 controls (66.6%)) were equal to zero, p = 0.354. The highest CAC scores were 128.1 in IGHD and 331.5 in controls. Two subjects were further evaluated. A 66-year-old IGHD female (with complaint of chest pain) underwent CT angiography and coronary angiogram, and a clinical three arterial lesion was found. The second, a 61-year-old control male exhibited normal exercise stress echocardiography. Both were recommended lifestyle modifications and statin use.

**Discussion**

Acquired adult-onset GHD is a model of metabolic syndrome, with abdominal obesity, insulin resistance and dyslipidemia (3, 4, 5). Nevertheless, the relationship between GHD and obesity includes several mechanisms and including some degree of GH resistance in obesity (27). Although GH replacement therapy has been shown to reduce this risk profile (6), it is still unclear if the high risk is due to untreated GHD, or to confounding factors often found in acquired GHD. Some of these factors such as hypogonadism, excessive glucocorticoid replacement, craniohypogonadism, radiotherapy (implicated in cerebrovascular mortality or de novo brain tumors) and very recently adrenal crisis (7, 8, 28, 29, 30, 31, 32, 33) have been implicated in the increased mortality risk associated with hypopituitarism (28). Accordingly, very recent national or multicenter studies have suggested that GHD deficiency in the context of hypopituitarism does not cause an increase in CV mortality despite increased general mortality risk (8, 29, 30).

As our previous studies indicated that this IGHD group has no premature clinical atherosclerosis (11, 15, 16, 17), we decided to assess subclinical atherosclerosis, by measuring the calcium content in the coronary arteries. Calcium score is an independent predictor of mortality in a multivariable model controlling for age, gender,
endocrine connections to increased risk ischemic heart disease (36). Conversely, IGF1 reduction in the general population has been linked to higher burden of classical CV risks throughout life (35). It is possible that the Portuguese ancestry influenced the calcium scores in both IGHD and controls. However, as calcium scores parallel the CV mortality rates in the three nations (34), we can conclude that IGHD subjects have an atherosclerosis risk that is similar to non-GHD asymptomatic normal individuals form the same region.

Both IGHD and control groups have low Framingham risk scores, despite the fact that the IGHD group is exposed to higher burden of classical CV risks throughout life (in this particular sample the prevalence of hypertension was not different between the two groups). One explanation for this finding is the increased insulin sensitivity of IGHD subjects despite visceral adiposity (12). Another possible explanation lies on the degree of IGF1 reduction. It is possible that different degrees of IGF1 deficiency may result in different effects on the vascular wall (15). Mild IGF1 reduction in the general population has been linked to increased risk ischemic heart disease (36). Conversely, very severe IGF1 reduction, as found in our IGHD model, may be protective against atherosclerosis. This fits with the increase longevity of animal models of GHD or GH resistance (37). Accordingly, when we treated a subset of these patients with submaximal doses of GH for 6 months, converting a severe in a mild IGF1 deficiency we observed the appearance of atherosclerotic plaques (17).

Calcium scores of patients with IGHD due to large homozygous deletions in the GH-1 gene, or with GH resistance (Laron dwarfism) are not available to compare the frequency of subclinical atherosclerosis in these models with ours. Longevity, influenced not only by clinical atherosclerosis, but also from other conditions such as cancer, infectious diseases and accidents, seems to be reduced in the former model (38), but is not in the latter (39). Differently from subjects with large deletions in the GH-1 gene (complete lack of GH secretion) and Laron dwarfism (lack of GH action), our IGHD subjects have very low, but detectable serum GH levels (10). This residual GH secretion may exert some metabolic actions, resulting in differences between these disease models.

One limitation of our study is that the age of our sample is relatively young. However, near 80% of the IGHD subjects are ≥35 years, age sufficient in other studies to identify individuals who have subclinical atherosclerosis when CV risk factors are present (40, 41). It is also possible that subjects with worse health status would not have volunteered.

Other factors that may influence the development of atherosclerosis include environmental factors and dietary habits. Both GHD and controls reside in the same area, which is becoming progressively more urbanized. While we have recently reported that GHD subjects consume in percentage more proteins, less carbohydrates and equal amount of lipids in comparison with the controls (42), it is difficult to predict the role of such dietary differences on the development of subclinical coronary atherosclerosis.

In conclusion, subjects with congenital isolated lifelong and untreated severe IGHD do not have accelerated subclinical coronary atherosclerosis when compared with the normal controls from the same region.

Declaration of interest
R Salvatori serves in the advisory board of Novo Nordisk, Novartis and Pfizer.

Funding
This research did not receive any specific grant from any funding agency in the public, commercial or not-for-profit sector.

Acknowledgements
The authors thank the ‘Associação do Crescimento Físico e Humano de Itabaianinha’ for their assistance and the Clinic CEMISE for Calcium scores measurements.
References

1. Aguiar-Oliveira MH & Salvatori R. Lifetime growth hormone (GH) deficiency: impact on growth, metabolism, body composition, and survival capacity. Chapter ID 160. In Handbook of Growth and Growth Monitoring in Health and Disease. Ed VR Peedy. New York, NY, USA: Springer Science Business Media, LLC, 2011.

2. Ghahri H, Cook DM, Saenger PH, Bengtsson BA, Feld S, Nippoldt TB, Rodbard HW, Seibel JA & Vance ML. American Associations of Clinical Endocrinologists medical guidelines for clinical practice for growth hormone use in adults and children – UPDATE. Endocrine Practice 2003 9 64–76. (doi:10.1858/EP.9.1.64)

3. de Boer H, Blok GJ & Van der Veen EA. Clinical aspects of growth hormone deficiency in adults. Endocrine Reviews 1995 16 63–86. (doi:10.1210/er.16.1.63)

4. Gola M, Bonadonna S, Doga M & Giustina A. Clinical review: Growth hormone and cardiovascular risk. Journal of Clinical Endocrinology and Metabolism 2005 90 1864–1870. (doi:10.1210/jc.2004-0545)

5. Colao A. The GH-IGF-I axis and the cardiovascular system: clinical implications. Clinical Endocrinology 2008 69 347–358. (doi:10.1111/j.1365-2265.2008.03292.x)

6. Gazzaruso C, Gola M, Karamouzis I, Giubbini R & Giustina A. Clinical review: GH receptor gene. Endocrinology and Metabolism 2007 95 713–717. (doi:10.1210/jc.2006-2265.2002.01570.x)

7. Gaillard RC, Mattsson AF, Akerblad AC, Bengtsson BA, Cara J, Feldt-Rasmussen U, Kohtowa-Haggstrom M, Monson JP, Saller B, Wilton P et al. Overall and cause-specific mortality in GH-deficient adults on GH replacement. European Journal of Endocrinology 2012 166 1069–1077. (doi:10.1530/EJE-11-0298)

8. Salvatori R, Hayashida CY, Aguiar-Oliveira MH, Phillips JA III, Souza AH, Gondo RG, Toledo SP, Conceição MM, Prince M, Maheshwari HD et al. Familial dwarfism due to a novel mutation of the growth hormone-releasing hormone receptor gene. Journal of Clinical Endocrinology and Metabolism 1999 84 917–923. (doi:10.1210/jcem.84.3.5599)

9. Aguiar-Oliveira MH, Gills MS, Barreto ES, Alcântara MR, Mirak-moid M, Souza AH, Martellini CE, Pereira FA, Salvatori R, Levine MA et al. Effect of Severe growth hormone (GH) deficiency due to a mutation in the GH-releasing hormone receptor on insulin-like growth factors (IGFs), IGF-binding proteins, and ternary complex formation throughout life. Journal of Clinical Endocrinology and Metabolism 1999 84 4118–4126. (doi:10.1210/jcem.84.6.11633)

10. Barreto-Filho JAS, Alcântara MRS, Salvatori R, Barreto MA, Souza ACS, Bastos V, Souza AH, Pereira RMC, Clayton PE, Gill MS et al. Familial isolated growth hormone deficiency is associated with increased systolic blood pressure, central obesity, and dyslipidemia. Journal of Clinical Endocrinology and Metabolism 2002 87 2018–2023. (doi:10.1210/jcem.87.5.84744)

11. Gomes-Santos E, Salvatori R, Ferrão TO, Oliveira CR, Diniz RD, Santana JA, Pereira FA, Barbosa RA, Souza AH, Melo EV et al. Increased visceral adiposity and cortisol to cortisone ratio in adults with congenital lifetime isolated GH deficiency. Journal of Clinical Endocrinology and Metabolism 2014 99 3285–3289. (doi:10.1210/je.2014-2132)

12. Oliveira CR, Salvatori R, Meneguz-Moreno RA, Aguiar-Oliveira MH, Pereira RM, Valença EH, Araújo VP, Farias NT, Silveira DC, Vieira JG et al. Adipokine profile and urinary albumin excretion in isolated growth hormone deficiency. Journal of Clinical Endocrinology and Metabolism 2010 95 693–698. (doi:10.1210/jc.2009-1919)

13. Oliveira CR, Salvatori R, Meneguz-Moreno RA, Aguiar-Oliveira MH, Pereira RM, Valença EH, Araújo VP, Farias NT, Silveira DC, Vieira JG et al. Adipokine profile and urinary albumin excretion in isolated growth hormone deficiency. Journal of Clinical Endocrinology and Metabolism 2010 95 693–698. (doi:10.1210/jc.2009-1919)

14. Oliveira CR, Salvatori R, Barreto-Filho JA, Rocha IE, Mari A, Pereira RM, Campos VC, Menezes M, Gomes-Santos E, Menegus-Moreno RA et al. Insulin sensitivity and -cell function in adults with lifetime, untreated isolated growth hormone deficiency. Journal of Clinical Endocrinology and Metabolism 2012 97 1013–1019. (doi:10.1210/jc.2011-2590)

15. Oliveira JL, Marques-Santos C, Barreto-Filho JA, Ximenes-Filho R, Bitto AV, Souza AH, Prado CM, Oliveira CR, Pereira RM, Vicente TA et al. Lack of evidence of premature atherosclerosis in untreated severe isolated growth hormone (GH) deficiency due to a GH-releasing hormone receptor mutation. Journal of Clinical Endocrinology and Metabolism 2006 91 2093–2099. (doi:10.1210/jc.2005-2571)

16. Souza AH, Farias MI, Salvatori R, Silva GM, Santana JA, Pereira FA, de Paula FJ, Valença EH, Melo EV & Barbosa RA. Lifetime, untreated isolated GH deficiency due to a GH-releasing hormone receptor mutation has beneficial consequences on bone status in older individuals, and does not influence their abdominal aorta calcification. Endocrine 2014 47 191–197. (doi:10.1007/s12020-013-0118-5)

17. Oliveira JL, Aguiar-Oliveira MH, D’Oliveira A, Pereira RM, Oliveira CR, Farias CT, Barreto-Filho JA, Anjos-Andre FA, Marques-Santos C, Nascimento-Junior AC et al. Congenital growth hormone (GH) deficiency and atherosclerosis: effects of GH replacement in GH-naive adults. Journal of Clinical Endocrinology and Metabolism 2007 92 4664–4670. (doi:10.1210/jc.2007-1636)

18. Aguiar-Oliveira MH, Oliveira ET, Pereira RM, Oliveira CR, Blackford A, Valença EH, Gomes-Santos E, Goin-Junior MB, Menegus-Moreno RA, Araujo VP et al. Longevity in untreated congenital growth hormone deficiency due to a homozygous mutation in the GHRH receptor gene. Journal of Clinical Endocrinology and Metabolism 2010 95 714–721. (doi:10.1210/jc.2009-0897)

19. Earls JP, Woodard PK, Abbara S, Akers SR, Araoz PA, Cummings KM, Cury RC, Dorbala S, Hoffmann U, Hsu JY et al. ACR appropriateness criteria asymptomatic patient at risk for coronary artery disease. Journal of the American College of Radiology 2014 11 12–19. (doi:10.2147/vhrm.s8753)

20. Park HE, Chun EJ, Choi SI, Lee SP, Yoon C-H, Kim H-K, Youn T-J, Kim Y-J, Choi DJ, Sohn D-W et al. Clinical and imaging parameters to predict cardiovascular outcome in asymptomatic subjects. International Journal of Cardiovascular Imaging 2013 29 1595–1602. (doi:10.1007/s10554-013-0235-5)

21. Warrhaj R & Nasir K. Subclinical cardiovascular disease assessment in persons with diabetes. Current Cardiology Reports 2013 15 358. (doi:10.1186/s11358-013-0358-2)

22. Detrano R, Guerci AD, Carr JJ, Bild DE, Burke G, Folsom AR, Liu K, Shea S, Szklo M, Blumenthal DA et al. Coronary calcium as a predictor of coronary events in four racial or ethnic groups. New England Journal of Medicine 2008 358 1336–1345. (doi:10.1056/NEJMoa072100)

23. Budoff MJ, McClelland RL, Detrano R, Wong N, Blumenthal RS, Kondos G & Kronmal RA. Coronary calcium predicts events better with absolute calcium scores than age-sex-race/ethnicity percentiles: MESA (Multi-Ethnic Study of Atherosclerosis). Journal of the American College of Cardiology 2009 53 345–352. (doi:10.1016/j.jacc.2008.07.072)

24. Sharma RK, Sharma RK, Voelker DJ, Singh VN, Pahuja D, Nash T & Reddy HK. Cardiac risk stratification: role of the coronary calcium score. Vascular Health and Risk Management 2010 6 603–611. (doi:10.2147/VHRM.S8753)

25. Budoff MJ, Shaw LJ, Liu ST, Weinstein SR, Moser TP, Tseng PH, Flores FR, Callister TQ, Raggi P & Berman DS. Long-term prognosis associated with coronary calcification: observations from a registry of 25,253 patients. Journal of the American College of Cardiology 2007 49 1860–1870. (doi:10.1016/j.jacc.2006.10.079)

26. D’Agostino RB, Grundy S, Sullivan LM & Wilson P. Validation of the framingham coronary heart disease prediction scores. Journal of the American Medical Association 2001 286 180–187. (doi:10.1001/jama.286.2.180)
27 Vijayakumar A, Yakar S & Leroith D. The intricate role of growth hormone in metabolism. Frontiers in Endocrinology 2011 2 32. (doi:10.3389/fendo.2011.00032)

28 Rosen T & Bengtsson BA. Premature mortality due to cardiovascular disease in hypopituitarism. Lancet 1990 336 285–283. (doi:10.1016/0140-6736(90)91812-O)

29 Van Bunderen CC, van Nieuwpoort IC, Arwert LI, Heymans MW, Franken AA, Koppeschaar HP, van der Lely AJ & Drent ML. Does growth hormone replacement therapy reduce mortality in adults with growth hormone deficiency? Data from the Dutch National Registry of growth hormone treatment in adults. Journal of Clinical Endocrinology and Metabolism 2011 96 3151–3159. (doi:10.1210/jc.2011-1215)

30 Burmer P, Mattsson AF, Johannsson G, Höybye C, Holmer H, Dahlqvist P, Berinder K, Engström BE, Erkman B, Erfurth EM et al. Deaths among adults patients with hypopituitarism: hypocortisolism during acute stress, and de novo malignant brain tumors contribute to an increased mortality. Journal of Clinical Endocrinology and Metabolism 2013 98 1466–1475. (doi:10.1210/jc.2012-4059)

31 Tomlinson JW, Holden N, Hills RK, Clayton RN, Bates AS, Sheppard MC & Stewart PM. Association between premature mortality and hypopituitarism. West Midlands Prospective Pituitary Study Group. Lancet 2001 357 425–431. (doi:10.1016/S0140-6736(00)04006-X)

32 Filipsson H, Monson JP, Koltowska-Haggström M, Mattsson A & Johannsson G. The impact of glucocorticoid replacement regimens on metabolic outcome and comorbidity in hypopituitary patients. Journal of Clinical Endocrinology and Metabolism 2006 91 3954–3961. (doi:10.1210/jc.2006-0524)

33 Bülow B, Hagmar L, Mikoczy Z, Nordström CH & Erfurth EM. Increased cerebrovascular mortality in patients with hypopituitarism. Clinical Endocrinology 1997 46 75–81. (doi:10.1046/j.1365-2265.1997.d01-1749.x)

34 Santos RD, Nasir K, Rumberger JA, Budoff MJ, Braunstein JB, Meneghello R, Barreiros M, Pereirinha A, Carvalhalo JA, Blumenthal RS et al. Difference in atherosclerosis burden in different nations and continents assessed by coronary artery calcium. Atherosclerosis 2006 187 378–384. (doi:10.1016/j.atherosclerosis.2005.09.017)

35 Pena SD, Pietro GD, Fuchshuber-Moraes M, Genro JP, Hutz MH & Kehdy FS. The genomic ancestry of individuals from different geographical regions of Brazil is more uniform than expected. PLoS ONE 2011 6 e17063. (doi:10.1371/journal.pone.0017063)

36 Juul A, Scheike T, Davidsen M, Gyllenborg J & Jorgensen T. Low serum insulin-like growth factor I is associated with increased risk of ischemic heart disease; a population-based case–control study. Circulation 2002 106 939–944. (doi:10.1161/01.CIR.0000027563.44593.CC)

37 Bartke A, Sun LY & Longo V. Somatotropic signaling: trade-offs between growth, reproductive development, and longevity. Physiological Reviews 2013 93 571–598. (doi:10.1152/physrev.00006.2012)

38 Besson A, Salemi S, Gallari S, Jenal A, Horn R, Mullis PS & Mullis RE. Reduced longevity in untreated patients with isolated growth hormone deficiency. Journal of Clinical Endocrinology and Metabolism 2003 88 3664–3667. (doi:10.1210/jc.2002-021938)

39 Laron Z. Lifespan and mortality of patients with Laron Syndrome in Chapter ID 41. In Laron Syndrome – from man to mouse. Lessons from Clinical and Experimental experience. Eds Z Laron & J Kopchick. Berlin, Heidelberg, Germany: Springer-Verlag, 2011.

40 Tzou WS, Douglas PS, Srivastava SR, Bond MG, Tang R, Chen W, Bereson GS & Stein JH. Increased subclinical atherosclerosis in young adults with metabolic syndrome. Journal of the American College of Cardiology 2005 46 457–463. (doi:10.1016/j.jacc.2005.04.046)

41 Dawson JD, Sonka M, Blecha MB, Lin W & Davis PH. Risk factors associated with aortic and carotid intimal medial thickness in adolescents and young adults: the muscatine offspring study. Journal of the American College of Cardiology 2009 53 2273–2279. (doi:10.1016/j.jacc.2009.03.026)

42 Oliveira-Santos AA, Salvadori R, Gomes-Santos E, Santana JA, Leal AC, Barbosa AA et al. Subjects with isolated GH deficiency due to a null GHRHR mutation eat proportionally more, but healthier than controls. Endocrine 2015 [in press]. (doi:10.1007/s12020-015-0670-2)

Received in final form 13 January 2016
Accepted 14 January 2016