Published in final edited form as:
Growth Horm IGF Res. 2012 ; 22(0): 102–107. doi:10.1016/j.ghir.2012.03.001.

The Association of Macro- and Micronutrient Intake with Growth Hormone Secretion

S. Denny-Brown, **T.L. Stanley**, **S.K. Grinspoon**, and **H. Makimura**
Eastern Virginia Medical School (S.D.B.), Norfolk, Virginia 23507, Program in Nutritional Metabolism and Neuroendocrine Unit (T.L.S., S.K.G., H.M.), and Pediatric Endocrine Unit (T.L.S.), Massachusetts General Hospital and Harvard Medical School, Boston, Massachusetts 02114

Abstract

Context—Growth hormone (GH) is known to be nutritionally regulated, but the effect of dietary composition on detailed GH secretion parameters has not previously been comprehensively evaluated.

Objective—The objective of the study was to determine whether specific macro- and micronutrients are associated with discrete parameters of GH secretion among subjects with wide ranges of body mass index.

Design—Detailed macro- and micronutrient intake was assessed by four-day food records while GH secretion was assessed by standard stimulation testing in 108 men and women in one study (Study 1), and by overnight frequent blood sampling in 12 men in another study (Study 2).

Results—Peak stimulated GH was positively associated with vitamin C (r=+0.29; *P*=0.003), dietary fiber (r=+0.27; *P*=0.004), arachidic acid (r=+0.25; *P*=0.008), and behenic acid (r=+0.30; *P*=0.002) intake in univariate analysis. Controlling for age, gender, race/ethnicity, visceral fat, HOMA-IR, total caloric intake and these four dietary factors in step-wise multivariate modeling, peak GH remained significantly associated with vitamin C and visceral fat (both P<0.05). In addition, vitamin C intake was associated with various parameters of endogenous GH secretion including basal GH secretion (r=+0.95; *P*<0.0001), GH half-life (r=+0.75; *P*=0.005), total GH production (r=+0.76; *P*=0.004), GH area-under-the-curve (r=+0.89; *P*=0.0001), mean $\log_{10}$ GH pulse area (r=+0.67; *P*=0.02), and overnight maximum (r=+0.62; *P*=0.03), nadir (r=+0.97; *P*<0.0001), and mean GH secretion (r=+0.89; *P*=0.0001).

Conclusions—These results suggest that certain micronutrients such as vitamin C intake are strongly and uniquely associated with stimulated and endogenous spontaneous GH secretion.

Keywords

diet; vitamin C; ascorbic acid; frequent sampling

Corresponding Author: Hideo Makimura, MD, PhD, Program in Nutritional Metabolism and Neuroendocrine Unit, Massachusetts General Hospital and Harvard Medical School, 55 Fruit St., LON 211, Boston, MA 02114, Tel: 617-726-8277, Fax: 617-724-8998, hmakimura@partners.org, **Reprint Requests:** Please address reprint requests to Dr. Hideo Makimura.

Disclosure: The authors have nothing to disclose

Introduction

Growth hormone (GH) secretion is nutritionally regulated. Metabolic stimuli including insulin, glucose, and free fatty acids are known to affect GH secretion, and may act through growth hormone releasing hormone (GHRH), somatostatin, ghrelin, and other pathways. Endogenous and stimulated GH secretion are reduced in the context of generalized and excess visceral adiposity[1–3] and are known to be enhanced by fasting[4]. Furthermore, protein intake, in particular gelatin, can acutely increase GH secretion in the short term[5,6]. However, the long-term contribution of various macro- and micronutrients on GH secretion has not been comprehensively investigated. While Merimee *et al.* previously demonstrated decreased summated GH secretion assessed by obtaining hourly blood samples over 24 hours, after a high-carbohydrate diet[7], this was a small study, involving only eight normal weight men, that did not control for covariates and was limited in its evaluation of GH secretion. Moreover, to our knowledge, the effect of micronutrient intake on GH secretion has not previously been studied in detail.

One micronutrient of interest is vitamin C. Plasma concentration of vitamin C is reduced in obesity[8, 9] and is inversely associated with waist-to-hip ratio, a measure of central adiposity, independent of BMI[10]. In addition, several studies have demonstrated an association between circulating vitamin C concentration and cardiovascular disease risk[11,12], paralleling the association of reduced GH secretion with abdominal adiposity and increased cardiovascular disease risk[3,13,14]. Furthermore, Tran CD et al. previously demonstrated a positive dose-response association between dietary vitamin C intake and IGF-1 concentrations[15], indicating a possible relationship between vitamin C intake and GH secretion.

We therefore investigated the role of dietary composition, including vitamin C intake, on GH secretion by analyzing four-day food and supplementation records of subjects from two previously completed clinical research studies in which detailed parameters of stimulated and endogenous spontaneous GH secretion were investigated. We hypothesized that intake of specific macro- and micronutrients would be associated with discrete parameters of both stimulated and endogenous spontaneous GH secretion.

Methods and Procedures

Study subjects

Study 1—One-hundred-and-eight generally healthy men and women from the Boston area were studied. Data from a subset of these subjects were previously published in a study evaluating the relationship between GH secretion and cardiovascular indices[14], however, detailed macro- and micronutrient intake of these subjects have not been reported. These subjects were adults, age 18 to 55 years, with a wide range of BMI. Subjects were without known pituitary disease. Exclusion criteria included a history of diabetes mellitus, thyroid disorders, chronic medical conditions such as HIV infection, or any medical condition known to affect the GH axis. Subjects taking medications known to affect GH secretion were excluded. Subjects with serum creatinine >1.5 mg/dl, hemoglobin <11 g/dl, or aspartate aminotransferase >2.5 times the upper limit of the normal range were also excluded.

Study 2—Twelve generally healthy men from the Boston area were studied. Data from these subjects were previously published in a study evaluating the effect of a short-term strategy to augment endogenous GH pulsatility using growth hormone releasing hormone[16]. Detailed micronutrient intake of these subjects has yet to be reported and we now compare macro- and micronutrient intake to parameters of endogenous GH secretion, obtained at

baseline, prior to any intervention in the study. These subjects were adult men, age 18 to 60 years, with BMI between 20–35 kg/m^2. Subjects were without known pituitary disease, history of cranial radiation, or severe renal disease, liver disease, or chronic illness. Additional exclusion criteria included use of corticosteroids, gonadal steroids, or anti-diabetic agents.

All subjects underwent written informed consent in compliance with the guidelines of the Subcommittee on Human Studies at the Massachusetts General Hospital prior to the administration of any study procedures.

Assessment of GH secretion

All subjects in Study 1 underwent standard GH stimulation testing with GHRH and arginine as previously reported[3,14]. Briefly, after an overnight fast, subjects were administered GHRH 1–29 [1 ug/kg] (Sermorelin acetate, Geref, Serono Laboratories, Inc., Norwell, Ma) intravenously followed by arginine hydrochloride [30 g/300 ml (max 30 g)]. GH levels were measured at 0, 30, 45, 60, 90, and 120 minutes. All subjects in Study 2 underwent overnight frequent blood sampling for GH at a frequency of every 10 minutes from 20:00 hours to 07:40 hours with the exception of one subject who underwent frequent sampling every 20 minutes. Parameters describing endogenous GH secretion including basal secretion, physiologic half-life, pulse frequency, and pulse area were determined by the automated deconvolution analysis program Auto-Decon[17]. For two GH profiles in which three consecutive samples were missing, the last GH value was carried forward × 1 to allow for analysis.

Laboratory methods

Serum GH was determined by the Beckman Access Ultrasensitive human GH assay, a paramagnetic particle, chemiluminescent immunoassay (Beckman Coulter, Chasta, MN) with an effective analytical sensitivity of 0.01 μg/liter. The intra-assay CV is 1.90–2.78% and the inter-assay CV is 1.77–2.65%. Fasting glucose was determined using standard methodology at our clinical laboratory. Insulin was measured using the paramagnetic particle, chemiluminescent Access immunoassay system (Beckman Coulter, Chasta, MN), with an analytical sensitivity of 0.03 IU/ml, and a precision of 3–5.6%. Serum IGF-1 was measured by EIA (Alpco Diagnostics, Inc., Salem, NH), with a detection limit of 2.3 μg/liter, an intra-assay CV of 6.6 to 9.7%, and an inter-assay CV of 11.3 to 13.7% and available for 85 subjects.

Anthropometric measurements

Height and body weight were obtained after an overnight fast. Total body fat percentage was determined by dual X-ray absorptiometry (DXA) testing using a Hologic-4500 densitometer (Hologic, Inc., Waltham, MA). In addition, 1-cm cross-sectional abdominal CT scans were performed at the level of L4 to assess the distribution of abdominal subcutaneous adipose tissue (SAT) and abdominal visceral adipose tissue (VAT) as previously described[18].

Assessment of nutritional intake

The absolute intake (grams/day) of macro- and micronutrients, including specific carbohydrates, amino acids, fatty acids, and vitamins, was determined using 4-day food records facilitated by a trained registered dietician during direct interview. Data were analyzed using Nutrition Data Systems for Research (NDSR) software with the NDSR 2008 data (Version 2, University of Minnesota, Minneapolis, MN). Dietary records were available for all 108 subjects in Study 1 and all 12 subjects in Study 2.

Statistical analysis

In Study 1, peak stimulated GH was related to demographic and anthropometric parameters, including age and BMI, as well as the intake of various macro- and micronutrients using univariate regression analysis with Pearson correlations. Univariate regression analysis was also performed to assess the relationship between IGF-1 and various macro- and micronutrients. Nutrient indices that showed a significant association with peak stimulated GH upon univariate analysis were examined further using multivariate regression models to evaluate the relationship between individual nutrients and peak stimulated GH, while adjusting for the effect of potential covariates whose role in the regulation of GH secretion has previously been established. Covariates that were controlled for in each model included age, gender, race, ethnicity, anthropometric measurements, HOMA-IR, and total caloric intake. Although total caloric intake has not previously been demonstrated to have a relationship with GH secretion, it was included in all multivariate regression modeling to discern effects of individual nutrients from overall caloric intake. Least squares regression modeling was performed using BMI and repeated using visceral fat as representative anthropometric measurements. In a final model, step-wise regression analysis was performed including all the individual nutrient indices shown to be independently related to peak stimulated GH in the prior multivariate models (fiber, vitamin C, behenic acid and arachidic acid), to avoid any potential inter-relatedness between dietary indices and determine which indices are most strongly related to peak GH stimulation. In Study 2, Pearson univariate regression analysis was used to relate parameters of endogenous spontaneous GH secretion including basal secretion, physiologic half-life, number of pulsatile secretion events, total production, percent pulsatile secretion, area-under-the-curve, mean $\log_{10}$ pulse area, and overnight maximum, nadir, and mean GH to nutrient variables that were found to be significantly related to peak stimulated GH in Study 1. Sensitivity analyses were performed excluding 4 subjects with vitamin C intake of >1,000 mg/day in Study 1. No sensitivity analyses was performed in Study 2 as all subjects had vitamin C intake of <1,000 mg/day. Statistical analysis was performed using JMP 9.0.0 (SAS Institute, Cary, North Carolina, USA). Statistical significance was defined as $P<0.05$.

Results

Clinical characteristics and nutrient intake values of Study 1 subjects

Subjects in Study 1 ranged in age from 18 to 55 years with a median age of 43 years (IQ range: 33–48.75 years). Subjects were 53.7% male and 58.3% Caucasian. BMI of subjects ranged from 19.3 to 62.8 kg/m^2 with median of 32.7 kg/m^2 (IQ range: 23.6–38.9 kg/m^2). Anthropometric, metabolic and nutritional intake values of subjects in Study 1 are presented in Table 1. The current US Recommended Dietary Allowance (RDA) for vitamin C is 75 mg/day for women and 90 mg/day for men for the ages of 19 to 60 years old[19]. Forty-eight subjects (44%) did not meet the recommended US RDA in our sample.

Univariate associations with peak stimulated GH in Study 1

Peak stimulated GH was negatively associated with age (r=−0.28; $P=0.004$), BMI (r=−0.62; $P<0.0001$), waist circumference (r=−0.72; $P<0.0001$), SAT (r=−0.53; $P<0.0001$), VAT (r=−0.66; $P<0.0001$) and percent body fat (r=−0.41; $P<0.0001$) as well as fasting glucose (r=−0.27; $P=0.005$), fasting insulin (r=−0.44; $P<0.0001$) and HOMA-IR (r= −0.37; $P=0.0002$).

Peak stimulated GH was positively associated with vitamin C (r=+0.29; $P=0.003$), dietary fiber (r=+0.27; $P=0.004$), arachidic acid (r=+0.25; $P=0.008$), and behenic acid (r=+0.30; $P=0.002$) intake in univariate analysis (Table 2). Peak stimulated GH was negatively associated with dietary cholesterol (r=−0.26; $P=0.006$), total trans fatty acids (r=−0.24;

P=0.01), elaidic acid (r=−0.24; *P*=0.01), and trans-linolelaidic acid (r=−0.24; *P*=0.01) intake in univariate analysis (Table 2).

Vitamin D, E and omega 3 fatty acids were not associated with peak stimulated GH. All other specific carbohydrates, amino acids, and fatty acids that were examined were not significantly associated with peak stimulated GH.

Univariate associations with IGF-1 in Study 1

IGF-1 was negatively associated with age (r=−0.41; P=0.0001) and BMI (r=−0.26; P=0.02). IGF-1 was positively associated with intake of dietary fibers (r=+0.24; P=0.03) but was not associated with macronutrients, or vitamin C, behenic or arachidic acid intake (all P>0.05).

Vitamin C and measures of central adiposity in Study 1

Dietary vitamin C intake was inversely associated with VAT as measured by cross-sectional CT scan (r=−0.20; *P*=0.04) and trended to an inverse association with waist circumference (r=−0.03; *P*=0.08) and waist-to-hip ratio (r=−0.18; P=0.08).

Multivariate analyses for peak stimulated GH in Study 1

Separate multivariate regression models assessing the relationship between individual dietary indices that were significantly associated with peak stimulated GH on univariate analyses, controlling for age, gender, race, ethnicity, VAT, HOMA-IR, and total caloric intake. In these models, peak GH remained significantly associated with vitamin C (β=0.03, *P*=0.007, R^2 for model=0.50, *P* for model<0.0001) (Table 3A), fiber (β=0.62, *P*=0.009, R^2 for model=0.50, *P* for model<0.0001) (Table 3B), arachidic acid (β=43.79, *P*=0.05, R^2 for model=0.48, *P* for model< 0.0001) (Table 3C), and behenic acid (β=33.92, *P*=0.04, R^2 for model=0.48, *P* for model< 0.0001) (Table 3D) intake. When controlling for BMI in lieu of VAT in the multivariate models, Vitamin C (β=0.02, *P*=0.03, R^2 for model=0.62, *P* for model<0.0001) and behenic acid (β=31.01, *P*=0.03, R^2 for model=0.62, *P* for model<0.0001) remained significant, while fiber (β=0.40, *P*=0.06, R^2 for model=0.61, *P* for model<0.0001) and arachidic acid (β=34.95, *P*=0.07, R^2 for model=0.61, *P* for model<0.0001) trended to statistical significance. Dietary cholesterol, total trans fatty acids, elaidic acid, and trans-linolelaidic acid (all *P*>0.1) intake were no longer significantly associated with peak stimulated GH after controlling for these covariates.

A combined forward step-wise multivariate model for peak stimulated GH was assessed including the four individual nutrients shown to be independently related to peak stimulated GH in the above multivariate models (vitamin C, fiber, behenic acid and arachidic acid) to determine which nutrient is the most strongly related to peak GH stimulation. Independent variables of age, gender, race/ethnicity, VAT, HOMA-IR, total caloric intake, and vitamin C, fiber, behenic and arachidic acid intake were simultaneously tested for inclusion in the model. Of the independent variables, only VAT and vitamin C were selected for inclusion by the modeling as significant parameters independently related to peak stimulated GH (*P*<0.05). In this combined model, only vitamin C, but not fiber, behenic or arachidic acid was significantly related to peak stimulated GH.

Sensitivity analyses excluding subjects with vitamin C intake >1,000 mg/day in Study 1

Four of the 108 subjects had vitamin C intake >1,000 mg/day. Sensitivity analyses excluding these four subjects did not significantly alter the results. Vitamin C intake remained significantly associated with peak stimulated GH (r=+0.29; P=0.01) amongst the remaining 104 subjects.

Clinical characteristics and nutrient intake values of Study 2 subjects

The subjects ranged in age from 21 to 58 years with median age of 49 years (IQ range: 35.75–55 years). All subjects were male and 83.3 % were Caucasian. BMI ranged from 20.9 to 33.6 kg/m^2 with median BMI of 27.6 kg/m^2 (IQ range: 24.3–32.1 kg/m^2). Details of their nutritional intake are presented in Table 4. Three subjects (25%) did not meet the recommended US RDA for vitamin C intake in Study 2.

The association of nutrient intake with parameters of endogenous spontaneous GH secretion in Study 2

The four nutrient indices, vitamin C, fiber, arachidic acid and behenic acid intake, that were identified as independently associated with peak stimulated GH on standard stimulation testing in Study 1, were evaluated for their association with parameters of endogenous GH secretion. Vitamin C intake was associated with basal GH secretion (r=+0.95; P<0.0001), GH half-life (r=+0.75; P=0.005), total GH production (r=+0.76; P=0.004), GH area-under-the-curve (r=+0.89; P=0.0001), mean $\log_{10}$ GH pulse area (r=+0.67; P=0.02), and overnight GH maximum (r=+0.62; P=0.03), nadir (r=+0.97; P<0.0001), and mean (r=+0.89; P=0.0001) in univariate analysis (Table 5). Fiber intake was associated with basal GH secretion (r=+0.76; P=0.004), mean $\log_{10}$ GH pulse area (r=+0.67; P=0.02), and overnight GH nadir (r=+0.60; P=0.04) in univariate analysis. Arachidic acid intake was negatively associated with the physiologic half-life of GH (r=−0.59; P=0.04) but no other parameters of endogenous GH secretion. Behenic acid intake was negatively associated with overnight maximal GH secretion (r=−0.61; P<0.05) and trended to significance with physiologic half-life (r=−0.56; P=0.06) and mean $\log_{10}$ GH pulse area (r=−0.54; P=0.07).

Discussion

We report the first association between various macro- and micronutrient intake variables and discrete measures of GH secretion in otherwise healthy men and women. Our data demonstrate a significant association between vitamin C and peak stimulated GH on standard clinical stimulation testing and various representative parameters of endogenous spontaneous GH secretion. The results suggest that decreased vitamin C intake may be independently associated with reduced GH secretion.

Previous studies evaluating different regulators of GH secretion have focused on various physiologic hormones including insulin, glucose, free fatty acids and neuropeptides such as GHRH, somatostatin, and various GH-releasing factors. While van Vught et al. have performed detailed studies evaluating the contribution of protein intake on acute GH stimulation[5, 6], limited studies have evaluated the role of macronutrient intake in long-term GH regulation. An earlier study in 35 elderly subjects was not able to detect any association between macronutrient intake and morning GH. However, in this study, GH was assessed after 2–3 hours of fasting and measured between 08:00 – 12:00 (noon) and neither standard clinical stimulation tests nor overnight frequent sampling was performed[20]. While one interventional study demonstrated suppression of summated GH secretion after an isocaloric high-carbohydrate diet[7], one week of a low carbohydrate, high protein diet had no effect on 24 hour GH concentrations measured every 20 minutes[21]. Similarly, two weeks of either high fat or high carbohydrate diet had no effect on fasting GH[22]. However, these studies investigating the relationship of macronutrient intake to GH are limited by their small size, the short duration of intervention and method of assessing GH.

Micronutrients, specifically retinoic acid or vitamin A, has been shown to play a role in GH secretion in previous studies. *In vitro* studies have demonstrated stimulation of GH secretion in human and rat pituitary cells by vitamin A[23, 24], and one clinical study has demonstrated a

positive correlation between plasma vitamin A levels and nocturnal GH secretion in children with impaired nocturnal GH secretion[25]. We now demonstrate a potential novel role for dietary vitamin C intake in regulating GH secretion in two independent clinical studies. In our first study, we demonstrate a significant positive association between vitamin C intake and peak stimulated GH secretion on standard stimulation testing in 108 otherwise healthy men and women that remains significant upon controlling for total caloric intake and variables whose role in GH secretion have previously been published (age, gender, race/ethnicity, visceral adiposity, and insulin resistance). The previously demonstrated negative association between GH and age and visceral adiposity was confirmed in our study. We therefore controlled for these possible covariates in the multivariate modeling and found the positive association between vitamin C and GH to remain significant. Furthermore, the positive association between vitamin C intake and GH secretion is confirmed by our second study, in which endogenous spontaneous GH secretion was examined by q10 minutes overnight frequent sampling. Our demonstration of a positive association between vitamin C intake and GH secretion is in concordance with and would help explain a previous study's finding of a positive dose-response association between dietary vitamin C intake and IGF-1 concentrations[15]. While we did not observe a direct relationship between dietary vitamin C intake and IGF-1 concentration in our study, this may be due to the smaller sample size in our study (108 subjects compared to the 1,542 subjects in the study by Tran et al.[15]).

This study was not designed to assess the mechanism of how dietary vitamin C intake affects GH secretion but rather as a hypothesis-generating study to identify macro- and micronutrients that may have a physiologic role in the regulation of GH. Nonetheless, previous studies suggest a physiological connection may exist between vitamin C intake and GH secretion. Vitamin C is well known as an antioxidant but it also functions as a co-factor for peptidylglycine α-amidating mono-oxygenase (PAM), which is responsible for amidation of various neuropeptides[26]. Tissue expression of PAM has been studied in animal models and very high tissue concentrations of PAM activity have been demonstrated in both the hypothalamus and pituitary[27,28]. Furthermore, PAM activity in rat serum and tissue homogenates as well as cultured rat anterior pituitary cells has been shown to be responsive to treatment with vitamin C[27,29]. In addition, vitamin C is actively transported across membranes using the sodium-ascorbate co-transporter (SVCT) 1 and 2[30]. SVCT2 in particular is expressed in neurons and endocrine cells[31], as well as hypothalamic tanycytes[32], allowing for high concentrations of local vitamin C compared to circulating vitamin C. While PAM activity has not been directly assessed in regards to regulators of GH secretion, GH releasing hormone (GHRH) is known to be a C-terminal amidated peptide[33–35], suggesting a possible role of the micronutrient vitamin C in regulating GH secretion via GHRH post-translational processing. SVCT2 may therefore allow higher local concentration of vitamin C in the hypothalamus leading to an increase in the activity of PAM resulting in greater efficiency of amidation and activation of GHRH, thereby increasing GH signaling. Although speculative, these prior studies in combination with the present study, suggest further investigations into the relationship between vitamin C and GH would be warranted.

The strengths of our study are its examination of detailed measures of stimulated and endogenous spontaneous GH secretion, the use of a relatively large population of 108 obese and non-obese men and women in study 1, and its validation in a separate more detailed physiology study that confirms the associations using a different method to assess GH secretion. Limitations include its observational design, our inability to account for food additives that may have an energetic or nutritional effect on GH secretion, and the use of self-administered food records. In addition, we were not able to measure specific micronutrient levels in the circulation to relate to GH parameters. Although self-administered dietary intake assessment tools are widely used, they can be associated with

some imprecision[36–40]. Specifically, subjects tend to underreport total caloric and protein intake; however, Bingham *et al.* demonstrated a significantly high correlation (Spearman rank correlation of 0.86) between plasma vitamin C level measured by liquid chromatography with electrochemical detection and recorded dietary intake of vitamin C, suggesting this micronutrient may be less affected by general underreporting seen in dietary recall[36]. As such, we were able to uncover significant relationships between GH secretion and vitamin C intake in two independent studies. In this study, vitamin C intake was significantly associated with VAT, a measure of central adiposity. Given the known association between GH and measures of central adiposity[3], this association may be mediated entirely by VAT. However, in multivariate regression modeling, we controlled for VAT and the relationship between dietary vitamin C intake and peak stimulated GH remained significant, making this unlikely. It is also possible that the relationships we demonstrate between GH secretion and vitamin C intake are a proxy for a healthy lifestyle, such as high fruit and vegetable intake, because we were unable to control for this variable in the present study. This study is an exploratory retrospective study. As such, future studies manipulating the dietary intake of vitamin C would be valuable in assessing their true contributions to GH secretion. Future studies employing Daily Recommended Intake percentages for nutrient intake values in order to take into account the change in nutrient requirements according to age and physical activity may also be of benefit.

In summary, we demonstrate for the first time that vitamin C intake is significantly associated with discrete parameters of stimulated and endogenous spontaneous GH secretion. Furthermore, this relationship appears independent of potential covariates such as age, gender, VAT, HOMA-IR, total caloric intake and other micronutrient intake. The associations between GH and vitamin C identified in this study suggest the potential utility of an interventional study to determine whether increasing vitamin C intake may affect endogenous GH secretion.

Acknowledgments

We gratefully acknowledge the MGH bionutrition and nursing staffs and the research volunteers for their participation in the study.

Funding: National Institutes of Health grant T32DK007028 to SDB, K23DK089910 to TLS, R01HL085268 to SKG, R01DK63639 to SKG, K24DK064545 to SKG, K23DK087857 to HM, and M01RR01066 and UL1RR025758, Harvard Clinical and Translational Science Center, from the National Center for Research Resources. The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Center for Research Resources or the National Institutes of Health.

Clinical Trials Registration: This study was registered at www.clinicaltrials.gov as NCT00562796 and NCT00850564.

REFERENCES

1. Riedel M, Hoeft B, Blum WF, et al. Pulsatile growth hormone secretion in normalweight and obese men: differential metabolic regulation during energy restriction. Metabolism. 1995; 44:605–610. [PubMed: 7752908]
2. Pijl H, Langendonk JG, Burggraaf J, et al. Altered neuroregulation of GH secretion in viscerally obese premenopausal women. J Clin Endocrinol Metab. 2001; 86:5509–5515. [PubMed: 11701729]
3. Makimura H, Stanley T, Mun D, et al. The effects of central adiposity on growth hormone (GH) response to GH-releasing hormone-arginine stimulation testing in men. J Clin Endocrinol Metab. 2008; 93:4254–4260. [PubMed: 18765508]
4. Ho KY, Veldhuis JD, Johnson ML, et al. Fasting enhances growth hormone secretion and amplifies the complex rhythms of growth hormone secretion in man. J Clin Invest. 1988; 81:968–975. [PubMed: 3127426]

5. van Vught AJ, Nieuwenhuizen AG, Veldhorst MA, et al. The effects of protein ingestion on GH concentrations in visceral obesity. Horm Metab Res. 2010; 42:740–745. [PubMed: 20582874]
6. van Vught AJ, Nieuwenhuizen AG, Veldhorst MA, et al. The effects of dietary protein on the somatotropic axis: a comparison of soy, gelatin, alpha-lactalbumin and milk. Eur J Clin Nutr. 2010; 64:441–446. [PubMed: 20216569]
7. Merimee TJ, Pulkkinen AJ, Burton CE. Diet-induced alterations of hGH secretion in man. J Clin Endocrinol Metab. 1976; 42:931–937. [PubMed: 773953]
8. Moor de Burgos A, Wartanowicz M, Ziemlanski S. Blood vitamin and lipid levels in overweight and obese women. Eur J Clin Nutr. 1992; 46:803–808. [PubMed: 1425534]
9. Aasheim ET, Hofso D, Hjelmesaeth J, et al. Vitamin status in morbidly obese patients: a cross-sectional study. Am J Clin Nutr. 2008; 87:362–369. [PubMed: 18258626]
10. Canoy D, Wareham N, Welch A, et al. Plasma ascorbic acid concentrations and fat distribution in 19,068 British men and women in the European Prospective Investigation into Cancer and Nutrition Norfolk cohort study. Am J Clin Nutr. 2005; 82:1203–1209. [PubMed: 16332652]
11. Gale CR, Martyn CN, Winter PD, et al. Vitamin C and risk of death from stroke and coronary heart disease in cohort of elderly people. BMJ. 1995; 310:1563–1566. [PubMed: 7787644]
12. Simon JA, Hudes ES, Browner WS. Serum ascorbic acid and cardiovascular disease prevalence in U.S. adults. Epidemiology. 1998; 9:316–321. [PubMed: 9583425]
13. Utz AL, Yamamoto A, Hemphill L, et al. Growth hormone deficiency by growth hormone releasing hormone-arginine testing criteria predicts increased cardiovascular risk markers in normal young overweight and obese women. J Clin Endocrinol Metab. 2008; 93:2507–2514. [PubMed: 18445664]
14. Makimura H, Stanley T, Mun D, et al. Reduced growth hormone secretion is associated with increased carotid intima-media thickness in obesity. J Clin Endocrinol Metab. 2009; 94:5131–5138. [PubMed: 19837914]
15. Tran CD, Diorio C, Berube S, et al. Relation of insulin-like growth factor (IGF) I and IGF-binding protein 3 concentrations with intakes of fruit, vegetables, and antioxidants. Am J Clin Nutr. 2006; 84:1518–1526. [PubMed: 17158438]
16. Stanley TL, Chen CY, Branch KL, et al. Effects of a Growth Hormone-Releasing Hormone Analog on Endogenous GH Pulsatility and Insulin Sensitivity in Healthy Men. J Clin Endocrinol Metab. 2011; 96:150–158. [PubMed: 20943777]
17. Johnson ML, Pipes L, Veldhuis PP, et al. AutoDecon: a robust numerical method for the quantification of pulsatile events. Methods Enzymol. 2009; 454:367–404. [PubMed: 19216935]
18. Rietschel P, Hadigan C, Corcoran C, et al. Assessment of growth hormone dynamics in human immunodeficiency virus-related lipodystrophy. J Clin Endocrinol Metab. 2001; 86:504–510. [PubMed: 11158000]
19. Panel on Dietary Antioxidants and Related Compounds, Standing Committee on the Scientific Evaluation of Dietary References Intakes, Food and Nutrition Board, Institute of Medicine. Dietary Reference Intakes of Vitamin C, Vitamin E, Selenium, and Carotenoids. Washington D.C.: National Academy Press; 2000.
20. Darling-Raedeke M, Thornton WH Jr, MacDonald RS. Growth hormone and IGF-I plasma concentrations and macronutrient intake measured in a free-living elderly population during a one-year period. J Am Coll Nutr. 1998; 17:392–397. [PubMed: 9710852]
21. Harber MP, Schenk S, Barkan AL, et al. Effects of dietary carbohydrate restriction with high protein intake on protein metabolism and the somatotropic axis. J Clin Endocrinol Metab. 2005; 90:5175–5181. [PubMed: 15972575]
22. McCargar LJ, Clandinin MT, Belcastro AN, et al. Dietary carbohydrate-to-fat ratio: influence on whole-body nitrogen retention, substrate utilization, and hormone response in healthy male subjects. Am J Clin Nutr. 1989; 49:1169–1178. [PubMed: 2658535]
23. Morita S, Fernandez-Mejia C, Melmed S. Retinoic acid selectively stimulates growth hormone secretion and messenger ribonucleic acid levels in rat pituitary cells. Endocrinology. 1989; 124:2052–2056. [PubMed: 2707148]

24. Djakoure C, Guibourdenche J, Porquet D, et al. Vitamin A and retinoic acid stimulate within minutes cAMP release and growth hormone secretion in human pituitary cells. J Clin Endocrinol Metab. 1996; 81:3123–3126. [PubMed: 8768885]
25. Evain-Brion D, Porquet D, Therond P, et al. Vitamin A deficiency and nocturnal growth hormone secretion in short children. Lancet. 1994; 343:87–88. [PubMed: 7903782]
26. Eipper BA, Milgram SL, Husten EJ, et al. Peptidylglycine alpha-amidating monooxygenase: a multifunctional protein with catalytic, processing, and routing domains. Protein Sci. 1993; 2:489–497. [PubMed: 8518727]
27. Eipper BA, Myers AC, Mains RE. Peptidyl-glycine alpha-amidation activity in tissues and serum of the adult rat. Endocrinology. 1985; 116:2497–2504. [PubMed: 3996324]
28. Lew RA, Clarke IJ, Smith AI. Distribution and characterization of peptidylglycine alpha-amidating monooxygenase activity in the ovine brain and hypothalamo-pituitary axis. Endocrinology. 1992; 130:994–1000. [PubMed: 1733739]
29. May V, Eipper BA. Regulation of peptide amidation in cultured pituitary cells. J Biol Chem. 1985; 260:16224–16231. [PubMed: 2999151]
30. Savini I, Rossi A, Pierro C, et al. SVCT1 and SVCT2: key proteins for vitamin C uptake. Amino Acids. 2008; 34:347–355. [PubMed: 17541511]
31. Hediger MA. New view at C. Nat Med. 2002; 8:445–446. [PubMed: 11984580]
32. Garcia Mde L, Salazar K, Millan C, et al. Sodium vitamin C cotransporter SVCT2 is expressed in hypothalamic glial cells. Glia. 2005; 50:32–47. [PubMed: 15625716]
33. Guillemin R, Brazeau P, Bohlen P, et al. Growth hormone-releasing factor from a human pancreatic tumor that caused acromegaly. Science. 1982; 218:585–587. [PubMed: 6812220]
34. Rivier J, Spiess J, Thorner M, et al. Characterization of a growth hormone-releasing factor from a human pancreatic islet tumour. Nature. 1982; 300:276–278. [PubMed: 6292724]
35. Bloch B, Baird A, Ling N, et al. Immunohistochemical evidence that growth hormone-releasing factor (GRF) neurons contain an amidated peptide derived from cleavage of the carboxyl-terminal end of the GRF precursor. Endocrinology. 1986; 118:156–162. [PubMed: 3079700]
36. Bingham SA, Cassidy A, Cole TJ, et al. Validation of weighed records and other methods of dietary assessment using the 24 h urine nitrogen technique and other biological markers. Br J Nutr. 1995; 73:531–550. [PubMed: 7794870]
37. Johnson RK, Goran MI, Poehlman ET. Correlates of over- and underreporting of energy intake in healthy older men and women. Am J Clin Nutr. 1994; 59:1286–1290. [PubMed: 8198052]
38. Martin LJ, Su W, Jones PJ, et al. Comparison of energy intakes determined by food records and doubly labeled water in women participating in a dietary-intervention trial. Am J Clin Nutr. 1996; 63:483–490. [PubMed: 8599310]
39. Horner NK, Patterson RE, Neuhouser ML, et al. Participant characteristics associated with errors in self-reported energy intake from the Women's Health Initiative food-frequency questionnaire. Am J Clin Nutr. 2002; 76:766–773. [PubMed: 12324289]
40. Prentice RL, Mossavar-Rahmani Y, Huang Y, et al. Evaluation and comparison of food records, recalls, and frequencies for energy and protein assessment by using recovery biomarkers. Am J Epidemiol. 2011; 174:591–603. [PubMed: 21765003]

Table 1

Characteristics and nutrient intake values of Study 1 (n=108). Values are presented as median (IQ range).

	All Subjects
N	108
Age (yr)	43 (33–48.75)
Gender, no. of males (%)	58 (53.7%)
Race, n (%)	
Caucasian	63 (58.3%)
Not Caucasian	45 (41.7%)
Body composition	
BMI (kg/m^2)	32.7 (23.6–38.9)
Waist circumference (cm)	106.2 (84.7–121.7)
SAT by Abd CT (cm^2)	332 (166–539)
VAT by Abd CT (cm^2)	117 (57–207)
% Total Fat by DEXA	31.0 (23.7–39.9)
Nutrient intake	
Calories (kcal)	2053 (1610–2563)
Carbohydrate (g)	259 (182–309)
Protein (g)	87 (72–113)
Fat (g)	79 (58–107)
Cholesterol (mg)	261 (179–411)
Saturated Fatty Acids (g)	25 (17–34)
Trans Fatty Acids (g)	3.9 (2.4–5.8)
Fiber (g)	16 (12–22)
Vitamin C (mg)	94 (52–156)
SFA 20:0 (arachidic acid) (g)	0.1 (0.07–0.15)
SFA 22:0 (behenic acid) (g)	0.05 (0.03–0.11)
TRANS 18:1 (elaidic acid) (g)	3.3 (1.9–4.8)
TRANS 18:2 (trans-linolelaidic acid) (g)	0.5 (0.3–0.7)
Growth hormone parameters	
Fasting GH (μg/L)	0.12 (0.03–0.31)
Peak Stimulated GH (μg/L)	10.6 (5.2–28.3)
IGF-1 (μg/L)	79.1 (64.5–101.3)

Table 2

Univariate analyses of peak stimulated GH to nutrient intake variables in all Study 1 subjects

Nutrient intake variable	R	*P*
Calories	−0.03	0.76
Carbohydrate	−0.002	0.99
Protein	−0.07	0.45
Fat	−0.05	0.61
Cholesterol	−0.26	0.006
Trans Fatty Acids	−0.24	0.01
Fiber	+0.27	0.004
Vitamin C	+0.29	0.003
SFA 20:0 (arachidic acid)	+0.25	0.008
SFA 22:0 (behenic acid)	+0.30	0.002
TRANS 18:1 (elaidic acid)	−0.24	0.01
TRANS 18:2 (trans-linolelaidic acid)	−0.24	0.01

Table 3

A. Multivariate modeling for peak stimulated GH controlling for age, gender, race, ethnicity, VAT, HOMA-IR, total caloric intake and vitamin C intake. R^2 for overall model =0.50 and P value for overall model <0.0001.

	B estimate	Standard error	*P*
Age	−0.31	0.21	0.14
Gender (male or not)	0.79	2.24	0.73
Race (Caucasian or not)	−0.45	1.98	0.82
Ethnicity (Hispanic or not)	−2.55	3.13	0.42
VAT	−0.13	0.02	<0.0001
HOMA-IR	−1.40	1.05	0.19
Total caloric intake	−0.0009	0.003	0.72
Vitamin C intake	0.03	0.01	0.007

B. Multivariate modeling for peak stimulated GH controlling for age, gender, race, ethnicity, VAT, HOMA-IR, total caloric intake and fiber intake. R^2 for overall model =0.50 and P value for overall model <0.0001.

	B estimate	Standard error	*P*
Age	−0.33	0.21	0.12
Gender (male or not)	1.69	2.30	0.47
Race (Caucasian or not)	−1.62	1.96	0.41
Ethnicity (Hispanic or not)	−4.00	3.16	0.21
VAT	−0.12	0.03	<0.0001
HOMA-IR	−1.56	1.06	0.15
Total caloric intake	−0.005	0.003	0.15
Fiber intake	0.62	0.23	0.009

C. Multivariate modeling for peak stimulated GH controlling for age, gender, race, ethnicity, VAT, HOMA-IR, total caloric intake and arachidic acid intake. R^2 for overall model =0.48 and P value for overall model <0.0001.

	B estimate	Standard error	*P*
Age	−0.24	0.21	0.17
Gender (male or not)	0.25	2.29	0.91
Race (Caucasian or not)	−1.85	2.01	0.36
Ethnicity (Hispanic or not)	−3.25	3.20	0.31
VAT	−0.13	0.03	<0.0001
HOMA-IR	−1.35	1.09	0.22
Total caloric intake	−0.002	0.003	0.46
SFA 20:0 (arachidic acid)	43.8	21.90	0.049

D. Multivariate modeling for peak stimulated GH controlling for age, gender, race, ethnicity, VAT, HOMA-IR, total caloric intake and behenic acid intake. R^2 for overall model =0.48 and P value for overall model <0.0001.

	B estimate	Standard error	*P*
Age	−0.21	0.21	0.32
Gender (male or not)	−0.03	2.29	0.99

D. Multivariate modeling for peak stimulated GH controlling for age, gender, race, ethnicity, VAT, HOMA-IR, total caloric intake and behenic acid intake. R^2 for overall model =0.48 and P value for overall model <0.0001.

	B estimate	Standard error	*P*
Race (Caucasian or not)	−2.16	2.03	0.29
Ethnicity (Hispanic or not)	−2.80	3.21	0.39
VAT	−0.13	0.03	<0.0001
HOMA−IR	−1.40	1.08	0.20
Total caloric intake	−0.0001	0.003	0.96
SFA 22:0 (behenic acid)	33.92	16.65	0.045

Table 4.

Nutrient intake values of subjects in Study 2 (n=12). Values are presented as median (IQ range).

	All Subjects (n=12)
Total energy (kcal)	2647 (2569–3005)
Carbohydrate (g)	342 (292–392)
Protein (g)	116 (83–135)
Fat (g)	99 (67–112)
Cholesterol (mg)	309 (146–468)
Saturated Fatty Acids (g)	29 (17–41)
Trans Fatty Acids (g)	3.6 (2.5–6.1)
Fiber (g)	22 (18–42)
Vitamin C (mg)	133 (78–254)
SFA 20:0 (arachidic acid) (g)	0.14 (0.08–0.19)
SFA 22:0 (behenic acid) (g)	0.06 (0.04–0.22)
TRANS 18:1 (elaidic acid) (g)	2.9 (2.1–5.0)
TRANS 18:2 (trans-linolelaidic acid) (g)	0.5 (0.4–0.8)

Table 5

Univariate analyses of vitamin C intake to parameters of endogenous spontaneous GH secretion among all Study 2 subjects (n=12).

	r	*P*
Basal GH secretion	+0.95	<0.0001
GH half-life	+0.75	0.005
Number of overnight GH secretion pulses	−0.30	0.35
Total GH production	+0.76	0.004
Percent pulsatile GH production	+0.13	0.68
GH area-under-the-curve	+0.89	0.0001
Mean $\log_{10}$ GH pulse area	+0.67	0.02
Overnight GH maximum	+0.62	0.03
Overnight GH nadir	+0.97	<0.0001
Overnight GH mean	+0.89	0.0001