Determinants of the growth hormone nadir during oral glucose tolerance test in adults

Katharina Schilbach¹, Christina Gar¹, Andreas Lechner¹, Shiva Sophia Nicolay¹, Laura Schwerdt¹, Michael Haenelt¹, Jakob Dal^{2,3}, Jens-Otto Lunde Jørgensen³, Sylvère Störmann¹, Jochen Schopohl¹ and Martin Bidlingmaier¹

¹Medizinische Klinik und Poliklinik IV, Klinikum der Universität München, Munich, Germany, ²Department of Endocrinology, Aalborg University Hospital, Aalborg, Denmark, and ³Department of Endocrinology and Internal Medicine, Aarhus University Hospital, Aarhus, Denmark

Correspondence should be addressed to M Bidlingmaier

Email
martin.bidlingmaier@med.uni-muenchen.de

Abstract

Objective: Growth hormone (GH) nadir (GH_{nadir}) during oral glucose tolerance test (OGTT) is an important tool in diagnosing acromegaly, but data evaluating the need to adjust cut-offs to biological variables utilizing today's assay methods are scarce. We therefore investigated large cohorts of healthy subjects of both sexes to define normal GH_{nadir} concentrations for a modern, sensitive, 22 kD-GH-specific assay.

Design: Multicenter study with prospective and retrospective cohorts (525 healthy adults: 405 females and 120 males).

Methods: GH concentrations were measured by the IDS-iSYS immunoassay after oral application of 75 g glucose.

Results: GH_{nadir} concentrations (µg/L) were significantly higher in lean and normal weight subjects (group A) compared to overweight and obese subjects (group B); (males (M): A vs B, mean: 0.124 vs 0.065, $P = 0.0317$; premenopausal females without estradiol-containing OC (OC-EE) (F_{PRE}): A vs B, mean: 0.179 vs 0.092, $P < 0.0001$; postmenopausal women (F_{POST}): A vs B, mean: 0.173 vs 0.078, $P < 0.0061$). Age, glucose metabolism and menstrual cycle had no impact on GH_{nadir}. However, premenopausal females on OC-EE (F_{PREOC}) exhibited significantly higher GH_{nadir} compared to all other groups (all $P < 0.0001$). BMI had no impact on GH_{nadir} in F_{PREOC} (A vs B, mean: 0.624 vs 0.274, $P = 0.1228$).

Conclusions: BMI, sex and OC-EE intake are the major determinants for the GH_{nadir} during OGTT in healthy adults. Using a modern sensitive GH assay, GH_{nadir} concentrations in healthy subjects are distinctly lower than cut-offs used in previous guidelines for diagnosis and monitoring of acromegaly.

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Introduction

Acromegaly is associated with increased morbidity and mortality and reduced quality of life (1, 2, 3). In patients with clinical signs of acromegaly and elevated IGF-I, investigation of GH suppression during OGTT (GH_{nadir}) is recommended as confirmatory test (4). Absolute values for GH_{nadir} indicative of insufficient suppression have changed during the last decades. Recommendations propose GH_{nadir} concentrations $\geq 1 \mu\text{g/L}$ to confirm the diagnosis of acromegaly. The value of post-operative GH_{nadir} to define disease control is less well established, but cut-offs of $1 \mu\text{g/L}$ and $0.4 \mu\text{g/L}$ have been published (5, 6). The

evolution of analytical methods with improvements in sensitivity and specificity as well as changes in calibration continues to affect reported GH concentrations, and thus, cut-offs from guidelines need to be updated accordingly (7, 8, 9, 10). Measuring GH following OGTT remains important since there are no other diagnostically relevant biomarkers in acromegaly apart from IGF-I (11, 12).

Because clinical symptoms develop slowly and are often unspecific, diagnosis is frequently delayed by several years, while complications are already prevalent (3, 13). Smaller pituitary adenomas are associated with higher

cure rates, and some symptoms resolve quickly after initiation of treatment (14, 15). Cumulative GH exposure is a determinant of morbidity and mortality in acromegaly (16). For all these reasons, earlier diagnosis and therapy are highly desirable. Adenoma size strongly influences the extent of GH excess and GH_{nadir} concentrations (17). In the early stage, smaller somatotrophic adenomas are likely to often be associated with only mild-to-moderate GH excess, which might escape diagnosis in biochemical testing. Accordingly, increasing the sensitivity for detection of the disease by lowering GH_{nadir} cut-offs has been discussed (10, 18, 19, 20). Measuring very low GH concentrations is possible with more recent GH assays, but reliable data on normal suppression, as measured with such assays, are scarce.

Furthermore, discrepant results from GH_{nadir} and IGF-I analysis can also delay diagnosis, and apart from assay problems, biological variables might be important (8). Sex, BMI and different cycle phase have been shown to influence the GH_{nadir}, but few studies systematically addressed this, and there is no consensus if and how cut-offs should be adjusted to such variables (9, 21, 22, 23, 24, 25, 26, 27).

As a first step to define more appropriate cut-offs specific for GH_{nadir} for a modern, highly sensitive and specific, widely available GH assay, we designed a study to assess GH_{nadir} during OGTT and the impact of biological variables in a larger number of subjects without pituitary disease (28). Because initial findings pointed to a pivotal role of sex, we extended our study to include a large cohort of females at various phases of the menstrual cycle or on oral contraceptives.

Subjects and methods

Subjects

Overall, 525 participants were included in the study. In a prospective study, we recruited 199 subjects (AcroCut cohort, 106 females, 93 males). Furthermore, we retrospectively analyzed samples from a study in female subjects (PPSDiab ('Prediction, Prevention and Subclassification of gestational and type 2 Diabetes') cohort, $n=299$), and from a study in male subjects (Danish cohort, $n=27$). None of the participants had a history of pituitary disease or other severe illness. The study protocols were approved by the Ethics Committee of the Medical Faculty of the Ludwig Maximilians Universität München, Munich, Germany (PC, AC) and the Ethics Committee System at Region Midt, Viborg, Denmark

(DC), respectively (approval-numbers: PPSDiab: 300-11, AcroCut: 228-16, Danish Cohort: 1-10-72-284-12). All participants provided written informed consent.

AcroCut cohort

The AC includes 199 (106 female and 93 male) adult participants. Exclusion criteria were known diabetes mellitus, impaired glucose metabolism, known pituitary disease, history of irradiation of the head, pregnancy, congenital adrenal hyperplasia, polycystic ovary syndrome, alcohol and drug abuse until 6 months prior to inclusion. Endocrinologists took medical history and performed physical examination in all subjects. Few subjects took antihypertensive medications (beta blocker: $n=7$, angiotensin II receptor blocker: $n=1$, angiotensin-converting inhibitors: $n=1$), acetylsalicylic acid ($n=2$), statins ($n=2$), proton pump inhibitors ($n=2$) or selective beta-2 adrenergic agonists ($n=2$). Anthropometrical data (waist circumference, BMI, body composition) were collected (29).

PPSDiab cohort (PC)

The PC was recruited from the diabetes center and the obstetrics department of the University Hospital (Klinikum der Universität München) in Munich, Germany. The cohort includes 299 premenopausal women, seen 3–16 months (mean: 9 months) after their first pregnancy, during which they had either gestational diabetes (GDM, $n=113$) or normal glucose metabolism ($n=186$) (30). Exclusion criteria were pre-pregnancy diabetes, chronic diseases requiring continuous medication, except for primary hypothyroidism, bronchial asthma, mild hypertension and gastroesophageal reflux.

Danish cohort (DC)

The healthy male subjects ($n=27$) were recruited from the general population in Aarhus, Denmark (adults between 35–65 years). Exclusion criteria were chronic diseases including diabetes mellitus, suspicion of pituitary disease, BMI $<18\text{ kg/m}^2$ and $>30\text{ kg/m}^2$ and intake of medication with known effect on GH secretion.

Methods

Oral glucose tolerance test

All subjects underwent a standard 75g OGTT (ACCU-CHEK® Dextrose O.G.-T. Saft 300mL, Roche). In all subjects ($n=325$), five blood samples were drawn from

an indwelling catheter (0, 30, 60, 90, 120 min). In one subgroup (AC, $n=199$) an additional sample was taken at 180 min. Almost all tests started between 7:00 and 10:00h after an overnight fast. All serum and plasma samples were centrifuged, aliquoted and stored until analysis at -20°C and -80°C as appropriate.

GH assay

Serum GH was measured using a 22 kDa-GH-specific automated chemiluminescence immunoassay (CLIA, IDS-iSYS, Immunodiagnostic Systems, Boldon, UK). Extensive information on performance characteristics and specificity of this assay was published elsewhere (28). Limit of quantification (LoQ) of the assay under routine conditions is $0.05\text{ }\mu\text{g/L}$. For calculations, GH values $<0.05\text{ }\mu\text{g/L}$ were arbitrarily set to $0.04\text{ }\mu\text{g/L}$. GH_{nadir} was defined as the lowest GH value after glucose ingestion. All samples with $\text{GH}_{\text{nadir}} \geq 0.4\text{ }\mu\text{g/L}$ were re-assayed after pre-treatment in heterophilic antibody and non-specific blocking tubes (Scantibodies Laboratory, Inc., Santee, CA, USA, Part Number: 31X762 and 31X761).

Other laboratory tests

Serum insulin (PC/AC: CLIA, LIAISON, DiaSorin, Saluggia, Italy; DC: ELISA, Dako Agilent) and plasma glucose (Gc, hexokinase method, Roche cobas® 8000 c702/c311) were measured in all samples. IGF-I (CLIA, IDS-iSYS, UK) was measured before glucose load. If IGF-I was above the assay-specific upper limit of normal (ULN) for age and sex, measurement was repeated to verify the concentration.

In PC and AC ($n=499$), we also measured triglycerides (TG, CHOD-PAP method, Roche cobas® 8000 c702), thyroid-stimulating hormone (TSH, CLIA, Roche cobas® 6000 e601), adrenocorticotrophic hormone (ACTH), luteinizing hormone (LH), follicle-stimulating hormone (FSH), prolactin (PRL), estradiol (E2, in women only) and progesterone (all CLIA, LIAISON, DiaSorin) before glucose intake. To rule out severe systemic disease, standard blood count, transaminases, gamma glutamyl transferase, creatinine and C-reactive protein were determined.

Anthropometric measurements

In PC and AC ($n=498$) height and waist circumference were measured to the nearest 1 cm. Waist-to-height ratio (WHtR) was calculated as waist circumference (cm)/height (cm). Body fat mass was determined by a bioelectrical impedance analysis scale (Tanita BC-418, Tanita Corporation, Japan).

Definition of groups for analysis, calculations and statistical analysis

Taking into account the main groups males (M), premenopausal females without OC-EE intake (F_{PRE}), premenopausal females with OC-EE intake (F_{PREOC}) and postmenopausal females (F_{POST}), we initially analyzed the three cohorts (PC, DC and AC) separately. As there were no differences in the findings between the cohorts regarding the group-specific GH_{nadir} , we combined the cohorts for further analysis. Each of the groups (M, F_{PRE} , F_{PREOC} , F_{POST}) consisted of subjects covering a wide range of BMIs.

In a separate analysis all premenopausal subjects without OC-EE intake ($n=325$) were stratified for phase of the menstrual cycle (follicular phase (FP, $n=58$), luteal phase (LP, $n=63$), periovulatory phase (POP, $n=35$), phase of menstrual cycle not clearly attributable (P_{unkn} $n=142$)) or intake of an oral contraceptive with gestagen monotherapy (OC-G, $n=19$) or use of hormone-releasing vaginal ring or intra-uterine device (IUD, $n=8$). Phase of menstrual cycle was determined based on anamnestic information and measurements of estradiol, progesterone, LH and FSH. In cases in which the menstrual cycle phase could not be assigned, subjects were allocated to the P_{unkn} group. Subjects were classified as taking OC only if they were currently taking the medication and not during the estrogen-free interval.

For analysis of GH_{nadir} in the four main groups, premenopausal females taking OC-G and females using a hormone-releasing vaginal ring or intra-uterine device (IUD) were included in the group of premenopausal females without OC-EE intake as there was no difference in GH_{nadir} between the groups (mean GH_{nadir} OC-G: $0.17\text{ }\mu\text{g/L}$ (standard deviation (s.d.) 0.14 , range: $0.04\text{--}0.54$, $n=19$), mean GH_{nadir} IUD: $0.1\text{ }\mu\text{g/L}$ (s.d. 0.04 , range: $0.04\text{--}0.15$, $n=8$), mean GH_{nadir} F_{PRE} without OC-G and IUD: $0.15\text{ }\mu\text{g/L}$ (s.d. 0.14 , $n=298$), $P=0.9582$, 0.4306 and 0.8166 between IUD and F_{PRE} without OC-G and IUD, IUD and OC-G and F_{PRE} without OC-G and IUD and OC-G, respectively). Tables 1 and 2 show details for all subjects in the analysis.

The Matsuda insulin sensitivity index (ISI, $\text{ISI} = 10\,000 / \sqrt{(\text{glucose}_0' \times \text{insulin}_0') \times (\text{glucose}_{\text{mean}(0',30',60',120')} \times \text{insulin}_{\text{mean}(0',30',60',120')})}$) and the homeostasis model assessment of insulin resistance (HOMA-IR) index ($\text{HOMA-IR} = (\text{glucose}_0' \times \text{insulin}_0') / 405$) were calculated. An $\text{ISI} < 5.0$ and a $\text{HOMA-IR} > 2.29$ were taken as an indication for impaired whole-body insulin sensitivity and insulin resistance, respectively (31). Evaluation and classification of the glucose metabolism were performed according to the American Diabetes Association (ADA) criteria from 2017 (32). Subjects were grouped into normal glucose tolerance (NGT), impaired fasting glucose (IFG),

Table 1 Characteristics of subjects presented as total numbers and percent or mean $\pm$ s.d. and range.

	Males (M)	*F _{PRE}	F _{PREOC}	F _{POST}	Differences between groups (P values)
Number, (n (%))	120 (22.8)	325 (62)	46 (8.7)	34 (6.5)	
Age (years)	41 $\pm$ 14 (20–76)	35 $\pm$ 5 (20–49)	27 $\pm$ 6 (21–42)	56 $\pm$ 4 (49–70)	M vs F _{PRE} : 0.1133, between all other groups: <0.0001
Anthropometric characteristics					
BMI (kg/m ²)	26.5 $\pm$ 4.6 (14.9–45.5)	25.1 $\pm$ 5.8 (17.3–49.9)	23.2 $\pm$ 4.5 (17.7–43.1)	27.6 $\pm$ 7.4 (18–53.1)	M vs F _{PRE} and F _{PREOC} : <0.0001 F _{PREOC} vs F _{POST} : 0.0026 F _{PRE} vs F _{POST} : 0.1016 F _{PRE} vs F _{PREOC} : 0.1213 M vs F _{POST} : >0.9999
Body fat content (%)**	20.0 $\pm$ 7.4 (6.3–38.9)	31.7 $\pm$ 7.9 (12–53.5)	28.3 $\pm$ 8.0 (10.9–52.7)	36.0 $\pm$ 7.9 (21.4–52)	M vs F _{PRE} , F _{PREOC} and F _{POST} : <0.0001 F _{PREOC} vs F _{POST} : 0.001 F _{PRE} vs F _{POST} : 0.0532 F _{PRE} vs F _{PREOC} : 0.0957
WtHR**	0.51 $\pm$ 0.07 (0.40–0.71)	0.48 $\pm$ 0.07 (0.36–0.74)	0.44 $\pm$ 0.04 (0.35–0.57)	0.51 $\pm$ 0.1 (0.37–0.8)	F _{PREOC} vs M and F _{POST} : <0.0001 F _{PREOC} vs F _{PRE} : 0.0001 M vs F _{PRE} : 0.002 F _{PREOC} vs F _{POST} : 0.2794 M vs F _{POST} : >0.9999
Glucose status					
Plasma glucose 0 min (mg/dL)**	92 $\pm$ 9 (63–127)	92 $\pm$ 9 (65–136)	88 $\pm$ 9 (74–108)	95 $\pm$ 8 (81–113)	F _{PREOC} vs F _{POST} : 0.0004 F _{PREOC} vs F _{PRE} : 0.0028 F _{PREOC} vs M: 0.0326 M vs F _{POST} : 0.2902 F _{PRE} vs F _{POST} : 0.3304 M vs F _{PRE} : >0.9999
NGT** (n (%))	70 (75)	247 (76)	38 (83)	18 (53)	
IFG** (n (%))	8 (9)	37 (11)	3 (7)	6 (18)	
IGT** (n (%))	10 (11)	26 (8)	2 (4)	5 (15)	
IFG + IGT** (n (%))	4 (4)	10 (3)	3 (7)	4 (12)	
Type 2 diabetes** (n (%))	1 (1)	6 (2)	0	1 (3)	
Insulin					
Insulin 0 min (μ U/mL)	7.6 $\pm$ 6.1 (<0.2–34.1)	8.4 $\pm$ 5.5 (0.6–47)	7.8 $\pm$ 3.1 (2.1–16.4)	9.4 $\pm$ 6.2 (1.4–34.2)	M vs F _{PRE} : 0.0491 M vs F _{POST} : 0.0595 between all other groups: >0.9999
Indices of glucose metabolism					
ISI**	6.3 $\pm$ 3.9 (0.9–24.3)	6.4 $\pm$ 3.5 (0.9–22.4)	6.4 $\pm$ 3.3 (2.0–19.8)	5.4 $\pm$ 3.6 (1.1–16.8)	F _{PRE} vs F _{POST} : 0.1671 F _{PREOC} vs F _{POST} : 0.3912 M vs F _{POST} : 0.5831 between all other groups: >0.9999
HOMA**	1.9 $\pm$ 1.4 (0.3–7.3)	2.0 $\pm$ 1.4 (0.2–10.2)	1.7 $\pm$ 0.7 (0.4–3.6)	2.2 $\pm$ 1.5 (0.3–8)	M vs F _{POST} : 0.6145 F _{PRE} vs F _{POST} : 0.9189 between all other groups: >0.9999
Triglycerides (mg/dL)**	108 $\pm$ 72 (39–488)	78.5 $\pm$ 40.4 (27–313)	95.3 $\pm$ 39.7 (34–191)	106.5 $\pm$ 58.3 (42–269)	M vs F _{PRE} : 0.0003 F _{PREOC} vs F _{PRE} : 0.0086 F _{PRE} vs F _{POST} : 0.0088 between all other groups: >0.9999

Continued

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Table 1 Continued.

	Males (M)	*F _{PRE}	F _{PREOC}	F _{POST}	Differences between groups (P values)
Prolactin (mIU/L)**	260 ± 135 (90–755)	350.9 ± 288.1 (32–2478)	381.5 ± 252.1 (132–1454)	223.6 ± 87.9 (97–442)	F _{PREOC} vs F _{POST} : 0.0035 M vs F _{PREOC} : 0.0066 F _{PRE} vs F _{POST} : 0.0972 F _{PREOC} vs F _{PRE} : 0.1714 M vs F _{PRE} : 0.2301 M vs F _{POST} : >0.9999
IGF-I					
IGF-I (ng/mL)	163 ± 58 (60–430)	177 ± 44 (77–325)	185 ± 54 (105–387)	127 ± 37 (56–228)	F _{POST} vs F _{PRE} and F _{PREOC} : <0.0001 M vs F _{POST} : 0.0012 M vs F _{PRE} : 0.0003 F _{PREOC} vs M: 0.0572 F _{PRE} vs F _{PREOC} : >0.9999 M vs F _{PRE} : <0.0001
IGF-I SDS	0.23 ± 0.87 (–2.33 to 2.02)	0.73 ± 0.9 (–1.49 to 3.24)	0.23 ± 0.98 (–1.15 to 2.88)	0.61 ± 0.93 (–1.6 to 2.37)	F _{PRE} vs F _{PREOC} : 0.0028 M vs F _{POST} : 0.2821 F _{PREOC} vs F _{POST} : 0.3882 Between all other groups: >0.9999

*Female participants taking OC-G or using IUD are included in the premenopausal group without OC-EE because there is no difference between the GH_{nadir} in subjects taking no OC or OC-G and using IUD; **data not available for the DC, n=27.

BMI, body mass index; F_{POST}, female, postmenopausal; F_{PRE}, female, premenopausal without OC-EE; F_{PREOC}, female, premenopausal with OC-EE; HOMA, homeostasis model assessment of insulin resistance; IFG, impaired fasting glucose; IFT/IGT, IFT and IGT; IGF-I, insulin-like growth factor-I; IGT, impaired glucose tolerance; ISI, Matsuda insulin sensitivity index; IUD, hormone-releasing vaginal rings or intra-uterine devices; NGT, normal glucose tolerance; OC-EE, estradiol-containing oral contraception; OC-G, oral contraception with gestagen monotherapy; SDS, standard deviation score; T2D, type 2 diabetes; WtHR, waist-to-height ratio.

impaired glucose tolerance (IGT), IFT and IGT (IFT/IGT) and type 2 diabetes (T2D) according to the plasma glucose during OGTT.

Statistical calculations were conducted using GraphPad Prism 7.0 (GraphPad Software) and IBM SPSS (IBM Analytics). For correlation analyses, we choose Spearmans test and for grouped analysis the Kolmogorov–Smirnov test and the Kruskal–Wallis test, respectively. For prediction of GH_{nadir}, sequential multiple regression analysis was performed with the following covariates in different models: age, sex, OC intake (OC-EE, OC-G, IUD), BMI, glucose metabolism (NGT, IFG, IGT, IFT/IGT, T2D), ISI, HOMA-IR, AUC_{insulin}, AUC_{glucose}, triglycerides, phases of menstrual cycle (FP, LP, POP), postmenopausal state and prolactin (33). Due to non-normal distribution, the data were log (Ln) transformed before multiple regression analysis. After transformation, all standard assumptions for multiple regression analysis were found. Under consideration of BMI, sex and OC-EE, individual fitted points were calculated with the following formula: $\text{Ln}_{\text{GH}_{\text{nadir}}} = -0.866 + (-0.413 \times \text{sex}) + (0.8282 \times \text{OC-EE}) + (-0.054 \times \text{BMI})$, sex: 0=female, 1=male, OC-EE: 0=no OC-EE, 1=OC-EE.

Results

Characteristics of the subjects

General health

All subjects were in good general condition and there was no indication for pituitary disease. In particular, participants did not report any signs or symptoms of acromegaly in a structured interview asking for chronic headache, increased perspiration, development of coarse facial features, tissue swelling or increase in shoe or ring size. Clinical and biochemical examinations indicated no acute or chronic illness. Diabetes was rare ($n=8$), and well controlled in the affected subjects.

IGF-I

In 491 subjects (94%) IGF-I was within the normal sex- and age-specific reference range (mean IGF-I standard deviation score (SDS) 0.57, s.d. 0.93). Thirty subjects (5% of all subjects, 1 M and 29 F) had an IGF-I above 2 SDS (max. SDS 3.24), but none of these subjects had a GH_{nadir} above 0.4 µg/L (mean GH_{nadir} 0.12, s.d. 0.09, range: 0.04–0.32). All subjects with an elevated IGF-I had a BMI

Table 2 Characteristics of female subjects regarding phases of menstrual cycle and oral contraception.

Characteristics	n (%)
Premenopausal subjects without OC intake, phases of menstrual cycle	
Follicular phase	58 (19)
Luteal phase	63 (21)
Ovulatory phase	35 (12)
Phase of menstrual cycle not clearly attributable	142 (48)
Total, n	298
Premenopausal subjects with OC intake or use of hormone-releasing vaginal rings or intra-uterine devices	
OC-EE	46 (63)
OC-G	19 (26)
IUD	8 (11)
Total, n	73
Postmenopausal subjects, n	34

IUD, hormone-releasing vaginal rings or intra-uterine devices; OC, oral contraception; OC-EE, estradiol-containing oral contraception; OC-G, oral contraception with gestagen monotherapy.

<25 kg/m². Two male subjects had an IGF-I below -2 SDS (-2.05 and -2.33). Both subjects were obese with a BMI of 35.5 and 45.5 kg/m², respectively.

Baseline GH

Mean baseline GH (GH_{basal}; 3.52 µg/L (s.d. 4.06 µg/L, range: 0.04–20)) was lower in M (0.82 µg/L, s.d. 1.62, range: 0.04–10.56) compared to F_{PRE} (4.06 µg/L, s.d. 4.01, range: 0.04–19.02, $P < 0.0001$), F_{PREOC} 7.62 µg/L (s.d. 5.05, range: 0.14–20, $P < 0.0001$) and F_{POST} 2.28 µg/L (SD 2.40, range: 0.04–9.68, $P = 0.0009$), respectively. GH_{basal} was not different between F_{PRE} and F_{POST} ($P = 0.219$), but higher in F_{PREOC} compared to all other women ($P = 0.0002$ vs F_{PRE} and $P < 0.0001$ vs F_{POST}).

GH_{nadir}

Mean GH_{nadir} (0.17 µg/L (s.d. 0.33, range: 0.04–5.46 µg/L)) was lower in M (0.09 µg/L, s.d. 0.1, range: 0.04–0.47) compared to F_{PRE} (0.15 µg/L, s.d. 0.14, range: 0.04–1.02) and F_{PREOC} (0.55 µg/L, s.d. 0.97, range: 0.04–5.46, all $P < 0.0001$), but not different compared to F_{POST} (0.12 µg/L, s.d. 0.1, range: 0.04–0.45, $P = 0.183$; Fig. 1). Among women, GH_{nadir} was lower in F_{PRE} vs F_{PREOC} and F_{POST} vs F_{PREOC} (all $P < 0.0001$), but not different between F_{PRE} and F_{POST} ($P > 0.999$).

In 99 and 93% of all subjects GH_{nadir} was <1.0 µg/L and <0.4 µg/L, respectively. In 50% of the males ($n = 60$), but only about 20% of the females, GH_{nadir} concentrations fell below the LoQ of the assay (<0.05 µg/L). Supplementary Table 1

(see section on [supplementary data](#) given at the end of this article) lists the percentage and BMI distribution of subjects in all groups with GH_{nadir} concentrations below traditional (1.0 and 0.4 µg/L) and arbitrarily determined new lower cut-offs.

GH_{basal} and GH_{nadir} were weakly correlated ($r_s = 0.722$, $P < 0.0001$, $R^2 = 0.1468$, $\text{GH}_{\text{nadir}} = 0.03126 \times \text{GH}_{\text{basal}} + 0.05725$), although there was significant scatter (Fig. 2).

Time point of GH_{nadir}

Taking into account only the time points between 0 min and 120 min, the majority of all subjects ($n = 319$, 61%) exhibited the GH_{nadir} 120 min after oral glucose intake. In 31 subjects (6%, 25 M, 5 F_{PRE} and 1 F_{POST}), the lowest GH value was observed already at baseline, while 29 (6%), 49 (9%) and 97 (18%) subjects exhibited the lowest GH concentration at 30, 60 and 90 min, respectively. If GH measurements would have been performed only before (0 min) and 120 min after oral glucose intake, 21 subjects (4%) would have been falsely classified as insufficient suppression by current criteria (GH_{nadir} >0.4 µg/L). Of these 21 subjects, 15, 5 and 1 subjects reached their nadir <0.4 µg/L at 90, 60 and 30 min, respectively.

In the AC cohort ($n = 199$), GH was also measured 180 min after glucose intake, and 22 subjects (11% of the 199 subjects) exhibited GH_{nadir} concentrations at this time point. Notably, baseline GH was high in this

Figure 1

GH_{nadir} concentrations (µg/L), median GH_{nadir} and interquartile range (IQR) in males (M), different phases of menstrual cycle (follicular phase (FP), luteal phase (LP), perioovulatory phase, with intake of oral contraception (OC) with gestagen monotherapy (OC-G) or estrogen-containing OC (OC-EE), with the use of hormone-releasing vaginal rings or intra-uterine devices and in postmenopausal women (F_{POST}). Only selected significance levels shown.

Figure 2

Correlation of GH_{basal} ($\mu\text{g/L}$) and GH_{nadir} ($\mu\text{g/L}$) in all subjects.

group (>5 and $>10 \mu\text{g/L}$ in 14 (63%) and 6 (27%) of the 22 subjects, respectively), which consisted mostly of slim, premenopausal women. In all the 22 subjects, the second lowest GH concentration was observed at 120 min, and only in two subjects, the GH concentration was $>0.4 \mu\text{g/L}$ at 120 min but fell to $<0.4 \mu\text{g/L}$ after 180 min.

Impact of biological factors and OC intake on GH_{nadir}

BMI and metabolic factors

Considering all subjects, BMI and GH_{nadir} were negatively correlated (Fig. 3, $r_s = -0.49$, $P < 0.0001$, $R^2 = 0.04037$, $\text{GH}_{\text{nadir}} = -0.01193 \times \text{BMI} + 0.472$).

There was a difference between the GH_{nadir} of all subjects with normal body weight (group A: BMI $<25 \text{ kg/m}^2$, $n=310$, mean GH_{nadir} : $0.222 \mu\text{g/L}$, s.d. 0.42, range: 0.04–5.46) and overweight/obese subjects (groups B: BMI 25 kg/m^2 , $n=215$, mean GH_{nadir} : $0.091 \mu\text{g/L}$, s.d. 0.098), $P < 0.0001$. This was also true in all subgroups (Table 3) except for premenopausal subjects on OC-EE (mean GH_{nadir} for group A and B $0.624 \mu\text{g/L}$ (s.d. 1.08, range: 0.04–5.46, $n=36$) and $0.274 \mu\text{g/L}$ (s.d. 0.31, range: 0.056–0.94, $n=10$), respectively, $P=0.1228$).

The full stepwise multiple regression model including sex, OC-EE intake and BMI to predict GH_{nadir} was highly significant ($R^2=0.304$, $F(3,520)=75.533$, $P < 0.0005$, adjusted $R^2=0.299$), indicating that sex, OC-EE intake and BMI together account for 30% of the variability of GH_{nadir} concentrations. BMI accounted for 12%, OC intake for 10% and sex for 8%, respectively. Using the data from the full stepwise multiple regression model,

we calculated individual fitted points for the normal GH_{nadir} corresponding to each BMI for males and females with and without OC-EE intake (Fig. 4A, B and C).

We also investigated influences of other metabolic factors (e.g. WtHR, HOMA-IR, ISI and body fat content) but did not find any relevant factors. Detailed information is given in Supplementary data 1.

Sex, OC intake, menstrual cycle and prolactin

In a stepwise regression model including OC-EE intake and sex, the influence of these variables on GH_{nadir} was 13% (higher GH_{nadir} with OC-EE intake, coefficient B 0.943) and 5% (lower GH_{nadir} in males, coefficient B -0.477), respectively. OC-G intake did not influence GH_{nadir} (mean GH_{nadir} with OC-G ($n=19$) $0.17 \mu\text{g/L}$, without OC-G ($n=307$) $0.15 \mu\text{g/L}$, $P=0.642$).

In premenopausal females not taking OC, we carefully determined the phase of the menstrual cycle, but there was no difference in GH_{nadir} between the different phases (Fig. 1, mean GH_{nadir} in FP, LP, POP was 0.14, 0.16 and $0.14 \mu\text{g/L}$, respectively, $P > 0.9999$ between all groups). GH_{nadir} also did not differ between pre- and postmenopausal women not on OC-EE (Fig. 1, mean GH_{nadir} in F_{PRE} and F_{POST} 0.15 and $0.12 \mu\text{g/L}$, respectively, $P > 0.9999$). In males, mean GH_{nadir} was lower compared to premenopausal women with or without OC-EE but did not differ from postmenopausal women (Fig. 1, mean GH_{nadir} in M, F_{PRE} , F_{PREOC} and F_{POST} 0.09, 0.15, 0.55 and

Figure 3

Correlation of BMI (kg/m^2) and GH_{nadir} concentrations ($\mu\text{g/L}$) in all subjects. Male: black, premenopausal females without estrogen-containing oral contraception: green, premenopausal females with intake of estrogen-containing oral contraception: orange and postmenopausal women: gray.

Table 3 GH_{nadir} (µg/L) in different sex- and BMI groups and potential GH_{nadir} cut-offs.

	Males		Females, premenopausal without OC-EE*		Females, postmenopausal		Females, premenopausal with OC-EE
	BMI <25 kg/m ²	BMI ≥25 kg/m ²	BMI <25 kg/m ²	BMI ≥25 kg/m ²	BMI <25 kg/m ²	BMI ≥25 kg/m ²	All
n	56	64	202	121	14	20	46
Mean	0.124**	0.065**	0.179***	0.092***	0.173****	0.078****	0.55
SD	0.121	0.056	0.153	0.069	0.112	0.056	0.975
Median (IQR)	0.06 (0.04–0.194)	0.04 (0.04–0.06)	0.134 (0.08–0.22)	0.066 (0.04–0.012)	0.14 (0.095–0.22)	0.065 (0.04–0.095)	0.28 (0.11–0.51)
Minimum	0.04	0.04	0.040	0.040	0.040	0.040	0.04
Maximum	0.470	0.357	1.015	0.374	0.454	0.294	5.462
Cut-off (µg/L, defined as mean + 2 SD)	0.366	0.177	0.486	0.229	0.396	0.190	2.50
Cut-off (µg/L/rounded to 1 digit)	0.4	0.2	0.5	0.2	0.4	0.2	2.5
n > cut-off	2 (4%)	2 (3%)	10 (5%)	10 (8%)	1 (7%)	1 (5%)	2 (4%)
Cut-off 0.4 µg/L and 0.2 µg/L for BMI <25 kg/m ² and ≥25 kg/m ² , respectively	0.4	0.2	0.4	0.2	0.4	0.2	0.4
n ≥ 0.4 and 0.2 for BMI <25 kg/m ² and ≥25 kg/m ² , respectively	2 (4%)	2 (3%)	14 (7%)	10 (8%)	1 (7%)	1 (5%)	18 (39%)
5% corresponds to n =	3	3	10	6	0.7	1	2
Cut-off if ≤5% lie above it	0.38	0.19	0.46	0.26	0.36	0.12	1.64

*Female participants taking OC-G or using IUD are included in the premenopausal group without OC-EE because there is no difference between the GH_{nadir} in subjects taking no OC or OC-G and using IUD; ***P* = 0.0317; ****P* < 0.0001; *****P* = 0.0061.

BMI, body mass index; IQR, interquartile range (Q1–Q3); IUD, hormone-releasing vaginal rings or intra-uterine devices; OC-EE, estradiol-containing oral contraception; OC-G, oral contraception with gestagen monotherapy.

0.12 µg/L, respectively, *P* < 0.0001 in M vs F_{PRE} and F_{PREOC} and *P* = 0.0946 in M vs F_{POST}). There was no correlation between estradiol concentration at baseline and GH_{nadir} in either all women (*r*_s = −0.04, *P* = 0.4225) or subgroups hereof (F_{PRE}, F_{PREOC}, F_{POST}). Analyzing all subjects together, prolactin and GH_{nadir} showed a weak positive correlation (*r*_s = 0.15, *P* < 0.0009). However, the correlation was not significant in subgroup analyses (M, F_{PRE}, F_{PREOC}, F_{POST}). After adjustment for other variables (sex, OC-EE and BMI) in a multiple regression analysis, prolactin had no impact on the GH_{nadir} (<0.01%, *P* = 0.627).

Age

In an univariate regression model, age showed a weak negative correlation (*r*_s = −0.17, *P* < 0.0001) with GH_{nadir}. However, this correlation disappeared after adjusting regression analysis for BMI, WtHR, ISI or HOMA-Index.

GH_{nadir} in clinically defined groups

As shown above, BMI, OC-EE use and sex are by far the most relevant confounders of GH_{nadir}, and all are easily available variables in daily clinical routine. However, the fitted centiles defining normal GH_{nadir} for each BMI from the stepwise multiple regression model (Fig. 4) are

clearly too complex for use in daily routine. We therefore developed a simple model of 'normal ranges for GH_{nadir}', which is based on sex (M, F_{PRE}, F_{POST}) and two BMI groups (BMI < and ≥25 kg/m²). We calculated the mean GH_{nadir} + 2 standard deviations (s.d.) and defined the resulting concentration as the 'upper limit of the normal range for GH_{nadir}' (ULN, Table 3). Using two significant decimals, the respective ULN for GH_{nadir} in M and F_{POST} is 0.4 µg/L if BMI is <25 kg/m² (A) and 0.2 if BMI is ≥25 kg/m² (B). For F_{PRE}, the ULN is 0.5 and 0.2 µg/L, respectively. In F_{PREOC}, where BMI plays no role for GH_{nadir}, the ULN is 2.5 µg/L regardless of the BMI. All data are summarized in Table 3.

Characteristics of subjects with GH_{nadir} ≥0.4 µg/L

Of the 525 subjects, only 35 (7%) had a GH_{nadir} ≥0.4 µg/L (but normal IGF-I). All but two of the 35 participants in this group were females, thereof 18 (55%) taking estradiol-containing OC. Mean BMI of all subjects with GH_{nadir} ≥0.4 µg/L was 21.3 kg/m² (s.d. 2.33, range: 17.6–27.3), 32 (91%) had a BMI <25 kg/m² and 12 (34%) had a BMI <20 kg/m² (Fig. 5). Only five subjects had a GH_{nadir} ≥1 µg/L, all of them were females (4 F_{PREOC}, 1 F_{PRE}), and all had a very low BMI (mean BMI 20.6 kg/m², s.d. 0.96, range: 19.2–21.9). Supplementary Table 2 shows details of all subjects with GH_{nadir} ≥0.4 µg/L.

Figure 4

Individual fitted points ($e^{\text{LnGH}_{\text{nadir}}}$) and 95% confidence interval. Formula: $\text{LnGH}_{\text{nadir}} = -0.866 + (-0.413 \times \text{sex}) + (0.8282 \times \text{OC-EE}) + (-0.054 \times \text{BMI})$, sex: 0 = female, 1 = male, OC-EE: 0 = no OC-EE, 1 = OC-EE. (A) males, (B) females without OC-EE, (C) females with OC-EE. BMI, body mass index; OC-EE, estrogen-containing oral contraception.

Figure 5

GH_{nadir} concentrations (µg/L), median GH_{nadir} and interquartile range (IQR) in males (M), premenopausal females without intake of estrogen (EE)-containing oral contraception (OC) (F_{PRE}), premenopausal females with OC-EE intake (F_{PREOC}) and postmenopausal women (F_{POST}). Red dots indicate subjects with BMI < 25 kg/m².

Discussion

In this study, we could demonstrate that BMI, OC use and sex are the three major determinants of the GH_{nadir} concentration in healthy subjects. Taken together, the three factors explain about 30% of the variability in GH_{nadir} concentrations (Fig. 4). Our data also provide further evidence that in healthy subjects, GH_{nadir} concentrations as measured by a modern, widely available, automated GH assay which fulfills recent consensus criteria regarding calibration, specificity and sensitivity are much lower than the current cut-offs mentioned in guidelines for the diagnosis and follow-up of acromegaly (7).

From studies on GH deficiency, it is known that BMI predicts GH response to GHRH arginine stimulation and that baseline GH tends to be lower in obese as compared to normal weight subjects (34, 35, 36, 37). We could show that also the degree of suppression of GH during OGTT highly depends on BMI (Figs 3 and 5). In our cohort, virtually all of the males and about 98% of the females with a BMI above 25 kg/m² exhibited GH_{nadir} < 0.4, while 12% of the subjects with a BMI < 25 kg/m² had GH_{nadir} concentrations above 0.4 µg/L (Fig. 3). BMI is easily available in clinical routine, and our data suggest that it must be taken into account in the interpretation of GH_{nadir} concentrations. In overweight and obese subjects without pituitary adenomas, lack of suppression below 0.4 µg/L is very unlikely, while insufficient suppression occasionally can be seen in very slim subjects.

OC-EE use is the second most important variable affecting GH_{nadir} concentrations in females, with significantly higher concentrations in F_{PREOC} compared to all other females. Oral EE intake has been shown to lead to higher GH concentrations compared to transdermal EE application (38). This effect is most likely due to the first-pass effect in the liver, leading to decreased hepatic IGF-I production and consecutively to a reduced negative feedback inhibition on GH secretion (39). Due to this phenomenon, GH-deficient women with OC-EE also require higher GH doses to achieve the same increase in IGF-I compared to females not on OC-EE (40). In line with this reasoning, we observe higher baseline GH concentrations in F_{PREOC} as compared to all other females. Notably, also IGF-I concentrations were lower in F_{PREOC} compared to F_{PRE} (Table 1). In contrast to all other groups, in the subgroup of F_{PREOC} BMI had no significant impact on GH_{nadir} concentrations (mean GH_{nadir} for BMI <25 kg/m² (*n*=36) and ≥25 kg/m² (*n*=10) 0.63 and 0.27 µg/L, respectively, *P*=0.1228), suggesting that the strong impact of OC-EE overrides the GH-lowering effect of BMI. Overall, our data suggest that OC-EE must be considered when acromegaly is suspected, and in borderline cases, it might be necessary to retest after discontinuation of estrogen treatment.

Finally, sex is a determinant of GH_{nadir}, although its relative contribution is smaller than that of BMI and OC use. The effect of sex is important in the premenopausal age, whereas after menopause the difference between males and females disappears. An earlier study also demonstrated a gender difference in subjects <30 years but not in older subjects (41). These findings point to a role of estrogens in the regulation of GH suppression. Estrogen binding to hypothalamic and pituitary estrogen receptors (ERα) and estrogen-induced reduction of somatostatin receptor expression leads to increased pituitary GH secretion (39). In line with this, it has been shown that stimulated GH release is increased in POP (42). This effect may cause the slightly higher GH_{nadir} concentrations seen in F_{PRE} compared to F_{POST}. Interestingly, however, fluctuations in endogenous estrogens throughout the menstrual cycle did not translate into systematic differences in GH_{nadir} concentrations in our large cohort of F_{PRE}. A previous publication has reported higher GH_{nadir} concentrations in the POP compared to FP and LP (22). In contrast to our study, however, the study was small (*n*=13), it is unclear how the phases of the menstrual cycle were determined and older GH assays were used. Therefore, we tend to conclude from our data that GH_{nadir} concentrations do not systematically change during the phases of the

menstrual cycle and that the lower BMI in F_{PRE} (Table 1) is the more likely explanation for the slightly higher GH_{nadir} concentration in F_{PRE} compared to F_{POST}.

In the initial regression analysis, we observed a significant negative correlation between GH_{nadir} and age. However, in contrast to a previous report from a smaller cohort, the correlation was no longer significant in our large cohort when adjusted for BMI or metabolic indices, which all are known to be age related (21). We believe that the difference in findings is due to the markedly older age of their participants (half of the group >53 years of age) and the smaller cohort size (*n*=213). In consideration of the long time from first symptoms to diagnosis of acromegaly and a reported mean age at diagnosis of 40–50 years, our cohort was intentionally designed to include younger subjects (43, 44). In another study with younger healthy subjects (mean age 40 years, *n*=46), age also did not correlate with GH_{nadir} (41).

Cut-offs for diagnostic tests are commonly derived from a comparison of test results in patients with the disease and in healthy subjects using receiver-operating characteristic (ROC) analysis. However, acromegaly usually develops slowly and with unspecific symptoms. Therefore, many patients are first diagnosed at an advanced stage of the disease, when high GH concentrations are seen. Therefore, a classical ROC analysis, based on comparison of GH_{nadir} in treatment-naïve patients with acromegaly and healthy subjects, might suffer from a bias, resulting in comparably high cut-off values. Such a strategy would inherently negatively affect sensitivity of the OGTT to detect early stages of the disease. In our study, we aimed therefore to determine the normal response of GH concentrations during OGTT by investigating a large, thoroughly characterized reference population and to derive cut-offs from the 'upper end of the normal response range' taking into account the most relevant biological variables. To calculate the cut-offs, we applied a strategy commonly used in laboratory medicine to define reference intervals: We calculated mean and SD for GH_{nadir} and defined the mean concentration plus 2 standard deviations as the 'upper limit of normal' for GH_{nadir}. This approach resulted in cut-offs of 0.2 µg/L in males and females not taking OC-EE with BMI ≥25 kg/m² and of 0.4 and 0.5 µg/L, respectively, in males and females not taking OC-EE with BMI <25 kg/m². The respective cut-off for premenopausal women taking OC-EE, however, would be 2.5 µg/L (Table 3). When applying these cut-offs to our own cohorts, 'insufficient suppression of GH' would have been diagnosed in 3–8% of the healthy participants'. Using 0.2 µg/L and 0.4 µg/L

in men and postmenopausal women, but 0.3 and 0.5 µg/L in premenopausal women not taking OC-EE and 1.6 in premenopausal women taking OC-EE would result in a correct classification as 'normal' in >95% of the healthy participants. In treatment-naïve patients with acromegaly from our outpatient clinic, the mean BMI was 28.1 kg/m² (range: 19.6–38.8 kg/m², *n*=54, unpublished data), and also publications from large registries confirm that most of the patients with acromegaly have BMIs ≥25 kg/m² (44, 45). Based on our findings on the impact of BMI on GH_{nadir} in healthy subjects, it is even more unlikely that in this BMI range, GH does not suppress to very low concentrations unless there is acromegaly. In our opinion this further supports the rationale of using lower GH_{nadir} cut-offs for diagnosis of acromegaly to achieve improved diagnostic sensitivity and earlier diagnosis. We acknowledge that such a strategy might be associated with the risk of compromising specificity in very slim patients. Though patients with acromegaly rarely have low BMI, the increased risk of false-positive OGTT results must be taken into account in specific cases.

As described in the results and summarized in Supplementary Table 2, we specifically analyzed the characteristics of the 35 participants in whom GH_{nadir} concentrations remained ≥0.4 µg/L. This group clearly consists exclusively of premenopausal females (only two males and one postmenopausal female with GH_{nadir} of 0.41, 0.47 and 0.45 µg/L, respectively) who were lean and/or on OC-EE.

For the daily clinical practice, we would suggest two different GH_{nadir} cut-offs to be used in conjunction with the modern, sensitive GH assay used in our study: 0.4 µg/L for normal weight patients (BMI <25 kg/m²) and 0.2 µg/L for overweight and obese patients (BMI ≥25 kg/m²). Of course our suggestions require that laboratories use modern, sensitive GH assays and that they report GH concentrations down to the LoQ. Although calculations suggest a slightly higher cut-off of 0.5 µg/L in normal weight premenopausal women (Table 3), using 0.4 µg/L also in this group for reasons of practicability in clinical routine is associated with only minor inaccuracy. In premenopausal females on OC-EE, however, the OGTT should be performed on the last day of the pill-free interval rather than using the very high cut-off established for this group (Table 3). If OC-EE is taken without a pill-free interval, a pause of at least 6 days is recommended. We were unable to recruit a sufficient number of postmenopausal females using hormone replacement therapy (HRT) because it is rarely prescribed in our population today. We would expect that also postmenopausal females on HRT exhibit higher GH_{nadir}

concentrations and therefore suggest the same tablet-free interval of at least 6 days prior to OGTT in this group.

Some authors had discussed the option to omit the OGTT from the diagnostic workup of acromegaly for cost and practicality reasons (46). However, the very weak correlation between baseline GH and GH_{nadir} concentrations seen in our large cohort of healthy subjects does not support this idea. Nevertheless, we wondered whether all time points of the standard protocol are required. Although individual GH_{nadir} concentrations could be found at any time point investigated in some individuals, the vast majority (85%) reached GH_{nadir} concentrations at baseline (time point 0), 90 or 120 min. Furthermore, most of the subjects with an individual GH_{nadir} at a different time point exhibited GH concentrations <0.4 µg/L also at 90 or 120 min, so classification as 'sufficient suppression' would not have changed when restricting sampling to baseline, 90 and 120 min. Only in about 1% of the subjects, time points 30 and 60 min were required to define a GH_{nadir} <0.4 µg/L. If there is a reason to reduce total blood volume and costs for GH analyses during OGTT, from our data it seems most appropriate to consider omitting the samples at these two time points. The additional samples taken at 180 min in a subset of our subjects did not reveal any diagnostic advantage in most of them. Only in slim, premenopausal women – frequently exhibiting very high baseline GH concentrations – the additional measurement at 180 min can be helpful to catch a late individual GH_{nadir}.

Conclusion

Our data suggest that for most healthy individuals, GH suppression measured by a modern sensitive GH assay yields concentrations far below those recommended in current clinical guidelines. The simple concept of sex- and BMI-adapted cut-offs presented here could easily be implemented for clinical routine and might serve as a first step to facilitate earlier detection of GH hypersecretion. However, we are aware that the proposed lower cut-offs derived from a normal population need to be verified in other cohorts, and the impact on sensitivity and specificity of the GH_{nadir} as a tool to diagnose and monitor acromegaly requires prospective evaluation in cohorts including patients with acromegaly.

Supplementary data

This is linked to the online version of the paper at <https://doi.org/10.1530/EJE-19-0139>.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of this study.

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