Development of a Method for the Determination of Pulsatile Growth Hormone Secretion in Mice

F. J. Steyn,* L. Huang,* S. T. Ngo, J. W. Leong, H. Y. Tan, T. Y. Xie, A. F. Parlow, J. D. Veldhuis, M. J. Waters, and C. Chen

School of Biomedical Science (F.J.S., L.H., J.W.L., H.Y.T., T.Y.X., C.C.), University of Queensland Center for Clinical Research (S.T.N.), and Institute for Molecular Bioscience (M.J.W.), The University of Queensland, Brisbane, Queensland 4072, Australia; National Hormone and Peptide Program (A.F.P.), Harbor-UCLA Medical Center, Torrance, California 90509; and Department of Medicine (J.D.V.), Endocrine Research Unit, Mayo School of Graduate Medical Education, Clinical Translational Science Center, Mayo Clinic, Rochester, Minnesota 55905

Measures of pulsatile GH secretion require frequent collection and analysis of blood samples at regular intervals. Due to blood volume constraints, repeat measures of circulating levels of GH in mice remain challenging. Consequently, few observations exist in which the pulsatile pattern of GH secretion in mice have been characterized. To address this, we developed a technique for the collection and analysis of circulating levels of GH at regular and frequent intervals in freely moving mice. This was achieved through the development of a sensitive assay for the detection of GH in small (2 μl) quantities of whole blood. The specificity and accuracy of this assay was validated following guidelines established for single-laboratory validation as specified by the International Union of Pure and Applied Chemistry. We incorporated an established method for tail-clip blood sample collection to determine circulating levels of GH secretion in 36 whole blood samples collected consecutively over a period of 6 h. Resulting measures were characterized by peak secretion periods and interpulse stable baseline secretion periods. Periods characterized by elevated whole blood GH levels consisted of multicomponent peaks. Deconvolution analysis of resulting measures confirmed key parameters associated with pulsatile GH secretion. We show a striking decrease in pulsatile GH secretion in mice after 12–18 h of fasting. This model is necessary to characterize the pulsatile profile of GH secretion in mice and will significantly contribute to current attempts to clarify mechanisms that contribute to the regulation of GH secretion. (***Endocrinology*** **152: 3165–3171, 2011)**

The release of GH in mammals occurs in a pulsatile manner and this is critical for its physiological function. Two central regulators maintain the rhythmic release of GH. Hypothalamic GHRH stimulates GH secretion, whereas somatostatin inhibits GH release (reviewed in Ref. 1). The feedback of GH on GHRH and somatostatin is supplemented with central and peripheral regulators to ensure that the release of GH meets physiological demands.

Determination of pulsatile GH secretion in individuals requires frequent collection of blood samples at regular intervals (5–20 min). Resulting measures allow extensive analysis of the physiology of GH release (2). Repeat measures of GH secretion in smaller mammals are limited due to technical difficulties associated with venous catheterization, whereas frequent collection of blood samples from the same animal is impeded by limited blood volume. This can be addressed by the isolation and reintroduction of red blood cell mass; however, a flux in blood volume and plasma content may adversely affect animal health and GH secretion. Consequently, few observations exist in which the pulsatile secretion of GH in mice have been measured (3, 4).

ISSN Print 0013-7227 ISSN Online 1945-7170
Printed in U.S.A.
Copyright © 2011 by The Endocrine Society
doi: 10.1210/en.2011-0253 Received March 4, 2011. Accepted May 2, 2011.
First Published Online May 17, 2011

* F.J.S. and L.H. contributed equally to this work.

Abbreviations: CV, Coefficient of variation; LOD, limit of detection; LOQ, limit of quantitation; mGH, mouse GH; PBS-T, PBS with Tween-20; rGH, rat GH.

Downloaded from https://academic.oup.com/endo/article/152/8/3165/2457288 by guest on 16 August 2022

Here we introduce a simplified method for frequent and repeat collection of blood samples in mice to obtain measures of GH secretion over a sampling period of 6 h. Deconvolution analysis of the resulting GH secretion profile confirms that this is sufficient to characterize parameters associated with the pulsatile secretion of GH. To illustrate the physiological importance of pulsatile measures of GH, we extended observations to characterize the impact of an overnight fast on GH secretory parameters.

Materials and Methods

Animals

Wild-type C57BL/6J male mice (7–8 wk old, 20.6–26.5 g) mice were used to validate measures of GH secretion. Mice were obtained from the University of Queensland Biological Resources, Australian Institute of Bioengineering and Nanotechnology. Mice were group-housed (n = 5) before the experiment in a 12-h light, 12-h dark cycle (on at 0600 h and off at 1800 h). Room temperature was maintained at 20 ± 2 C. Animals had free access to food (19.6% protein, 4.6% fat; Specialty Feeds, Glen Forrest, Australia) and water, unless otherwise specified. Experimental procedures were performed in accordance with the University of Queensland Animal Ethics Committee.

Development of a sensitive GH assay

To measure GH we developed and validated a sensitive sandwich ELISA. A 96-well plate (Corning Inc., Corning, NY) was coated with 50 μl capture antibody [National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK)-anti-rat GH (rGH)-IC-1 (monkey), AFP411S, NIDDK-National Hormone and Pituitary Program (NHPP, Torrance, CA) at a final dilution of 1:40,000] and incubated overnight at 4 C. To reduce nonspecific binding, each well was subsequently incubated with 200 μl blocking buffer [5% skim milk powder in 0.05% PBS with Tween-20 (PBS-T, 0.05%)]. A standard curve was generated using a 2-fold serial dilution of mouse GH (mGH) (mGH reference preparation, AFP-10783B, NIDDK-NHPP) in PBS-T supplemented with BSA (0.2%, PBS-T/BSA). Bound standards and samples were incubated with 50 μl detection antibody (rabbit antiserum to rGH, AFP5672099, NIDDK-NHPP, at a final dilution of 1:40,000). The bound complex was incubated with 50 μl horseradish peroxidase-conjugated antibody (antirabbit, IgG; GE Healthcare Ltd., Little Chalfont, Bucks, UK; at a final dilution of 1:2000). Addition of 100 μl O-phenylenediamine (00-2003; Invitrogen, Carlsbad, CA) substrate to each well resulted in an enzymatic colorimetric reaction. This reaction was stopped by addition of 50 μl 3 M HCl, and the absorbance was read at a wavelength of 490 nm with a Rainbow 96 monochromatic microplate reader. The concentration of GH in each well was calculated by regression of the standard curve.

Antibody pair

Measures reflecting the sensitivity and selectivity of the ELISA in detecting GH secretion are attributed to antibody specificity and affinity. The immunogen for developing both the rabbit and monkey antisera was highly purified iodination-grade GH (NIDDK-rGH-I-5) derived from frozen rat pituitary glands. Titer of anti-rGH antibodies and cross-reactivity was assessed by RIA. For the rabbit antiserum, cross-reactivity to rPRL, rTSH, rLH, and rFSH was less than 0.1% with 50% binding of rGH at a final dilution of 1:1 million. For the monkey antiserum, cross-reactivity to rPRL, rFSH, rLH, rTSH, and vasopressin was less than 0.01%. For rGH, 100% binding of was observed at a final dilution of 1:1.5 million.

Validation of the GH assay

Validation of the GH ELISA followed guidelines established by the International Union of Pure and Applied Chemistry (IUPAC) (5). ELISA characteristics were assessed using terminal blood samples. Mice were anesthetized with a single ip injection of sodium pentobarbitone (32.5 mg/ml; Virbac Animal Health, Milperra, New South Wales, Australia), and terminal cardiac blood samples (800 μl) were collected using a heparinized syringe (100 IU/ml).

Linearity was assessed by analysis of 12 serially diluted mGH sample sets, evenly spaced over a concentration range of 0.03–1.0 ng/ml. To extend measures beyond the expected range of detection of pulsatile GH in whole blood samples (3, 4), a second nonlinear regression analysis was performed. This nonlinear regression assessed the range of serially diluted mGH samples from 0.03–8.0 ng/ml.

The accuracy of the GH ELISA was assessed by spike recovery analysis of whole blood samples supplemented with known amounts of mGH (0.25, 1.0, and 2.0 ng/ml). Recovery in treated *vs.* untreated samples were assessed using multiple repeats (n = 4–10), and validated over two assays. Repeatability precision was determined by inclusion of quality controls at the beginning and end of each assay. Quality control stocks were generated using culture media collected from the GH-secreting GH3 cell line. Cells were maintained as detailed previously (6). Aliquots of media were stored at −80 C for inclusion in subsequent assays. Intraassay variability was assessed by repeat analysis (n = 10) of a single whole blood sample and the same sample supplemented with 2.0 ng/ml mGH. Interassay variability was assessed by repeat measures of a single sample (1:6 dilution of stock culture media collected from GH3 cell lines) across six separate assays.

The limit of detection (LOD) was assessed by analysis of an extension of the lowest portion of the standard curve and represents the lowest amount of GH that can be significantly detected. Repeatable measures (n = 6) above background were considered and the final concentration calculated as follows: $LOD = X + (3 \times SD)$, where X is the average measure. The limit of quantitation (LOQ) represents the lowest amount of measured GH that can be reliably quantified. Measures were repeated six times and the final concentration calculated as follows: $LOQ = X + (10 \times SD)$.

Validation of a tail-tip whole blood sample collection method

A modified approach to a tail-clip blood collection procedure as described earlier (7) was used for repeat collection of whole blood samples. Due to the small volume of blood collected (4 μl/sample), potential variability between samples may greatly impact final measures of GH. We determined the variability between samples by repeat analysis (n = 10) of whole blood fractions (4 μl) collected from terminal blood samples. To verify whether measures of whole blood GH reflect the amount of GH

Downloaded from https://academic.oup.com/endo/article/152/8/3165/2457288 by guest on 16 August 2022

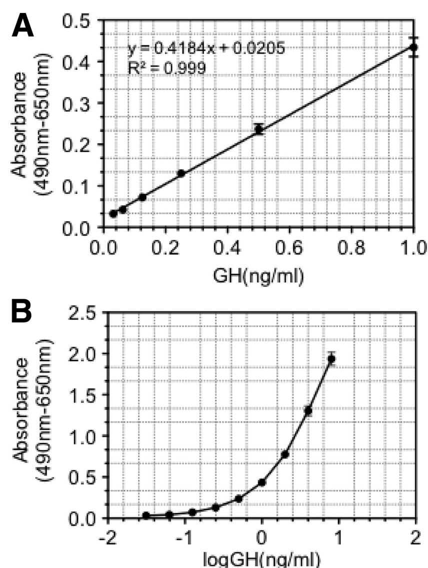

FIG. 1. Mean linear and nonlinear calibration curves as assessed by repeat analysis of mGH standards ranging from 0.03–8.0 ng/ml. The absorbance (color intensity, corrected for nonspecific background) is plotted on the x-axis against known mGH values (standards) on the y-axis. A, The linearity of dilution within a dilution range of 0.03–1.0 ng/ml is matched by an R^2 value of 0.999. The range of y-intercept using the linear scale (when x = 0.0) is from 0.3996–0.4349. The SD of the vertical distances of all data points within the linear regression (S_{yx}) is 0.005. B, For the nonlinear regression, the R^2 value for repeats ranged from 0.9996–1.0000, with a nonlinear S_{yx} of 0.002–0.16.

observed in plasma, we determined the recovery of GH in whole blood samples relative to plasma volume using terminal blood samples (n = 5). At the time of collection, whole blood and plasma weight was recorded. Measures of GH recovery in whole blood *vs.* plasma were corrected for red blood cell (1125 kg/m^3) and plasma (1025 kg/m^3) density.

Measures of pulsatile GH secretion

Pulsatile secretion of GH was assessed under basal conditions (n = 7) and after a 12-h overnight fast (n = 7). Pulse measures were compared with one-off measures of GH collected from animals that were not pulse bled. These measures represent control and fasting samples collected at the start (0 h, n = 6 per group) and after 18 h (n = 6 per group) of fasting. A cardboard tube was placed in the cage 2 d before sample collection and used during the blood collection procedure to assist with animal handling. Control animals had free access to food and water for the duration of the experiment. For fasting, food was removed at 1800 h.

Starting at 0630 h, 36 sequential tail-tip blood samples were collected from each mouse at 10-min intervals. At the time of sampling, mice were placed inside the cardboard tube and held by the base of the tail. The distal 1-mm portion of the tail was excised using a surgical blade, and a small volume of blood was allowed to pool on the tip of the tail. Using a 10-μl Gilson pipette, a 4-μl sample of whole blood was collected and transferred to 116 μl of 0.05% PBS-T. Samples were mixed and placed on dry ice. After blood collection, gentle pressure was applied to the wound to stem blood flow, and the animal was returned to the home cage. For repeat samples, the surface of the original wound was disrupted; the tail wound was briefly immersed in physiological saline (0.9% sodium chloride; Baxter, Old Toongabbie, New South Wales, Australia), and if necessary, the remaining scab was removed using the edge of a sharp blade. In general, repeat excision of the tail tip was unnecessary. Care was taken to collect samples from free flowing blood. When necessary, gentle pressure was applied along the base to the tip of the tail to assist blood flow. Collection of samples by experienced operators was performed in less than 30 sec. Samples were transferred to −80 C for storage before analysis.

Statistical analysis

Data are presented as mean ± SEM. Differences between groups were identified by Student's *t* test. All measures (exclud-

Downloaded from https://academic.oup.com/endo/article/152/8/3165/2457288 by guest on 16 August 2022

TABLE 1. Validation of the sensitive GH assay and the tail-tip blood sample collection method

	GH (ng/ml), mean ± SEM	SD	GH (ng/ml)	Recovery (%), mean ± SEM	CV (%)
Accuracy/recovery					
0.25 ng/ml	0.24 ± 0.01			97.12 ± 2.00	4.13
1.0 ng/ml	0.89 ± 0.01			88.75 ± 0.93	2.11
2.0 ng/ml	1.88 ± 0.02			98.42 ± 2.54	3.87
Repeatability precision					
Intraassay CV (whole blood)	1.80 ± 0.02				3.20
Intraassay CV (whole blood + 2 ng/ml GH)	3.85 ± 0.04				3.29
Interassay CV				3.31 ± 0.12	8.73
LOD/LOQ					
LOD	0.027 ± 0.001	0.004	0.038		
LOQ			0.065		
Pipetting variability					
Sample 1	1.88 ± 0.04				3.87
Sample 2	3.85 ± 0.04				3.29
Whole blood GH content					
Whole blood				58.25 ± 5.57	
Whole blood (corrected for plasma volume)				107.80 ± 7.55	

Recovery of GH in whole blood samples was quantified by supplementing samples with 0.2, 1.0, and 2.0 ng/ml mGH. Intraassay CV were determined using whole blood samples and whole blood samples supplemented with mGH (2 ng/ml). The interassay CV was determined using culture media derived from the GH3 cell line. LOD [mean + (3 × SD)] and LOQ [mean + (10 × SD)] were determined by extension of the low end of the standard curve. Pipetting variability was determined via repeat analysis of whole blood fractions of terminal whole blood samples.

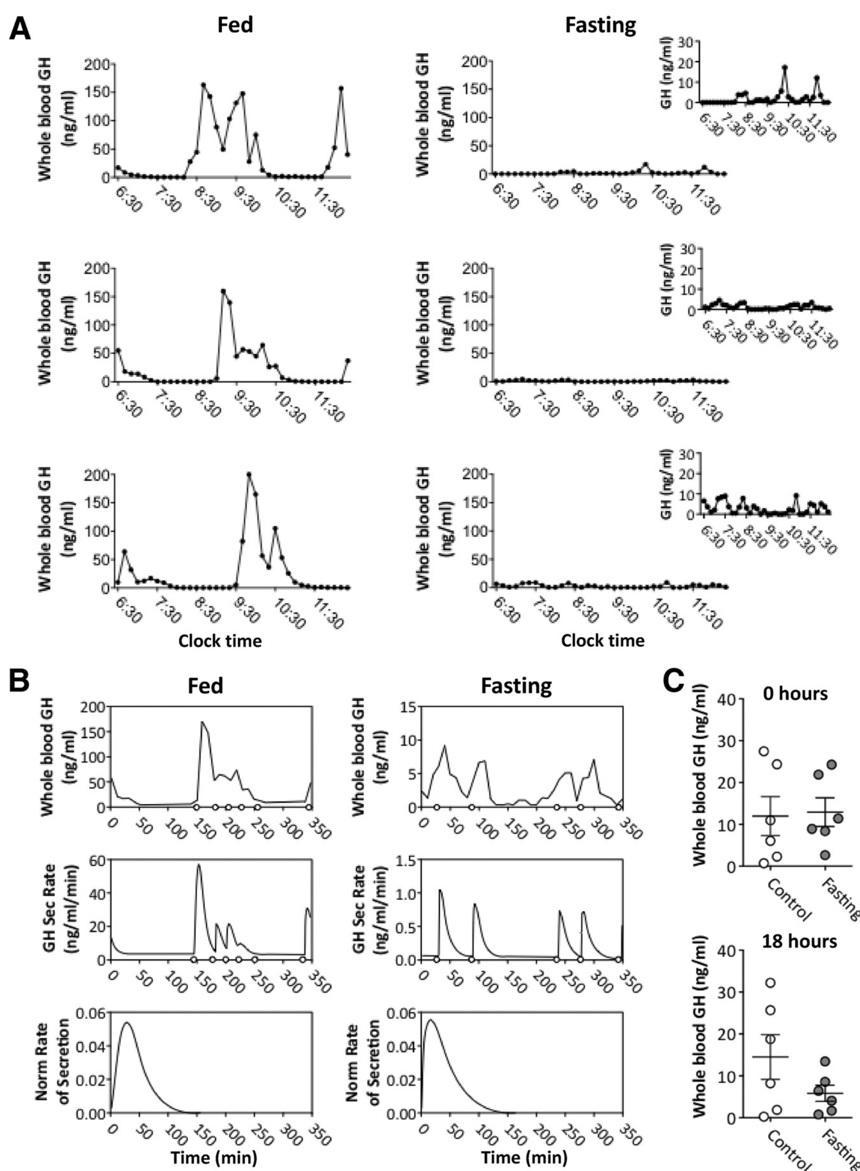

FIG. 2. Measures of GH secretion in control and fasting animals. A, Representative examples of pulsatile GH secretion in control (fed) and fasting animals. Samples were collected for 6 h at 10-min intervals starting at 0630 h. In controls, a regular periodicity of pulsatile GH secretion was characterized by peak secretion periods (between 0830 and 1030 h) and interpulse stable low baseline secretion periods. Peaks approached but rarely exceeded 200 ng/ml. A decrease in GH secretion in mice was observed after a 12-h overnight fast (*insets* illustrate GH secretion on a reduced y-axis). B, Representative examples of output figures after deconvolution analysis showing whole blood concentrations of GH, GH secretory rate, and the normalized rate of GH secretion in fed and fasting animals. The onset of pulses is indicated by an open circle (○). C, One-off measures of GH collected at the beginning of the experiment (0 h) and after 18 h of fasting. An overall reduction in GH secretion occurred after an 18-h fast; however, this did not reach significance.

ing deconvolution analysis) were performed using GraphPad Prism version 5.0b (GraphPad Inc., San Diego, CA). The threshold level for statistical significance was set at $P < 0.05$.

Kinetics and secretory patterns of pulsatile GH secretion were determined by deconvolution analysis following parameters established previously (8, 9). Measures include the number and mass of secretory bursts (the mass per pulse) and the subject-specific (slow) half-life. In addition, we report basal, pulsatile, and total GH secretion and the mode of secretory bursts (time in minutes from pulse onset to the peak of the burst). The orderliness of regularity of serial GH serum concentrations was calculated by approximate entropy analysis (10).

Results

Validation of the GH assay

Linearity was assessed by repeat analysis of standards ranging from 0.03–1.0 ng/ml (Fig. 1A). The linearity of dilution within this range is matched by an R^2 value of 0.999 (where R^2 represents a measure of goodness-of-fit of linear regression). This range was extended (0.03–8.0 ng/ml) to allow nonlinear regression analysis for determination of GH concentration over a larger range (Fig. 1B). The theoretical range of detection using this nonlinear standard curve in a 2-μl whole blood sample (correcting for a dilution factor of 1:30) is 0.9–240 ng/ml.

Validation of the GH assay by measures of recovery, repeatability precision, and LOD and LOQ of GH is summarized in Table 1. On average, recovery of GH using a sample range from 0.25–2.0 ng/ml exceeded 89%. The intraassay coefficient of variation (CV) corresponding to whole blood concentrations and whole blood samples supplemented with mGH were 3.2 and 3.29% respectively. The interassay CV calculated from six independent assay runs was 8.73%. The LOD and LOQ were 0.038 and 0.065 ng/ml, respectively.

Validation of the tail-tip sampling procedure

Validation of the tail-tip sampling procedure is summarized in Table 1. The pipetting variability of an experienced operator was determined via repeat analysis of whole blood fractions containing 1.88 ± 0.04 and 3.85 ± 0.04 ng/ml GH. The CV within 10 repeats at either concentration was less than 4%. We observed 58.25 ± 5.57% recovery of GH in whole blood samples when compared with plasma. To account for differences in plasma content, recovery of GH content in whole blood was adjusted relative to the plasma volume. Recovery of GH in whole blood after repeat analysis of whole blood relative to isolated plasma volume was 107.80 ± 7.55%.

Downloaded from https://academic.oup.com/endo/article/152/8/3165/2457288 by guest on 16 August 2022

TABLE 2. Deconvolution analysis and approximate entropy analysis of GH in 2-μl whole blood tail-tip samples collected from fed (control) and overnight fasted mice

	Fed control	Fasted 12 h	*P* value
Number of secretory bursts/6 h	4.57 ± 0.69	4.85 ± 0.55	0.75
Mass of GH secreted/burst (MPP, ng/ml)	301.30 ± 59.85	11.52 ± 3.01	0.00[a]
Mean secretory burst half-life duration (min)	6.90 ± 1.23	5.60 ± 1.31	0.48
Basal secretion rate (ng/ml · 6 h)	56.60 ± 22.95	29.41 ± 7.07	0.28
Pulsatile GH secretion rate (ng/ml · 6 h)	1200.86 ± 188.02	61.71 ± 22.78	0.00[a]
Total GH secretion rate (ng/ml · 6 h)	1257.43 ± 206.89	91.14 ± 28.24	0.00[a]
Mode of secretory bursts (min)	6.58 ± 1.36	6.76 ± 1.59	0.93
Approximate entropy (1, 0.35)	0.46 ± 0.06	0.72 ± 0.10	0.04

Samples were collected at 10 min intervals between 0630 h and 1230 h. Data are presented as mean ± SEM.

[a] $P < 0.001$.

Downloaded from https://academic.oup.com/endo/article/152/8/3165/2457288 by guest on 16 August 2022

Measures of pulsatile GH secretion in mice after an overnight fast

Representative examples of GH pulses in fed and fasting mice are illustrated in Fig. 2A. The pattern of GH secretion in control mice during the initial hours after the start of the light phase exhibited a regular periodicity characterized by peak secretion periods (occurring between 0830 and 1030 h) and interpulse stable baseline secretion periods. Periods characterized by elevated whole blood GH levels consisted of multicomponent peaks lasting from 2–2.5 h. Peaks approached but rarely exceeded 200 ng/ml. Representative examples of output figures after deconvolution analysis are illustrated in Fig. 2B. Parameters assessed are detailed in Table 2. A striking decrease in pulsatile GH secretion in mice was observed after the overnight fast. This is characterized by a significant reduction in the mass of GH secreted per burst, the pulsatile and total GH secretion rate, and an increase in the irregularity of GH pulses. Single measures of GH collected at the start and at the end of the fasting period reflect variable amounts of GH between mice (Fig. 2C). No differences in mean GH between control (11.98 ± 4.65 ng/ml) and animals designated for fasting (12.92 ± 3.43 ng/ml) were observed at 0 h. We observed an overall reduction in GH secretion after fasting (18 h); however, this did not reach significance (Fig. 2C, 14.51 ± 5.35 *vs.* 5.82 ± 1.92 ng/ml, $P = 0.16$).

Discussion

Due to challenges encountered during frequent blood sample collection in mice, measures of pulsatile GH secretion in transgenic mouse models designed to specifically investigate the GH axis do not exist. To avoid complications associated with venous catheterization and low blood volume, we optimized methods to limit blood loss and surgical intervention. By combining high-affinity anti-GH antibodies, we are able to generate a very sensitive and specific sandwich ELISA for the detection of mGH in 2-μl whole blood samples. This ELISA can be easily reproduced without the need for expensive equipment or considerations for the use of radioactive material, as is the case for RIA. The specificity and accuracy of the ELISA was validated following guidelines established by IUPAC (5). Blood samples were collected using a modified approach to an established tail-clip procedure. This method of blood collection compares favorably with techniques designated as less stressful (7), an important consideration because stress decreases GH secretion in rodents (11). Furthermore, restriction of tail tip amputation to less than 4 mm does not appear to have detrimental long-term physiological consequences (12).

Observation of pulsatile GH secretion in mice confirms a regular periodicity characterized by peak secretion periods and interpulse stable baseline secretion periods. This pattern is similar to that observed earlier (3, 4) and compares well with measures obtained from rats (13). Periods characterized by elevated whole blood GH levels consist of multicomponent peaks as is commonly observed in rats. This represents an improvement on previous measures in mice (3, 4) and likely results from the increased sensitivity and greater resolution of the pulse method. Although one-off measures may be used to define properties of pulsatile GH secretion in mice, methods such as regression plot analysis require significantly larger sample sets (11). Using our method, blood collection is limited to a small number of animals that can be retained for future sampling. This is central to the incorporation of proper measures allowing an extension of experimental analysis and repeat measures, while conforming to ethical considerations deemed essential in modern research practices (namely reduction, refinement, and replacement).

Using this procedure, we determined the impact of fasting on the pulsatile secretion of GH in mice. Unlike rats (14), one-off measures of GH suggest an overall increase in the secretion of GH during early periods of fasting in

mice (15). These effects are observed after 24 h of fasting (starting at 0700 h). However, we observed an overall decrease in the secretion of pulsatile GH in mice after a 12-h overnight fast (starting at 1800 h). Deconvolution analysis confirmed a significant reduction in the mass of GH secreted per burst and the pulsatile and total GH secretion rate. Our matched measures of GH collected in one-off samples generally agree with this observation. Unlike pulse measures, however, the perceived decrease in circulating levels of GH in this sample set does not reach significance. This further illustrates the difficulties associated with single point measures in defining GH secretion. Our observations suggest that the reduction in GH secretion in mice after fasting occurs much earlier than previously thought. Previous studies in which a fasting-induced increase in GH secretion in mice was characterized occurred in response to a 24-h fast (16). It is possible that the differing results are due to differences in experimental approach. Documented measures of pulsatile GH secretion in mice after a 12-h overnight fast do not exist, whereas one-off measures suggesting an increase in GH secretion may not accurately reflect an overall change in pulsatile GH secretion. Early observations in rats relying on one-off measures of GH generated similar conflicting observations (17–20). It was not until the analysis of pulsatile GH secretion in fasting rats that the impact of fasting on GH secretion was clarified (14). Extension of pulsatile measures of GH secretion will clarify the impact of fasting on GH secretion in mice. Additionally, we need to consider that handling stress may have further contributed to the reduction in GH secretion after fasting. The GH axis is particularly sensitive to stress (11, 21), with inappropriate handling causing an immediate reduction in circulating levels of GH (11). Observations in control animals confirmed peak levels of GH after repeat handling, and thus, it appears that our modifications to the tail-clip blood collection technique to limit handling and sample collection duration sufficiently reduced stress and allows the identification of reliable GH measures. Confirmation of this, however, requires further validation.

In conclusion, we introduced a method for the routine analysis of pulsatile secretion of GH in mice. This method allows the analysis of pulsatile secretion of GH in transgenic mouse lines and thus will provide valuable information necessary to characterize the mechanisms that regulate GH secretion in mice.

Acknowledgments

We gratefully acknowledge the assistance and support from staff and animal technicians at the University of Queensland Biological Resources, Australian Institute of Bioengineering and Nanotechnology.

Address all correspondence and requests for reprints to: Prof. Chen Chen, School of Biomedical Science, University of Queensland, St. Lucia 4072, Australia. E-mail: chen.chen@uq.edu.au.

L.H. is a recipient of an overseas postgraduate research scholarship from the China Scholarship Council (China) and subsidy scholarship by the University of Queensland. Research was supported by the Australian National Health and Medical Research Council and The University of Queensland.

Disclosure Summary: The authors have nothing to disclose.

Downloaded from https://academic.oup.com/endo/article/152/8/3165/2457288 by guest on 16 August 2022

References

1. **Müller EE, Locatelli V, Cocchi D** 1999 Neuroendocrine Control of Growth Hormone Secretion. Physiol Rev 79:511–607
2. **Giustina A, Veldhuis JD** 1998 Pathophysiology of the neuroregulation of growth hormone secretion in experimental animals and the human. Endocr Rev 19:717–797
3. **MacLeod JN, Pampori NA, Shapiro BH** 1991 Sex differences in the ultradian pattern of plasma growth hormone concentrations in mice. J Endocrinol 131:395–399
4. **Pampori NA, Shapiro BH** 1994 Effects of neonatally administered monosodium glutamate on the sexually dimorphic profiles of circulating growth hormone regulating murine hepatic monooxygenases. Biochem Pharmacol 47:1221–1229
5. **Thompson M, Ellison SLR, Wood R** 2002 Harmonized guidelines for single-laboratory validatioin of methods of analysis. Pure Appl Chem 74:835–855
6. **Yang SK, Parkington HC, Epelbaum J, Keating DJ, Chen C** 2007 Somatostatin decreases voltage-gated Ca2+ currents in GH3 cells through activation of somatostatin receptor 2. Am J Physiol Endocrinol Metab 292:E1863–E1870
7. **Abatan OI, Welch KB, Nemzek JA** 2008 Evaluation of saphenous venipuncture and modified tail-clip blood collection in mice. J Am Assoc Lab Anim Sci 47:8–15
8. **Keenan DM, Chattopadhyay S, Veldhuis JD** 2005 Composite model of time-varying appearance and disappearance of neurohormone pulse signals in blood. J Theor Biol 236:242–255
9. **Keenan DM, Iranmanesh A, Veldhuis JD** 2011 Analytical construct of reversible desensitization of pituitary-testicular signaling: illustrative application in aging. Am J Physiol Regul Integr Comp Physiol 300:R349–R360
10. **Veldhuis JD, Johnson ML, Veldhuis OL, Straume M, Pincus SM** 2001 Impact of pulsatility on the ensemble orderliness (approximate entropy) of neurohormone secretion. Am J Physiol Regul Integr Comp Physiol 281:R1975–R1985
11. **Xu J, Bekaert AJ, Dupont J, Rouve S, Annesi-Maesano I, De Magalhaes Filho CD, Kappeler L, Holzenberger M** 2011 Exploring endocrine GH pattern in mice using rank plot analysis and random blood samples. J Endocrinol 208:119–129
12. **Arras M, Rettich A, Seifert B, Käsermann HP, Rülicke T** 2007 Should laboratory mice be anaesthetized for tail biopsy? Lab Anim 41:30–45
13. **Tannenbaum GS, Martin JB** 1976 Evidence for an endogenous ultradian rhythm governing growth hormone secretion in the rat. Endocrinology 98:562–570
14. **Tannenbaum GS, Rorstad O, Brazeau P** 1979 Effects of prolonged food deprivation on the ultradian growth hormone rhythm and immunoreactive somatostatin tissue levels in the rat. Endocrinology 104:1733–1738
15. **Park S, Peng XD, Frohman LA, Kineman RD** 2005 Expression analysis of hypothalamic and pituitary components of the growth hor-

mone axis in fasted and streptozotocin-treated neuropeptide Y (NPY)-intact (NPY+/+) and NPY-knockout (NPY−/−) mice. Neuroendocrinology 81:360–371
16. **Luque RM, Park S, Kineman RD** 2007 Severity of the catabolic condition differentially modulates hypothalamic expression of growth hormone-releasing hormone in the fasted mouse: potential role of neuropeptide Y and corticotropin-releasing hormone. Endocrinology 148:300–309
17. **Friedmann RC, Reichlin S** 1965 Growth hormone content of the pituitary gland of starved rats. Endocrinology 76:787–788
18. **Meites J, Fiel NJ** 1965 Effect of starvation on hypothalamic content of "somatotropin releasing factor" and pituitary growth hormone content. Endocrinology 77:455–460
19. **Dickerman E, Negro-Vilar A, Meites J** 1969 Effects of starvation on plasma GH activity, pituitary GH and GH-RF levels in the rat. Endocrinology 84:814–819
20. **Trenkle A** 1970 Effect of starvation on pituitary and plasma growth hormone in rats. Proc Soc Exp Biol Med 135:77–80
21. **Balcombe JP, Barnard ND, Sandusky C** 2004 Laboratory routines cause animal stress. Contemp Top Lab Anim Sci 43:42–51

Downloaded from https://academic.oup.com/endo/article/152/8/3165/2457288 by guest on 16 August 2022

Renew Your Subscription Now!

Don't miss a single issue of our highly-cited, high-impact factor journals.

www.endo-society.org