Regulation and Adaptation of Endocrine Axes at High Altitude

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Abstract

As a model of extreme conditions, eight healthy women, part of a 40-member Nepal mountain-climbing expedition, were monitored for dynamic endocrine adaptations. Endocrine measurements were made at frequent intervals over a 6-10 hours period at four altitudes: 450 m, 4800 m (Base Camp), 6050 m and again at 4800 m (on descent) after an acclimatization period (4800 mA). Quantified hormones were growth hormone (GH), prolactin (PROL), Cortisol (Cort), Thyroid-stimulating hormone (TSH), and free thyroxine. These hormones are important to the anabolic/catabolic balance of the body, and are vital to growth, homeostasis, hypothalamic inhibition, regulation of stress and metabolism. A key secondary question was the degree to which acclimatization can stabilize hormonal disruption. Based upon statistical false discovery rates, the present analyses unveil marked adaptive changes in the thyroid axis at the level of pulsatile secretion of the pituitary hormone TSH and its downstream product, free thyroxine; strong effects upon the mass of GH, TSH, Cortisol and PROL secretion per burst; and prominent pulsatile frequency disruption and recovery for PROL and cortisol. Since pulsatility changes reflect de facto perturbations in hypothalamo-pituitary control mechanisms, the present data introduce the concept of both frequency and amplitude-dependent adaptive control of brain-pituitary neuroendocrine signals under conditions of extreme altitude exertion and exposure.

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1. Introduction

Stress-related successful adaptations or failures of critical endocrine systems are contributors to health or morbidity and mortality across diverse ethnic, age, occupational and health groups in both sexes. For example, physiological adaptations of the hypothalamic-pituitary-adrenal axis (HPA) to major internal and external stressors are fundamental to maintain homeostasis and attendant health and longevity. However, major stressors especially when combined are not ethical to apply to normal individuals. One context of massive and multifactorial stress is ascent to high altitude under combined stresses of hypoxia, heavy physical exertion, psychological stress, sleep deprivation, cold exposure and dietary changes. The acute hypoxic setting may per se be deleterious both acutely and in the long-term. Catabolic manifestations of weight loss and sarcopenia are frequent in hypoxemic patients with COPD (14), or otherwise healthy subjects exposed to high altitude (6, 7, 26). Likewise, sustained heavy physical exertion is strongly catabolic. In mountaineering, hypoxia and exhaustion are likely exacerbated by psychological stress, sleep deprivation, high variation in temperature and high nutritional demand. In contrast, physical exertion drives the growth hormone axis which is anabolic, and cold exposure drives the thyroid axis, also anabolic. How the aggregate of these factors affect endocrine systems is unknown.

In principle, disruption of endocrine regulation could mediate high-altitude sickness and might even explain the condition of high-altitude deterioration, a severe catabolic state ultimately leading to death. Few studies have investigated changes in hormone secretion at high altitudes. Thyroid hormones were found to increase, but thyroid-stimulating hormone (TSH) was preserved in single-sample studies at around 3500 to 4300 m (1, 2). In other studies, cortisol concentrations rose during

66 acute hypoxic exposure (21, 22). Changes reported in other endocrine systems have been
67 contradictory (3, 13, 16, 21).

68 A large observational study of the authors, performed in the same expedition with both male
69 and female subjects at various altitudes up to 7000 m under standardized conditions revealed that
70 adrenal, thyroid and gonadal axes are affected by increasing altitude. Adrenal axis and prolactin
71 were activated and thyroid axis suppressed at very high altitude >4.800 m. Acclimatisation at 4800
72 m led to normalization of adrenal but not of thyroid axes (27). However, this and none of the
73 previous studies counted for physiological pulse patterns of pituitary and adrenal hormone
74 secretion, which constitutes an integral part of hormonal regulation (25).

75 To assess the pathophysiologic impact of progressive altitude ascent on hypothalamo-pituitary
76 function in a more comprehensive manner, we undertook systematic analysis of the pulsatile
77 patterns of growth hormone (anabolic), prolactin (hypothalamic dopamine monitor), cortisol
78 (catabolic), TSH and free thyroxine (anabolic) secretion in 8 women across successive altitudes
79 from 450 m to a very high altitude of 6050 m. In addition, we tested the hypothesis that
80 acclimatization to 4800 m on the descent leads to a stabilization of endocrine changes.

81

82 ## 2. Materials and Methods

83 ### 2.1 Course of the expedition and participants

84 The medical research expedition involved Mount Himlung Himal (7126 m) in Nepal. Blood
85 sampling and comprehensive cardiorespiratory assessment were performed at four altitudes: 450
86 m (baseline); 4800 m (Base Camp 1) on Day 7; 6050 m (Camp 2) on Day 13; and, again at 4800
87 m on Day 19 (or 20) after an acclimatization period (4800 mA). The ascent protocol was in

accordance with standard practices, so to allow for adequate acclimatization and to minimize the risk of severe altitude illness.

Eight healthy female subjects who were part of this 40-member medical research expedition volunteered for repeated blood sampling. All individuals were from a lowland environment, but had basic mountaineering experience. None of the female subjects had any neurologic, cardiac or respiratory disease, diabetes mellitus type I or II, or the need for any regular medication, particularly thyroid hormones, corticosteroids and other medication which could possibly affect hormone analysis. Comprehensive details of the protocol and subject recruitment are published elsewhere (4, 17, 27).

For each of the eight women, growth hormone (GH), prolactin (PROL), Cortisol (Cort), Thyroid stimulating hormone (TSH) and free (unbound) thyroxine (fT4) were assayed at each study site over approximately six hr of frequent (10-20 min intervals) blood sampling.

2.2 Experimental Protocol

At each of the four altitudes, blood sampling was performed at a targeted frequency of every 10-20 min for a targeted interval of six hours (10 min for first two hours; 20 min for the last four hours). Meeting these targets varied slightly from individual to individual and within individual across different altitudes due to the complexity and changing nature of the environment. The strength of observing the same individuals at the four altitudes is that paired comparisons can be achieved, allowing for greater statistical power and precision.

Blood samples of 1.2 ml were withdrawn from a peripheral venous needle. All blood samples were centrifuged immediately for 10 min at 2000 g (EBA 20, Hettich AG, Bäch, Switzerland). Aliquots were frozen to -40° to -60°C on-site and kept frozen at -80°C until analysis. The assays

were as follows: (1) GH was measured on a IMMULITE 2000 XPI (Siemens, Erlangen, Germany) using a solid-phase, two-site chemiluminescent immunometric assay (IMMULITE 2000 Growth Hormone, Siemens, Erlangen, Germany) with normal values <7.0 ug/l; (2) prolactin was quantified using a homogeneous, sandwich chemiluminescent immunoassay (LH FLEX reagent, Siemens, Erlangen, Germany) on a Dimension Vista System (Siemens, Erlangen, Germany) with normal values 2.2 – 28 ug/l; (3) cortisol was assessed by competitive immunoassay (Advia Centaur Cortisol Assay, Siemens, Erlangen, Germany). Detection range 13.80 – 2069 nmol/l and normal values 140-700 nmol/l; (4) TSH was measured by a homogeneous, sandwich chemiluminescent immunoassay (TSH FLEX reagent and Dimension Vista System, Siemens, Erlangen, Germany) with normal values of 0.4-4.0 mlU/l; and, (5) fT4 was assayed via a homogeneous, sequential chemiluminescent immunoassay (fT4 FLEX reagent, Siemens, Erlangen, Germany) using Dimension Vista System (Siemens, Erlangen, Germany) with normal values of 9.9-19.3 pmol/l.

2.3Statistical Modeling

2.3.1 Hormone dynamics

Changes in physiological hormone regulation are monitored most often at the concentration level, but are mediated by changes at the levels of unobserved underlying secretion rates and kinetics, here termed hormone dynamics. Two of the authors (D.M. Keenan, J.D. Veldhuis) have developed over the past 20 years methods to recover hormone secretion and elimination rates from sequential measurements of time-varying concentrations (15, 25). For each hormone and each subject, the basic model from which summary statistics for hormone dynamics can be calculated is as follows:

First, for each hormone (per subject), there are m (unobserved) secretory pulse times to be estimated:

$$T_m = (T^{(1)}, T^{(2)}, \ldots, T^{(m)}) \quad \text{(m also unknown)}$$

The estimated length of times between pulse times, i.e., interpulse intervals (IPI), for each person, hormone and altitude were modeled as a Weibull renewal processes (the IPI's are IID Weibull random variables). There are two pulse-related parameters: pulse frequency λ (approximately, the reciprocal of the Weibull mean) and a pulse regularity parameter γ. The regularity parameter is 1 for a Poisson process, and increases as the IPI's become more regular (the coefficient of variation is inversely related to γ). Starting at pulse time $T^{(k)}$, an accumulated mass $M^{(k)}$ of hormone is released:

$$M^{(k)} = (\eta_0 + \eta_1 \times (T^{(k)} - T^{(k-1)}) + A^{(k)} \qquad (1)$$

which is assumed to be a linear function of the IPI plus a random effect. The random effect allows for desensitization and inherent biological variation, modeled as IID $N(0, \sigma_A^2)$. The mass is released at a time-varying rate (mass per unit distribution value per unit time):

$$\psi(s) \propto s^{\beta^{(1)}\beta^{(3)}-1} e^{-\left(\frac{s}{\beta^{(2)}}\right)^{\beta^{(3)}}}, s \geq 0 \quad \text{(a normalized rate of release)} \qquad (2)$$

given as a three-parameter Gamma density. The resulting secretion rate is then the sum of two components: a (constant) β_0 basal secretion rate and the pulsatile secretion rate:

$$Z(s) = \beta_0 + \sum_{T^{(k)} \leq s} M^{(k)} \psi\left(s - T^{(k)}\right) \qquad \text{(Secretion Rate at time s)} \qquad (3)$$

A biexponential elimination rate is necessary to properly model the kinetics; specifically, a single exponential decay results in an overestimation of the secretion (15). The two fractional elimination rates are a fast rate $\alpha^{(1)}$, which captures the rapid effects of diffusion and advection, and a slow rate $\alpha^{(2)}$, which describes the removal from the blood. The result of the two processes, secretion and kinetics, are the time-evolving (true) hormone concentrations:

$$X(t) = \left(ae^{-\alpha^{(1)}t} + (1-a)e^{-\alpha^{(2)}t}\right) + \int_0^t \left(ae^{-\alpha^{(1)}(t-s)} + (1-a)e^{-\alpha^{(2)}(t-s)}\right) Z(s)ds \quad (4)$$

with that which is observed by frequent blood sampling and hormone assay being:

$$Y_i = X(t_i) + \varepsilon_i,\ i = 1, \ldots, n \quad \text{(the observed concentrations)} \quad (5)$$

where the ε_i 's are IID $N(0, \sigma_\epsilon^2)$ and denote general randomness and measurement error.

Thus, for each subject and each of the five hormones, there is a parameter space:

$$\theta = (\alpha^{(1)}, \alpha^{(2)}, \beta_0, \beta^{(1)}, \beta^{(2)}, \beta^3, \eta_0, \eta_1, \sigma_A^2, \sigma_\epsilon^2) \quad (6)$$

Estimates of the components of the parameter set produce ten summary statistics. The first four statistics, are "somewhat independent" of one another – this will be important in an interpretation of the False Discovery Rate (FDR) adjustment for multiple P-values:

(1) Mass Per Pulse (MPP); (2) Pulse Frequency (scaled to #/24 hrs); (3) Total Secretion (basal plus pulsatile) (scaled to 24 hrs); (4) Basal Secretion. The remaining six statistics are: (5) Total Pulsatile Secretion (scaled to 24 hrs); (6) Fractional Basal Secretion (scaled to 24 hrs); (7) Pulse Regularity; (8) Mode of burst-like Release; (9) Fast Half-Life; (10) Slow Half-Life.

2.3.2 Statistical hypotheses

We focus on Six Hypothesis Groups (the first two are the primary ones). For each Hypothesis Group there are 10 summary variables for each of the five hormones $(10\ x\ 5 =)50$ potential hypothesis tests. Moreover, in a strict hypothesis-testing context, statistics are formulated as the alternate hypotheses. For example, Hypothesis Group 1 is that 4800 m acclimatization results in a reduction in the basic summary variables, in comparison to 4800 m. (The reduction being back towards that of the Baseline.) The resulting P-values will be the evidence that this is true, i.e., that the complement (Acclimatization not resulting in a reduction) is false.

H1: 4800 m vs (or, more precisely, >) 4800 m Acclimatized (i.e., acclimatization results in a reduction in the key summary statistics).

H2: 6050 m vs (>) 4800 m (i.e., the increased altitude causes an increase in the summary statistics).

H3: 4800 m vs (>) 450 m (i.e., the increased altitude causes an increase in the summary statistics compared with Baseline).

H4: 4800 m Acclimatization vs (>) 450 m (i.e., the increased altitude, even after acclimatization, causes an increase in the summary statistics compared with Baseline).

H5: 6050 m vs (>) 450 m (i.e., the increased altitude causes an increase in the summary statistics compared with Baseline).

H6: 6050 m vs (>) 450 m Acclimatization (i.e., the increased altitude causes an increase in the summary statistics compared with 4800 m Acclimatization).

As stated above, there 10 summary variables for each of the five hormones $(10 \text{ x } 5 = 50 \text{ variables})$, with each potentially part of each of six hypotheses ($\mathbf{H1 - H6}$). How to test multiple hypotheses has always been a difficult statistical question. The one mainstay, though, has always been that hypotheses that were precisely formulated prior to the observation of the data should not require an additional allowance of randomness to account for their prior non-selection. This allowance has historically taken the form of multiple comparisons, Studentized range, Bonferroni adjustments and, most importantly for modern statistics, the False Discovery Rate (FDR). We state this, inasmuch as our four key summary statistics and our two most important hypothesis groups (**H1** and **H2**), for the five hormones, were obvious prior hypotheses. It is not as if they were devised based upon the observed data, and that should therefore be considered in evaluating their P-values (**Figure 4**, described below).

3. Results

In the present study, eight women were evaluated over 6-10 hr periods with blood sampling every 10-20 min, at four altitudes: base camp (450m), 4800m, 6050m and again at 4800m (on descent) with a period for acclimatization. Note that in the figures, 4800 mA is placed next to 4800 m for ease of comparison, even though chronologically realized after 6050 m on the descent. The focus of the present study is on changes in anabolic-catabolic balance under extreme altitude. Five hormones were measured from each blood sampling: GH, PROL, Cortisol, TSH and fT4. Because of the extreme conditions, the length of blood sampling for different individuals at a given altitude and the same individual at different altitudes varied by necessity. Hence, to place summary statistics on comparable scales, measures that involved time were scaled to their corresponding 24-hr values (e.g., pulse frequency, and basal and total pulsatile secretion).

In **Figure 1**, all of the blood-sampled concentrations, across the four altitudes (columns) and the five hormones (rows), are displayed for the eight subjects. The concentration scale for any one hormone is the same across altitudes, for ease of comparison. Prominent pulsatility is evident in all hormones. The general similarity across altitude reflects the fundamental difficulty in the detection of changes in the dynamics. Because each individual is followed across the four altitudes, one can remove individual variation via differencing between two altitudes (i.e., pairing). Without doing this it would be difficult to identify differences across altitude.

Our two most important hypotheses (**H1-H2**) concern, respectively, acclimatization (at 4800m) and the final increase in altitude from 4800m to 6050m. To test these and the other hypotheses, one must recover, from the concentrations, the unobserved hormonal secretions, removing the effects of hormone elimination. In **Figure 2a** the analytically recovered (estimated) time-varying hormone secretion rates are displayed, the result of the statistical deconvolution

methods described in Methods. One visual consequence is that changes in dynamical regulation are not easily detected in one-dimensional plots, except for the rather dramatic drop in GH secretory-burst size (mass) at 4800 m after acclimatization.

Four key outcome statistics are: Mass Per Pulse (MPP), Pulse Frequency (scaled to 24 hr), Total Secretion (scaled to 24 hr) and the Basal Secretion (scaled to 24 hr). In **Figure 2b**, these four summary statistics (mean (dashed line), median (solid line)) are displayed, subject by subject (different colors), across the different altitudes. Because there are differences in the units of measurement for each hormone, for comparison purposes, we have scaled each of the four statistics by their maximum (across subjects). An overall pattern is visually revealed suggesting the effects of acclimatization, especially in Mass Per Pulse (MPP) in each of the five hormones, the left-most subplot. MPP values, for each hormone, are significantly increased at 4800m and 6050m, with those at 4800mA (acclimatization) brought back towards baseline (450m). This is borne out in the hypothesis testing displayed in **Figure 5**, discussed below, suggesting that acclimatization can stabilize (at least certain aspects of) hormonal disruption due extreme altitude.

In **Figures 3-4,** a more precise and detailed display of the above *four* principal summary statistics is presented, with certain novel and significant patterns visually highlighted for all five hormones and all eight subjects. In each subplot (**a-b**) of **Figures 3-4**, there are two columns. The left column displays (asterisks) all of the values, for the eight subjects, of the four principal summary statistics, hence allowing for an interpretation of the distributions. Visual comparisons of baseline (450 m), 4800 m and 4800 mA are quite dramatic with respect to distinct hormone changes in MPP, pulse frequency and total and basal secretion. For most hormones, MPP goes up and then down (with acclimatization), becoming potentially lower than baseline (**Figure 3a**). Pulse frequency (except for GH and fT4) does the reverse (**Figure 3b**), going down in the transition

from 450 m to 4800 m and then rising dramatically at 6050 m. At 4800 m, pulse frequency is similar to that at 6050 m. The data indicate a physiological effect of the 450 m to 4800 m transition for both secretory burst mass and number, which is distinct to hormone type. The rise in MPP and/or frequency contributes to an increase in overall pulsatile secretion (the product of burst mass and frequency), which with basal secretion sums to total secretion.

The right column of each subplot (**a-b**) of **Figure 3-4** depicts intraindividual hormone differences corresponding to each hypothesis (1-6). It is these paired values which are the basis for the hypothesis testing (**Figure 5**). For some summary statistics, the appropriate hypothesis to test would be to one side (e.g., an increase) as opposed to the other direction (e.g., a decrease). For others it would be opposite. To account for this, we have calculated the P-value in both one-sided directions and taken the minimum of the two; if a two-sided test were deemed appropriate, it would be double this value.

For Total Secretion (**Figure 4a**), the most dramatic changes are in the thyroid axis. Total TSH and fT4 secretion increase significantly with altitude, with a return to baseline values (450 m) after acclimatization at 4800 m. On the other hand, GH, PROL and Cortisol's total secretion at 450 m, 4800 m and 6050 m are not so different, although values at 4800 mA are reduced. Basal (non-pulsatile) (**Figure 4b**) similarly has an intriguing pattern. Basal secretion rates at baseline 450 m and at 4800 mA are similar, whereas values decrease at altitudes of 4800 m and 6050 m. This occurs concomitantly with a rise in pulsatile secretion [**Figure 7a-b**].

Figure 5 provides P values for each hormone and each hypothesis. In **Figure 5a**, for the four key summary variables, we plot the P-values over the six hypotheses, quantifying what was summarized above concerning **Figures 3-4**. The testing was done using a t-test (7 degrees of freedom (df)). For Hypothesis 1 (comparing 4800 m to 4800 mA), all five hormones show highly

significant decreases in MPP (**$P<10^{-6}$, for each**). Cortisol pulse frequency increases are highly significant for 6050 m compared to 450 m and 4800 m altitudes (). All hormones except PROL significantly decreased their Basal Secretion due to acclimatization (**$P<.05$**). A striking additional outcome is the significance of fT4 Total Secretion comparisons for all six Hypotheses (**$P<10^{-4}$**, in all but H2). TSH Total Secretion comparisons are highly significant for the three elevations (4800 m, 6050 m, 4800 mA) compared to baseline (450 m) (**$P<10^{-4}$, for each**).

Thus, hormone- and altitude-specific adaptations in hypothalamo-pituitary regulation were inferable in the women climbers studied here. Foremost were highly significant changes in the burst-like mode of intermittent fT4 and TSH secretion over time. For both hormones in the thyroidal axis, analytically estimated pulse frequency was altitude-independent, whereas the mass of hormone released per pulse and the time-invariant basal secretion rate increased significantly after ascension from 450 m to 4800 m, and analogously at the extreme elevation of 6050 m compared with 450 m. Both secretion features decreased in magnitude with acclimatization (4800 mA) compared with 4800 m). Elevated thyroid hormone output at high altitude is consistent with the cold stress so anticipated, and the whole-body adaptation to the same. Our data show further that the pituitary and hypothalamus are involved, since both TSH and fT4 rose, framing the consideration that feedback upon the hypothalamus and pituitary gland is muted under these conditions. Otherwise, the rise in fT4 of purely thyroidal origin would be expected to quench TSH release, which did not occur (22).

Figure 5b displays a False Discovery Rate (FDR) plot (six subplots) for each of the six Hypothesis Groups (**H1-H6**) at the α=.05 level. In each, the 20 P-values: 20=4 (statistics) x 5 (hormones) are plotted on a log scale with differing symbols and colors. The importance of **Figure 5b** is the additional reassurance of the statistical significance of the results in **Figure 5a**, described

above. Moreover, when one has hypotheses specified *a priori*, as with our four primary summary statistics, a traditional comparison to $given\ \alpha$ $(\mathrm{e.g.},.05)$ level of significance is justified. The merit of FDR is in the case where hypotheses may have been selected after the data has been obtained.

Thus, for the test of Acclimatization (**Hypothesis 1**), all hormones except fT4 exhibit a decrease in pulsatile secretion. PROL shows a dramatic decrease in the mode of burst-like secretion (faster release). Both thyroid hormones, TSH and fT4, exhibit increases in basal secretion when altitudes 4800 m, 4800 mA and 6050 m are compared with 450 m (**Hypotheses 3-5**). Other variables are different for one altitude comparison, but not for others, and we have not elaborated on those occurrences. In **Figure 5c**, we make the same assessments as in **Figure 5a**, but use a sign-test rather than a t-test. From **Figures 3-4**, one can see that some of the paired difference distributions have occasional values larger than the rest. The sign test will remove any undue influence of such extreme values. The results of significance (i.e., P-values) are quite similar to those of **Figure 5a** (and hence to **Figure 5b**).

Figures 6-7 present the remaining six outcome variables. The format is the same as in **Figures 3-5**. The six summary statistics are Total Pulsatile Secretion, Fractional Basal of Total Secretion, pulse regularity (a Weibull distribution parameter), mode of secretory release (for the waveform ψ) and, the fast and slow half-lives of elimination. For statistical evaluations of these latter six outcomes variables, **Figure 7a** depicts P-values using a t-test (7 df) over the six hypotheses. For Hypothesis 1(Acclimitization at 4800 m), both total pulsatile secretion and the fraction of basal to total secretion are highly significant (**$P<10^{-3}$**) for GH, PROL, Cort and TSH, with fT4 being just above the P=.05 level. In **Figure 7b**, a FDR plot is given for each of the six Hypothesis Groups (**H1-H6**) at the α=.05 level. Its importance is that it substantiates the above mentioned highly

317 significant **Hypothesis 1** results. **Figure 7c** represents statistical inferences using a sign-test rather
318 than a t-test. In all 3 assessments, results are quite similar.
319

320 ## 4. Discussion

321 Hypothalamo-pituitary dependent adaptations in endocrine regulation due to extreme altitude
322 conditions were evaluated in 8 women, by sampling blood every 10-20 min over a 6-10 hr period
323 at four altitudes (450 m, 4800 m, 4800 m Acclimatization, and 6050 m). Five representative
324 anabolic, catabolic, and hypothalamically restrained (PROL) hormones: GH, PROL, Cort, TSH
325 and fT4, were assayed in each sample of each hormone time series to quantify hormonal pulse
326 amplitude and frequency (25). Because the same individuals were evaluated at each altitude, and
327 the outcome statistics were summarized at each altitude, paired comparisons were made for each
328 subject and altitude. Other studies (27) have been conducted in which changes in the levels of the
329 reproductive hormones (LH, testosterone) were the focus. One difficulty with such studies is the
330 individual non-synchronous differences (per female participant) in menstrual cycle phases.
331 Consequently, in the present investigation, the focus is on anabolic-catabolic hormone balance (or
332 imbalance) under extreme altitude conditions. Data in this unique setting are sparse, due to the
333 substantial challenges earlier in obtaining frequently sampled hormone time series under the severe
334 physical and psychological stresses of mountaineering.

335 Foremost of the changes in dynamical response to extreme altitude were the highly significant
336 changes in the burst-like mode of intermittent fT4 and TSH secretion over time. Secondary major
337 outcomes of the analysis were changes in hypothalamically regulated pulse frequencies of PROL
338 and Cort. Both pulse frequencies decreased after climbing from 450 m (baseline) to 4800 m, and
339 then increased with acclimatization at 4800 m, thus returning toward baseline levels. After the

extreme condition change from 4800 m to 6050 m, both pulse frequencies increased further. A change in pituitary-target gland pulse frequencies is taken as prima facie evidence of hypothalamic adaptations, inasmuch as both the pituitary gland and the adrenal gland are devoid of intrinsic pulsatility when study ex vivo or after hypothalamic disconnection in vivo (10-12, 19, 24).

Another major finding was that the mass of hormone secreted per burst for all four of PROL, Cort, TSH and GH fell significantly during acclimatization at 4800 m (**Hypothesis 1**), as did the fraction of basal to total secretion. Since pulsatile pituitary-target organ hormone release in each of these endocrine axes is modulated by hypothalamic neurotransmitter drive (GH, TSH, Cort) or restraint (PRL under dopaminergic inhibition), the present data provide the first clear evidence that exertion, hypoxia, nutrition, stress and fatigue associated variously with strenuous high-altitude ascent strongly control the brain and pituitary gland in healthy human beings.

Adaptations in pituitary-hormone pulse frequency are particularly notable mechanistically. This is because the pituitary gland per se acquires its timing (frequency and spacing) of secretory bursts from hypothalamic signals (10-12, 19, 24) rather than from intrinsic pulsatility of pituitary tissue. Thus, a change in hormone pulse number denotes a de facto change in hypothalamic neurotransmitter regulation of the pituitary gland. Notably, both prolactin and cortisol pulse frequencies slowed significantly at 4800 m compared with 450 m. With continued adaptation to higher altitude including 6800 m, both pulse frequencies rose again. These patterns strongly support hypothalamic neurotransmitter adaptations, since prolactin is under dopamine restraint primarily, where cortisol is under noradrenergic stimulation via the peptides ACTH-releasing factor (CRH) and vasopressin (AVP) (18). There is evidence for even more complex multifactorial regulation of prolactin and ACTH-cortisol pulse timing, via neurotransmitters such as GABA, NMDA, serotonin, acetylcholine among others. Whatever the dominant prolactin and ACTH pulse

363 onset-determining pathway in humans, the presently observed pulse slowing for prolactin and
364 cortisol point to altitude/exertion effects on such pulse-regulating inputs to the pituitary. However,
365 adaptive recovery of pulse frequency at sustained high altitude provides important evidence that
366 pulse slowing is not permanent.

367 Changes in pituitary-hormone secretory pulse size (burst mass, or serum hormone pulse height)
368 are jointly determined by pulse frequency (higher frequencies yielding smaller pulses) and by
369 hypothalamic secretagogues or inhibitors controlling pituitary secretion directly. This dogma is
370 well articulated for the GH axis, where in both GHRH and ghrelin (secretogogues) augment,
371 whereas somatostatin suppresses the size of GH pulses (11). Moreover, systemic blood-borne
372 hormones and metabolites further enhance or diminish pituitary responses to brain signals.
373 Accordingly, pulse size is the result of four major regulatory interactions: hypothalamic
374 neurotransmitter amount and type (stimulation or inhibition), and timing (frequency), and intrinsic
375 pituitary responsiveness as modified by blood-borne signals (e.g., free fatty acids as a circulating
376 negative effector of GH pulse size). Thus, interpretation of pituitary-hormones burst-mass changes
377 is more complicated. For example, the smaller size of pulses of prolactin, cortisol, TSH and GH
378 during adaptation to 4800 m would plausibility reflect greater dopamine inhibition (prolactin, TSH
379 and GH), lesser noradrenergic drive (Cort and GH), more hypothalamic somatostatin inhibition
380 (prolactin, TSH and GH, and to a lesser degree, ACTH/Cort), and/or higher IGF-F restraint (GH)
381 (10-12, 19, 23, 24).

382 Whatever the final mechanisms eventually proven in further experimental models, the present
383 pulsatility data allow for the first time a clear inference that combined hypothalamic-pituitary
384 mechanisms mediate the prominent endocrine changes associated with high altitude exposure.
385 Detailed neuropharmacological and neuroendocrine interventional experiments will be needed to

elucidate the molecular causes for the observed changes in all of TSH (frequency and amplitude), as well as prolactin, Cort and GH (primarily amplitude).

The control of low basal (non-pulsatile) hormone secretion is not well understood (9). A plausible and testable hypothesis is that sustained neurotransmitter drive serves to increase intracellular pituitary hormone accumulation, and thus secondary elevate interpulse release as basal secretion. This assumes that basal secretion is due to constitutive unregulated hormone release, as distinct from pulsatile secretagogue-induced burst-like release of predocked secretory granules (25).

These findings also raise the question as to whether the hormonal changes are linked to the cardiorespiratory and metabolic changes which can be found in hypobaric hypoxia (18). In a previous paper (27) it was demonstrated that total hormone concentrations are closely linked with altitude but not with parameters of oxygen transportation such as O_2 saturation and pO_2 values. It was therefore concluded that there must be other factors such as disturbed sleep or physical stress, which induce these changes. It was hypothesized that the physiological changes at high altitude might possibly be influenced or might even be partly under control of the endocrine system. This present study does verify this hypothesis due to both the modeling at the level of secretion (and not concentrations), as well utilizing the study design for pairing. Moreover, the present results support and extend the findings of (27) that the concentrations of several hormones, especially stress related hormones such as cortisol, prolactin and thyroid hormone, change with altitude and that acclimatization almost normalizes these changes. A side consequence of the present study reveals that not only the serum hormone concentrations changes with altitude and normalizes with acclimatization (as shown in (27)) but also the hormone pulse frequency and basal and pulsatile secretion patterns.

Furthermore, this study revealed that endocrine changes are far more complex then described in (27). The increase of hormone concentrations with altitude seem to be a result of a marked increase of MPP whereas pulse frequency decreased. This is of relevance as even subtle changes of MPP and frequency can have marked endocrinological and thereby physiological effects. The functional effect of pituitary and adrenal hormones is not only due to their serum concentration but also to their secretion pattern. In women for instance physical and psychological stress and diseases can reduce the frequency of LH and FSH release, which lead to amenorrhoea even though total blood concentrations are not substantially affected (20).

Thus, the observed changes in basal hormone secretion suggest first that endocrine regulation at high altitude and during acclimatization is far more complex than previously thought and second that altitude also effects pituitary hormone processing (5, 8). This hypothesis would be consistent with known suppression of pituitary hormone synthesis by intracellular messengers, such as hypoxia inducible factor (8).

Figure Legends

Figure 1. The concentration profiles are plotted for the eight subjects, for each hormone and each altitude. The rows are the five hormones (GH, PROL, Cortisol, TSH, fT4) and the columns are the four altitudes (450 m, 4800 m, 4800 m Acclimatization, 6050 m). Within each subplot are the profiles for the eight subjects delineated by color. The y-axis scale for each hormone is the same across altitudes, to enhance comparisons.

Figure 2. **2a.** The recovered secretion rates (mass/distribution volume/min) are plotted for the eight subjects, for each of the five hormones (rows) and altitudes (columns). **2b.** Four key patterns, across the hormones, that were detected. Plotted are four statistics that summarize information about the secretion and kinetic information, as altitudes change. The four are the sample means (across 8 subjects) of mass per pulse (MPP), pulse frequency, total secretion and the fraction of Basal to total secretion. To place all five hormones on a common plot, for each hormone, the means are normalized by their maximum (over the four altitudes).

Figure 3. Of the ten summary statistics designed to extract distinct structure from the recovered secretion and elimination rates, the first four (mass per pulse (MPP), pulse frequency, total secretion and basal secretion) are most fundamental; these are displayed in **Figures 3-4**. In **Figures 3a-b**, Mass Per Pulse (MPP, **3a**) and Pulse Frequency (**3b**), are plotted. In each subplot(**3a-b**), there are two columns. In the left column are the summary statistic values for the eight subjects (black asterisk) at each of the four altitudes, for the five hormones (GH, PROL, Cortisol, TSH, fT4). The means (dashed line) and medians (solid line) at each altitude, for each hormone, are linearly connected. In the right column, the differences in the statistic, for each

subject, for each hypothesis are calculated and plotted as a function of **Hypotheses 1-6**. For example, in **Hypothesis 1**, the difference in the values at 4800 m and 4800 m Acclimatization are calculated. The means (dashed line) and medians (solid line) are plotted versus Hypotheses 1-6. A dashed box is drawn around the results for Hypotheses 1-2 to emphasis that they were a priori formulated-hypotheses of particular importance.

Figure 4. In **Figures 4a-b** are plotted the two summary statistics: total secretion (**4a**) and basal secretion (**4b**). *The legend format is the same as in* **Figure 3**, with two columns in each subplot. That is, in the left column are the summary statistic values for the eight subjects (black asterisk) at each of the four altitudes, for the five hormones (GH, PROL, Cortisol, TSH, fT4). The means (solid line) and medians (dashed line) at each altitude, for each hormone, are linearly connected. In the right column, the differences in the statistic, for each subject, for each hypothesis are calculated and plotted as a function of **Hypotheses 1-6**.

Figure 5. 5a. For Hypothesis 1-6, and each of the four summary statistics in **Figures 3-4**: mass per pulse (MPP), pulse frequency, total secretion and fraction of basal to total secretion, a t-test was performed and a P-value was calculated for each null hypothesis that there is no change due to the difference in the two altitudes (e.g., between 4800 m and 450 m). The P-value is calculated to each side and the minimum of the two values is the resulting plotted P-value. If one wishes to consider two-sided P-values, one would just double the plotted value. What are plotted are the log P-values versus Hypotheses 1-6, for the five hormones (designated by differing colors) with the log P-values linearly connected across the hypotheses. A dashed box is drawn around the results for Hypotheses 1-2 to emphasis that they were a priori formulated-hypotheses of particular

478 importance. A dashed horizontal line is plotted at log (.05). For hypotheses that were a priori
479 formulated for the five hormones, as were Hypotheses 1-2 and the present four summary statistics,
480 one can argue that comparisons to log (.05) is justified. **5b.** For each hypothesis (1-6), there are
481 5 hormones and 4 summary statistics (20=4 x 5), and one can consider the multiple comparisons
482 effect on the log P-values. For each hypothesis (1-6), a False Discovery Rate (FDR) plot is
483 presented (at $\alpha = .05$). There are four symbols (square, x, circle, asterisk) and five colors for the
484 hormones (Red, Blue, Black, Green, Cyan). The solid black line denotes the boundary curve for
485 significance (at α=.05), assuming no a priori selection of relevant hypotheses. The red dashed
486 curve corresponds to the situation where the underlying statistics are correlated; this curve is given
487 only for illustrative purposes, since independence is reasonable in the present case: MPP, pulse
488 frequency, basal secretion and total secretion could all individually go up or down.). FDR analysis
489 is presented as a secondary justification for the general results of **5a**, where Hypotheses 1-2 and
490 the present four statistics were formulated a priori to the data. It can be viewed as supportive
491 evidence for the results enclosed is the dashed boxes in **5a**. **5c.** As alternative evidence, a sign test
492 is now performed and the resulting log P-values are plotted. A dashed box is drawn around the
493 results for **Hypotheses 1-2** to emphasis that they were a priori formulated-hypotheses of particular
494 importance.
495
496 **Figure 6.** Ten summary statistics, designed to extract distinct structure from the recovered
497 secretion and elimination rates. In **Figures 3-4**, four of the summary statistics were plotted. In
498 **Figure 6**, the remaining six summary statistics are presented. The six statistics are: total pulsatile
499 secretion (**6a**), fraction of basal to total secretion (**6b**), pulse regularity (**6c**), mode of release (**6d**),
500 fast half-life (**6e**) and slow half-life (**6f**). *The legend format is the same as in* **Figures 3-**4. In

each subplot(**4a-f**), there are two columns. In the left column are the summary statistic values for the eight subjects (black asterisk) at each of the four altitudes, for the five hormones (GH, PROL, Cortisol, TSH, fT4). The means (dashed line) and medians (solid line) at each altitude, for each hormone, are linearly connected. In the right column, the differences in the statistic, for each subject, for each hypothesis are calculated and plotted as a function of Hypotheses 1-6.

Figure 7. **7a.** For Hypothesis 1-6, and each of the six summary statistics in **Figure 6**: total pulsatile secretion, fraction of basal to total secretion, pulse regularity, mode of release, fast half-life and slow half-life, a t-test was performed and a P-value was calculated for each null hypothesis. In Hypotheses 1-6, the null hypothesis in each, is that there is no change due to the difference in the two altitudes (e.g., between 4800 m and 450 m). As in **Figure 5**, the P-value is calculated to each side (under the null hypothesis) and the minimum of the two values is the resulting plotted P-value. What are plotted are the log P-values versus Hypotheses 1-6, for the five hormones (designated by differing colors) with the log P-values linearly connected across the hypotheses. A dashed box is drawn around the results for Hypotheses 1-2 to emphasis that they were a priori formulated-hypotheses of particular importance. A dashed horizontal line is plotted at log (.05). For Hypotheses 1-2, which were a priori formulated for the five hormones, one could (potentially) argue that comparisons to log (.05) is justified. This argument is not as strong however as that for the four summary statistics in **Figure 4**5 **7b.** For each hypothesis (1-6), there are 5 hormones and 6 summary statistics (30=6 x 5), and one can consider the multiple comparisons effect on the log P-values in **Figure 7a**. A False Discovery Rate (FDR) plot is presented (at $\alpha = .05$). There are six symbols (square, triangle, x, hexagon, circle, asterisk) and five colors for the hormones (Red, Blue, Black, Green, Cyan). The dashed curve is for the correlated case and the solid for the

uncorrelated case, which is appropriate. **Figure 7b** is viewed as supportive evidence for the results enclosed is the dashed boxes in **Figure 7a**. **7c.** As alternative evidence to that Presented in **Figure 7a**, where a t-test was performed, a sign test is now performed and the resulting log P-values are plotted. The results are very similar to those of **Figure 7a**.

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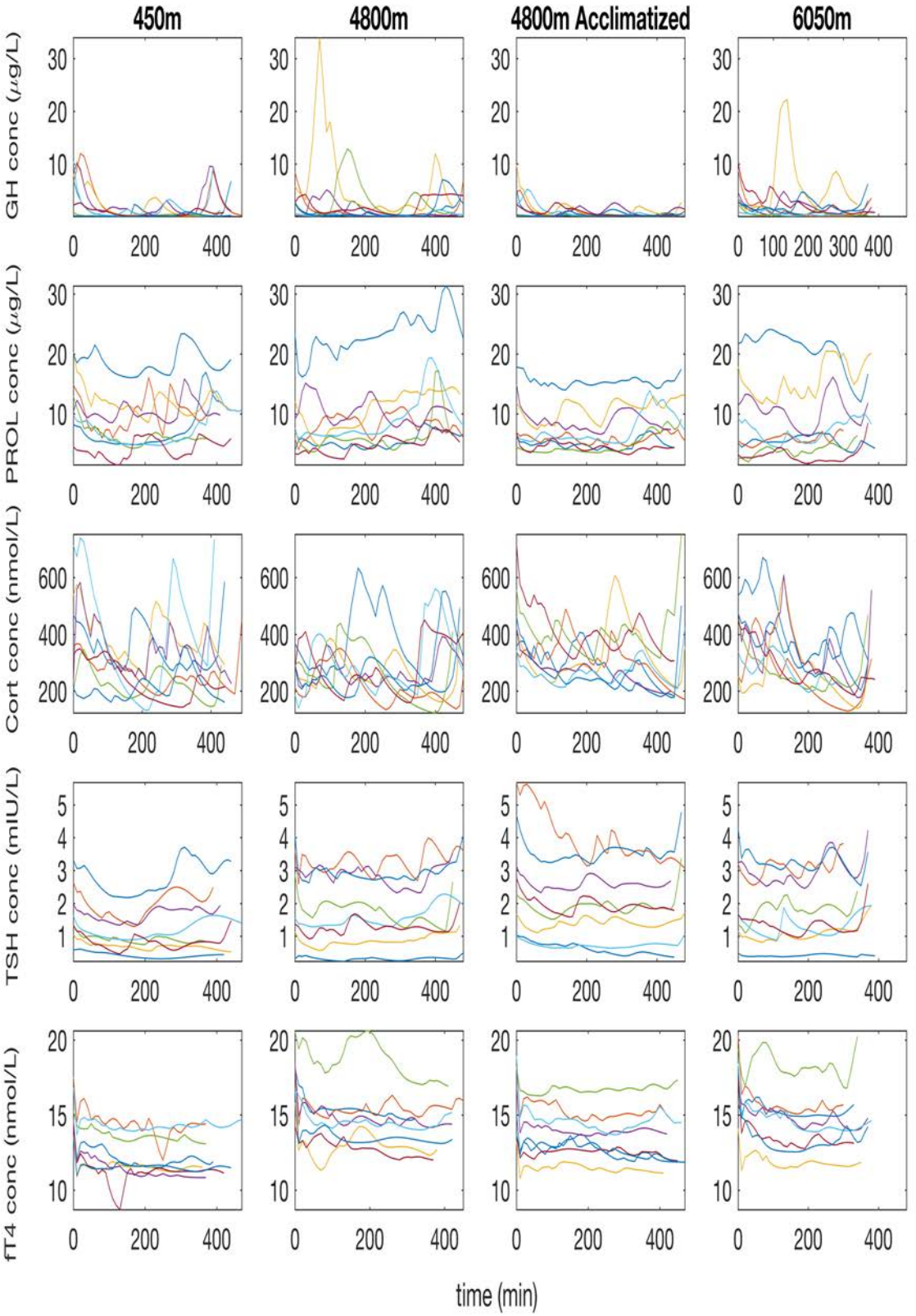
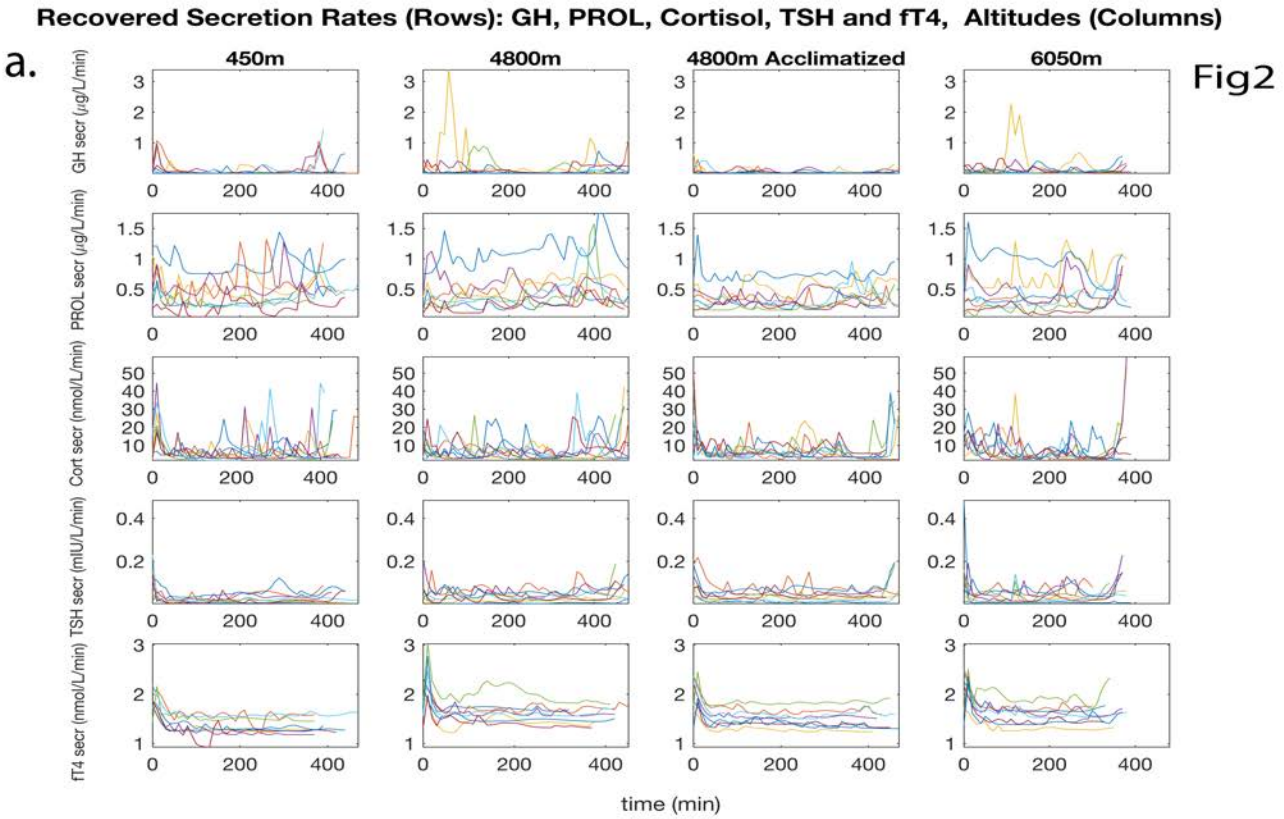
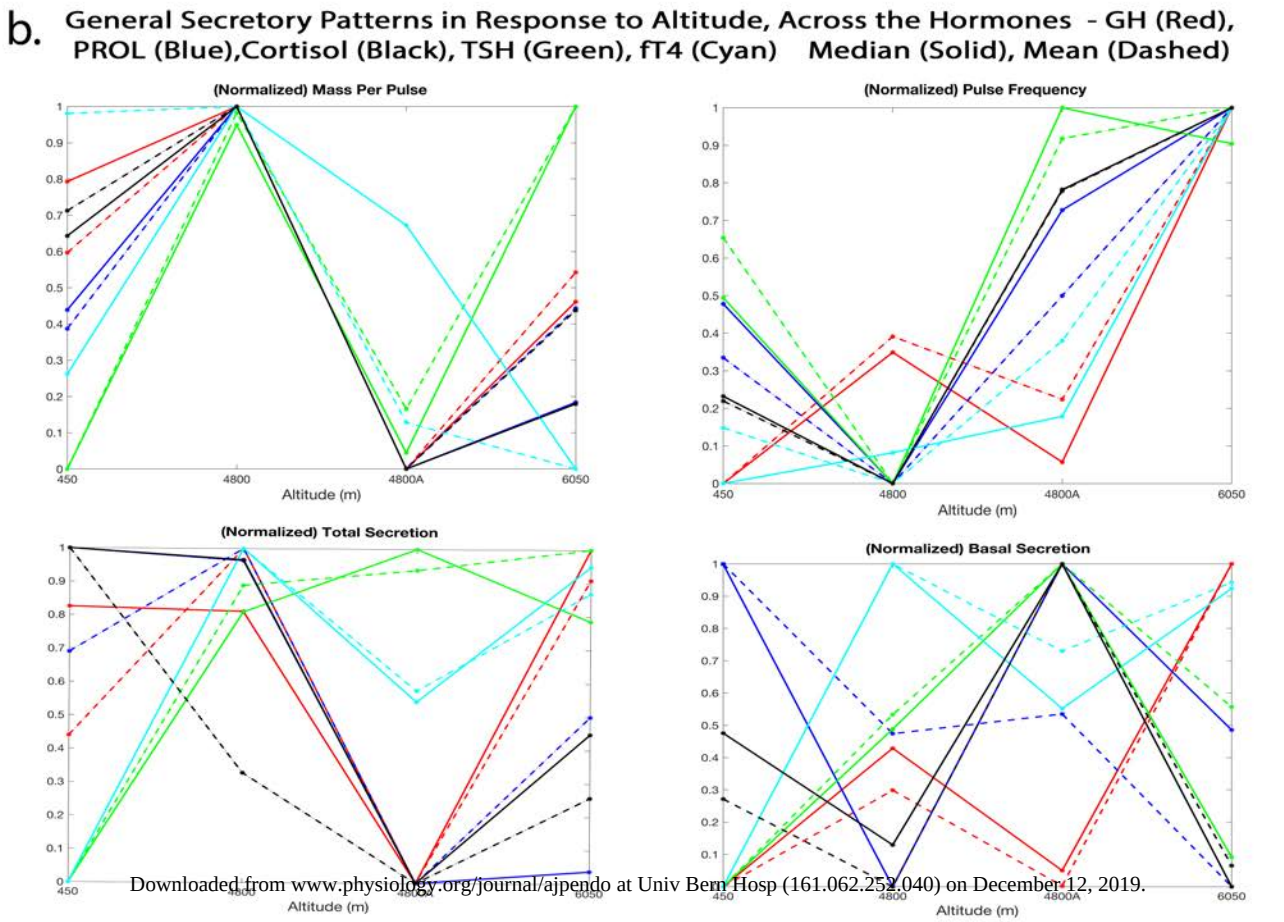
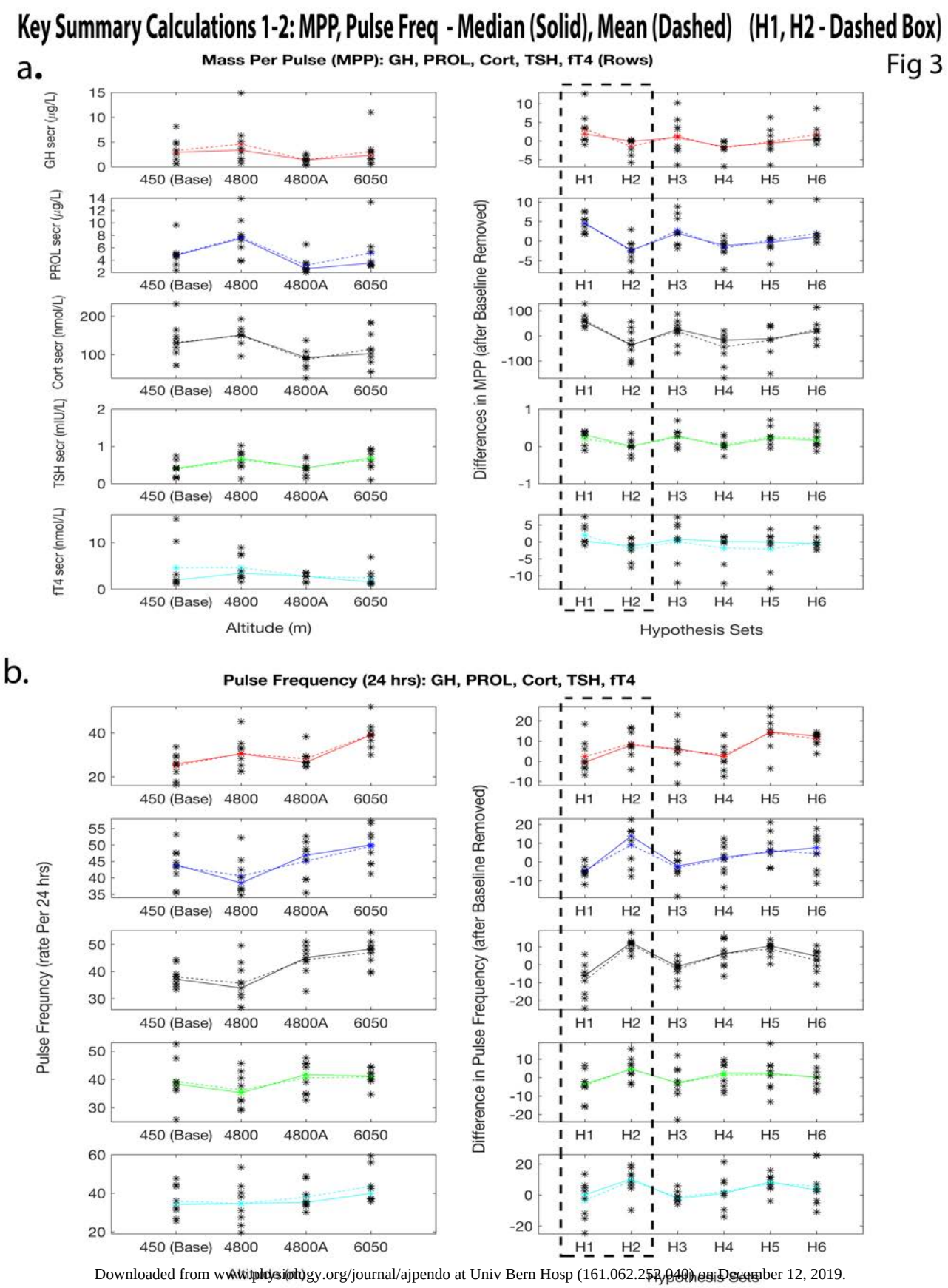


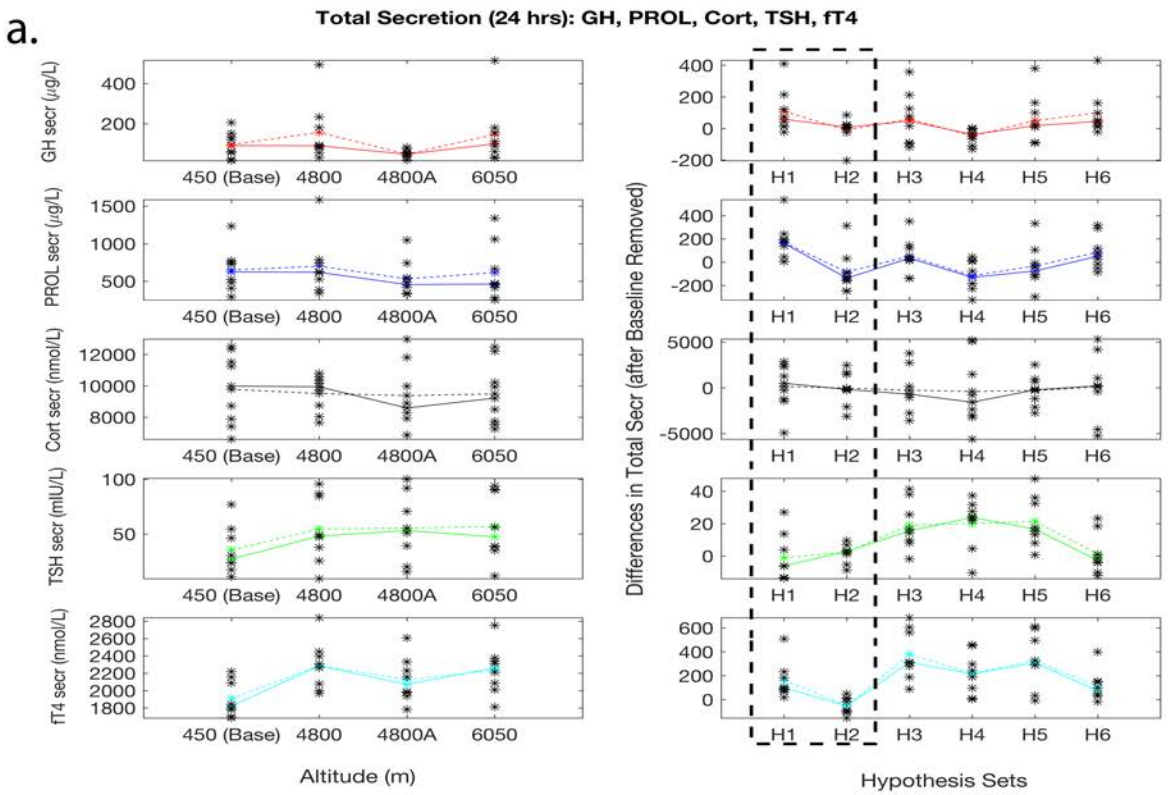


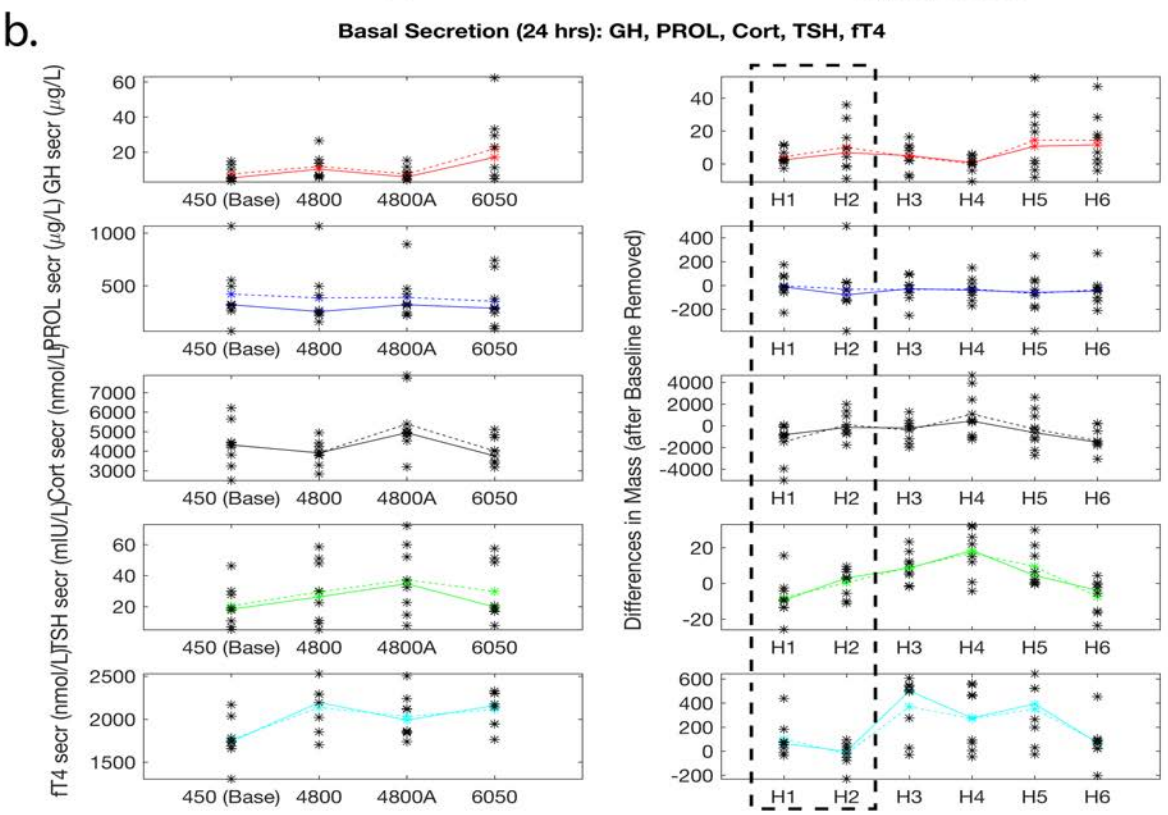


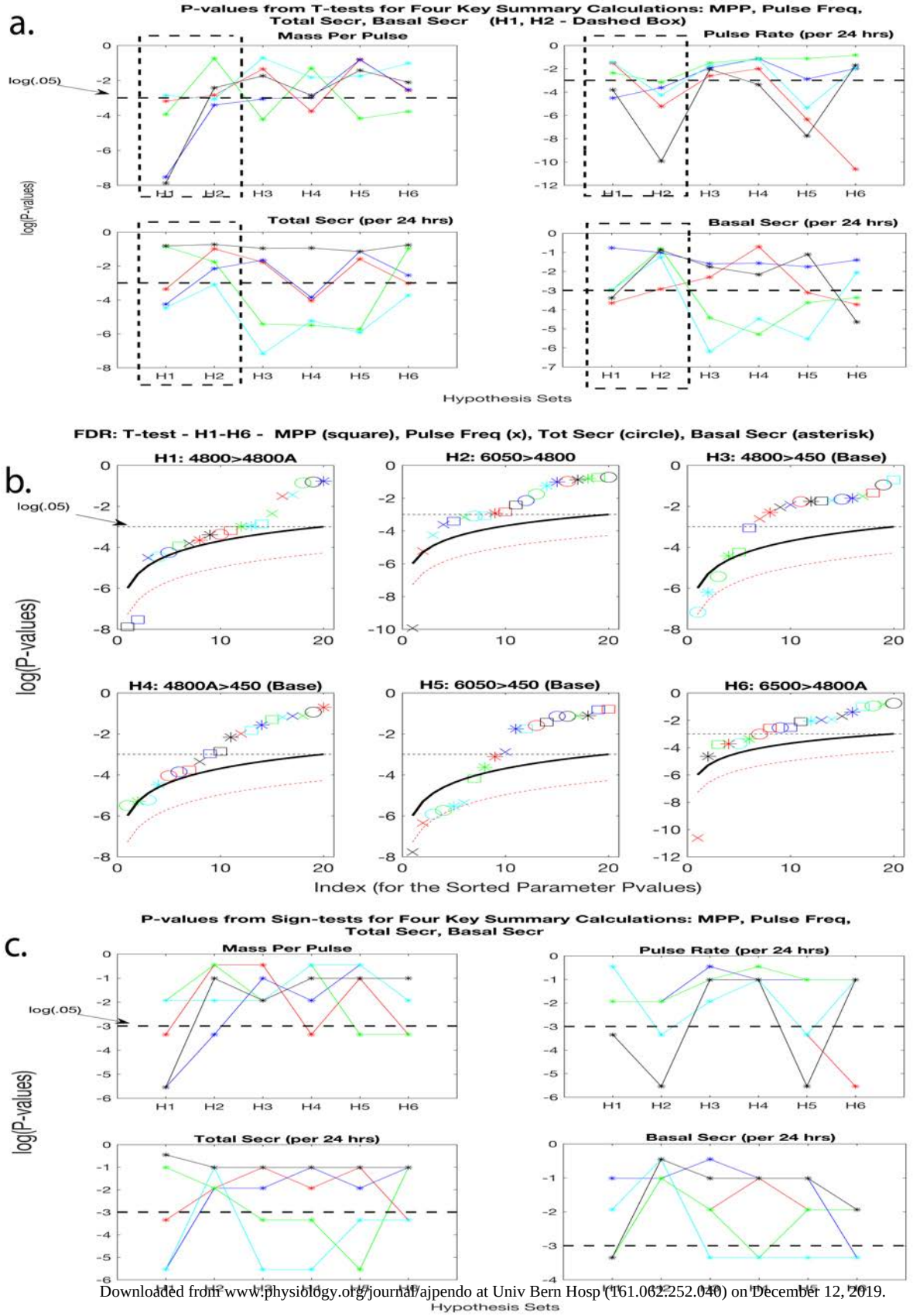


Key Summary Calculations 3-4: Total Secr, Basal Secr - Median (Solid), Mean (Dashed) (H1, H2 - Dashed Box)

Fig 4

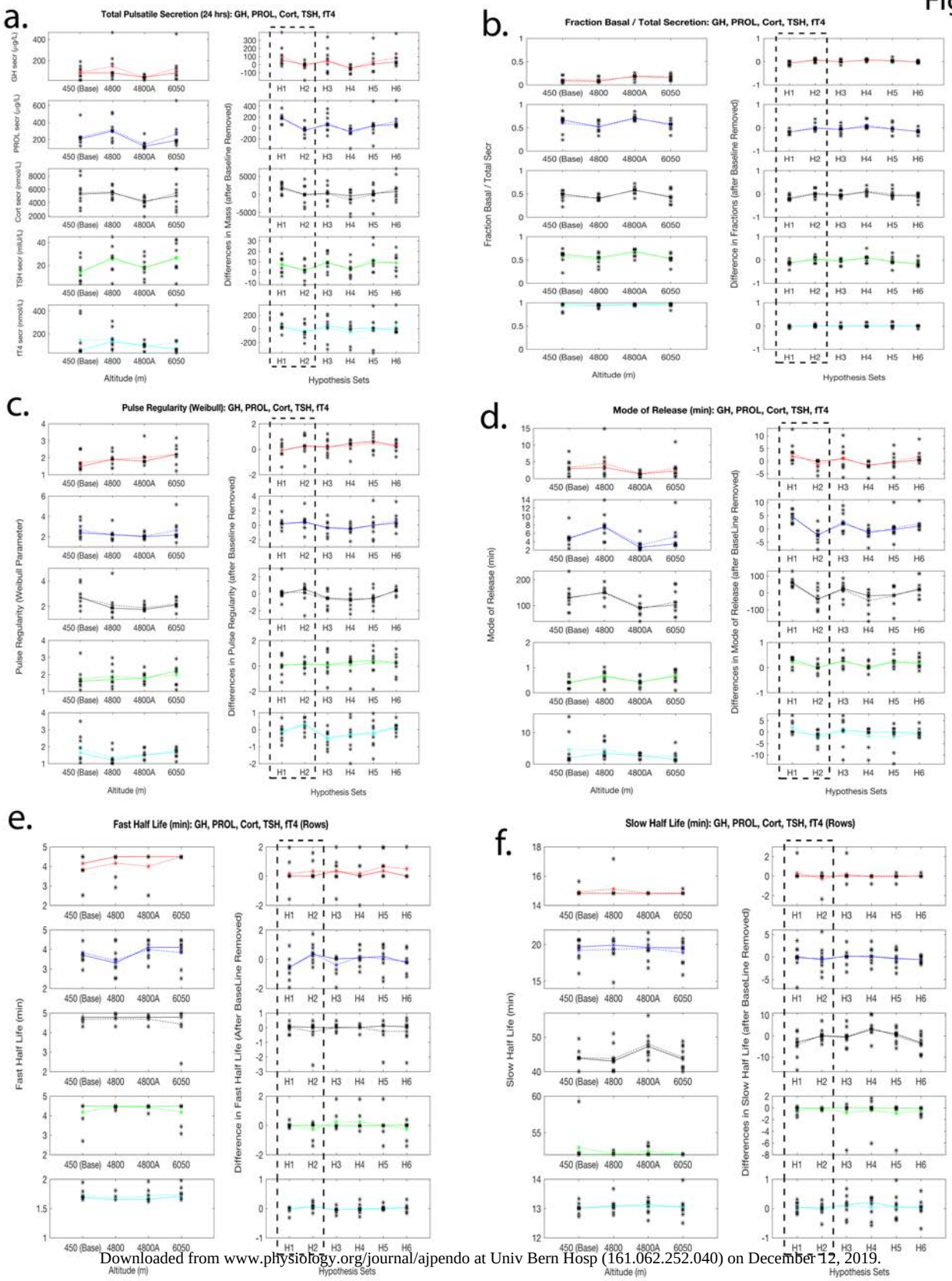






Six Additional Summary Calculations: Total Pul, Frac Basal/Tot Secr, Pulse Regularity, Mode of Release, Halflives - Median (Solid), Mean (Dashed) (H1, H2 - Dashed Box)

Fig 6



GH(Red), PROL(Blue), Cort(Black), TSH(Green), fT4(Cyan)

Fig 7