

1 Regulation and Adaptation of Endocrine Axes at High Altitude

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22 **Abstract**

23 As a model of extreme conditions, eight healthy women, part of a 40-member Nepal mountain-
24 climbing expedition, were monitored for dynamic endocrine adaptations. Endocrine
25 measurements were made at frequent intervals over a 6-10 hours period at four altitudes: 450 m,
26 4800 m (Base Camp), 6050 m and again at 4800 m (on descent) after an acclimatization period
27 (4800 mA). Quantified hormones were growth hormone (GH), prolactin (PROL), Cortisol (Cort),
28 Thyroid-stimulating hormone (TSH), and free thyroxine. These hormones are important to the
29 anabolic/catabolic balance of the body, and are vital to growth, homeostasis, hypothalamic
30 inhibition, regulation of stress and metabolism. A key secondary question was the degree to which
31 acclimatization can stabilize hormonal disruption. Based upon statistical false discovery rates, the
32 present analyses unveil marked adaptive changes in the thyroid axis at the level of pulsatile
33 secretion of the pituitary hormone TSH and its downstream product, free thyroxine; strong effects
34 upon the mass of GH, TSH, Cortisol and PROL secretion per burst; and prominent pulsatile
35 frequency disruption and recovery for PROL and cortisol. Since pulsatility changes reflect de
36 facto perturbations in hypothalamo-pituitary control mechanisms, the present data introduce the
37 concept of both frequency and amplitude-dependent adaptive control of brain-pituitary
38 neuroendocrine signals under conditions of extreme altitude exertion and exposure.

39

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43

44 **1. Introduction**

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

61 In principle, disruption of endocrine regulation could mediate high-altitude sickness and might
62 even explain the condition of high-altitude deterioration, a severe catabolic state ultimately leading
63 to death. Few studies have investigated changes in hormone secretion at high altitudes. Thyroid
64 hormones were found to increase, but thyroid-stimulating hormone (TSH) was preserved in single-
65 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

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

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

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

100

101 **2.2 Experimental Protocol**

102 At each of the four altitudes, blood sampling was performed at a targeted frequency of every 10-
103 20 min for a targeted interval of six hours (10 min for first two hours; 20 min for the last four
104 hours). Meeting these targets varied slightly from individual to individual and within individual
105 across different altitudes due to the complexity and changing nature of the environment. The
106 strength of observing the same individuals at the four altitudes is that paired comparisons can be
107 achieved, allowing for greater statistical power and precision.

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

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

123

124 **2.3 Statistical Modeling**

125 **2.3.1 Hormone dynamics**

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

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

134
$$T_m = (T^{(1)}, T^{(2)}, \dots, T^{(m)}) \quad (m \text{ also unknown})$$

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

142
$$M^{(k)} = (\eta_0 + \eta_1 \times (T^{(k)} - T^{(k-1)})) + A^{(k)} \quad (1)$$

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

146
$$\psi(s) \propto s^{\beta^{(1)}\beta^{(3)}-1} e^{-\left(\frac{s}{\beta^{(2)}}\right)^{\beta^{(3)}}}, s \geq 0 \quad (\text{a normalized rate of release}) \quad (2)$$

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

149
$$Z(s) = \beta_0 + \sum_{T^{(k)} \leq s} M^{(k)} \psi(s - T^{(k)}) \quad (\text{Secretion Rate at time } s) \quad (3)$$

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

155

156 $X(t) = \left(a e^{-\alpha^{(1)}t} + (1 - a) e^{-\alpha^{(2)}t} \right) + \int_0^t \left(a e^{-\alpha^{(1)}(t-s)} + (1 - a) e^{-\alpha^{(2)}(t-s)} \right) Z(s) ds$ (4)

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

158 $Y_i = X(t_i) + \varepsilon_i, i = 1, \dots, n$ (the observed concentrations) (5)

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

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

161 $\theta = (\alpha^{(1)}, \alpha^{(2)}, \beta_0, \beta^{(1)}, \beta^{(2)}, \beta^3, \eta_0, \eta_1, \sigma_A^2, \sigma_\varepsilon^2)$ (6)

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

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

169

170 **2.3.2 Statistical hypotheses**

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

178

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

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

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

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

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

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

190

191 As stated above, there 10 summary variables for each of the five hormones ($10 \times 5 =$
192 50 variables), with each potentially part of each of six hypotheses (**H1 – H6**). How to test
193 multiple hypotheses has always been a difficult statistical question. The one mainstay, though,
194 has always been that hypotheses that were precisely formulated prior to the observation of the
195 data should not require an additional allowance of randomness to account for their prior non-
196 selection. This allowance has historically taken the form of multiple comparisons, Studentized
197 range, Bonferroni adjustments and, most importantly for modern statistics, the False Discovery
198 Rate (FDR). We state this, inasmuch as our four key summary statistics and our two most
199 important hypothesis groups (**H1** and **H2**), for the five hormones, were obvious prior hypotheses.
200 It is not as if they were devised based upon the observed data, and that should therefore be
201 considered in evaluating their P-values (**Figure 4**, described below).

202 3. Results

203 In the present study, eight women were evaluated over 6-10 hr periods with blood sampling
204 every 10-20 min, at four altitudes: base camp (450m), 4800m, 6050m and again at 4800m (on
205 descent) with a period for acclimatization. Note that in the figures, 4800 mA is placed next to 4800
206 m for ease of comparison, even though chronologically realized after 6050 m on the descent. The
207 focus of the present study is on changes in anabolic-catabolic balance under extreme altitude. Five
208 hormones were measured from each blood sampling: GH, Prol, Cortisol, TSH and fT4. Because
209 of the extreme conditions, the length of blood sampling for different individuals at a given altitude
210 and the same individual at different altitudes varied by necessity. Hence, to place summary
211 statistics on comparable scales, measures that involved time were scaled to their corresponding
212 24-hr values (e.g., pulse frequency, and basal and total pulsatile secretion).

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

220 Our two most important hypotheses (**H1-H2**) concern, respectively, acclimatization (at
221 4800m) and the final increase in altitude from 4800m to 6050m. To test these and the other
222 hypotheses, one must recover, from the concentrations, the unobserved hormonal secretions,
223 removing the effects of hormone elimination. In **Figure 2a** the analytically recovered (estimated)
224 time-varying hormone secretion rates are displayed, the result of the statistical deconvolution

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

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

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

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

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

260 For Total Secretion (**Figure 4a**), the most dramatic changes are in the thyroid axis. Total TSH
261 and fT4 secretion increase significantly with altitude, with a return to baseline values (450 m) after
262 acclimatization at 4800 m. On the other hand, GH, Prol and Cortisol's total secretion at 450 m,
263 4800 m and 6050 m are not so different, although values at 4800 mA are reduced. Basal (non-
264 pulsatile) (**Figure 4b**) similarly has an intriguing pattern. Basal secretion rates at baseline 450 m
265 and at 4800 mA are similar, whereas values decrease at altitudes of 4800 m and 6050 m. This
266 occurs concomitantly with a rise in pulsatile secretion [**Figure 7a-b**].

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

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

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

290 **Figure 5b** displays a False Discovery Rate (FDR) plot (six subplots) for each of the six
291 Hypothesis Groups (**H1-H6**) at the $\alpha = .05$ level. In each, the 20 P-values: $20 = 4$ (statistics) \times 5
292 (hormones) are plotted on a log scale with differing symbols and colors. The importance of **Figure**
293 **5b** is the additional reassurance of the statistical significance of the results in **Figure 5a**, described

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

298 Thus, for the test of Acclimatization (**Hypothesis 1**), all hormones except fT4 exhibit a
299 decrease in pulsatile secretion. Prol shows a dramatic decrease in the mode of burst-like
300 secretion (faster release). Both thyroid hormones, TSH and fT4, exhibit increases in basal
301 secretion when altitudes 4800 m, 4800 mA and 6050 m are compared with 450 m (**Hypotheses 3-**
302 **5**). Other variables are different for one altitude comparison, but not for others, and we have not
303 elaborated on those occurrences. In **Figure 5c**, we make the same assessments as in **Figure 5a**,
304 but use a sign-test rather than a t-test. From **Figures 3-4**, one can see that some of the paired
305 difference distributions have occasional values larger than the rest. The sign test will remove any
306 undue influence of such extreme values. The results of significance (i.e., P-values) are quite similar
307 to those of **Figure 5a** (and hence to **Figure 5b**).

308 **Figures 6-7** present the remaining six outcome variables. The format is the same as in **Figures**
309 **3-5**. The six summary statistics are Total Pulsatile Secretion, Fractional Basal of Total Secretion,
310 pulse regularity (a Weibull distribution parameter), mode of secretory release (for the waveform
311 ψ) and, the fast and slow half-lives of elimination. For statistical evaluations of these latter six
312 outcomes variables, **Figure 7a** depicts P-values using a t-test (7 df) over the six hypotheses. For
313 Hypothesis 1 (Acclimatization at 4800 m), both total pulsatile secretion and the fraction of basal to
314 total secretion are highly significant ($P < 10^{-3}$) for GH, Prol, Cort and TSH, with fT4 being just
315 above the $P = .05$ level. In **Figure 7b**, a FDR plot is given for each of the six Hypothesis Groups
316 (**H1-H6**) at the $\alpha = .05$ 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

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

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

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

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

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

394 These findings also raise the question as to whether the hormonal changes are linked to the
395 cardiorespiratory and metabolic changes which can be found in hypobaric hypoxia (18). In a
396 previous paper (27) it was demonstrated that total hormone concentrations are closely linked with
397 altitude but not with parameters of oxygen transportation such as O₂ saturation and pO₂ values. It
398 was therefore concluded that there must be other factors such as disturbed sleep or physical stress,
399 which induce these changes. It was hypothesized that the physiological changes at high altitude
400 might possibly be influenced or might even be partly under control of the endocrine system. This
401 present study does verify this hypothesis due to both the modeling at the level of secretion (and
402 not concentrations), as well utilizing the study design for pairing. Moreover, the present results
403 support and extend the findings of (27) that the concentrations of several hormones, especially
404 stress related hormones such as cortisol, prolactin and thyroid hormone, change with altitude and
405 that acclimatization almost normalizes these changes. A side consequence of the present study
406 reveals that not only the serum hormone concentrations changes with altitude and normalizes with
407 acclimatization (as shown in (27)) but also the hormone pulse frequency and basal and pulsatile
408 secretion patterns.

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

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

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432 **Figure Legends**

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

438

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

446

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

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

460
461 **Figure 4.** In **Figures 4a-b** are plotted the two summary statistics: total secretion (**4a**) and basal
462 secretion (**4b**). *The legend format is the same as in Figure 3*, with two columns in each subplot.
463 That is, in the left column are the summary statistic values for the eight subjects (black asterisk) at
464 each of the four altitudes, for the five hormones (GH, PROL, Cortisol, TSH, FT4). The means
465 (solid line) and medians (dashed line) at each altitude, for each hormone, are linearly connected.
466 In the right column, the differences in the statistic, for each subject, for each hypothesis are
467 calculated and plotted as a function of **Hypotheses 1-6**.

468
469 **Figure 5. 5a.** For Hypothesis 1-6, and each of the four summary statistics in **Figures 3-4**: mass
470 per pulse (MPP), pulse frequency, total secretion and fraction of basal to total secretion, a t-test
471 was performed and a P-value was calculated for each null hypothesis that there is no change due
472 to the difference in the two altitudes (e.g., between 4800 m and 450 m). The P-value is calculated
473 to each side and the minimum of the two values is the resulting plotted P-value. If one wishes to
474 consider two-sided P-values, one would just double the plotted value. What are plotted are the log
475 P-values versus Hypotheses 1-6, for the five hormones (designated by differing colors) with the
476 log P-values linearly connected across the hypotheses. A dashed box is drawn around the results
477 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 ($20=4 \times 5$), 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 $\alpha=.05$), 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 in 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 emphasize 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

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

506

507 **Figure 7. 7a.** For Hypothesis 1-6, and each of the six summary statistics in **Figure 6**: total pulsatile
508 secretion, fraction of basal to total secretion, pulse regularity, mode of release, fast half-life and
509 slow half-life, a t-test was performed and a P-value was calculated for each null hypothesis. In
510 Hypotheses 1-6, the null hypothesis in each, is that there is no change due to the difference in the
511 two altitudes (e.g., between 4800 m and 450 m). As in **Figure 5**, the P-value is calculated to each
512 side (under the null hypothesis) and the minimum of the two values is the resulting plotted P-value.
513 What are plotted are the log P-values versus Hypotheses 1-6, for the five hormones (designated by
514 differing colors) with the log P-values linearly connected across the hypotheses. A dashed box is
515 drawn around the results for Hypotheses 1-2 to emphasis that they were a priori formulated-
516 hypotheses of particular importance. A dashed horizontal line is plotted at log (.05). For
517 Hypotheses 1-2, which were a priori formulated for the five hormones, one could (potentially)
518 argue that comparisons to log (.05) is justified. This argument is not as strong however as that for
519 the four summary statistics in **Figure 45 7b.** For each hypothesis (1-6), there are 5 hormones and
520 6 summary statistics (30=6 x 5), and one can consider the multiple comparisons effect on the log
521 P-values in **Figure 7a.** A False Discovery Rate (FDR) plot is presented (at $\alpha = .05$). There are
522 six symbols (square, triangle, x, hexagon, circle, asterisk) and five colors for the hormones (Red,
523 Blue, Black, Green, Cyan). The dashed curve is for the correlated case and the solid for the

524 uncorrelated case, which is appropriate. **Figure 7b** is viewed as supportive evidence for the results
525 enclosed in the dashed boxes in **Figure 7a. 7c**. As alternative evidence to that Presented in **Figure**
526 **7a**, where a t-test was performed, a sign test is now performed and the resulting log P-values are
527 plotted. The results are very similar to those of **Figure 7a**.

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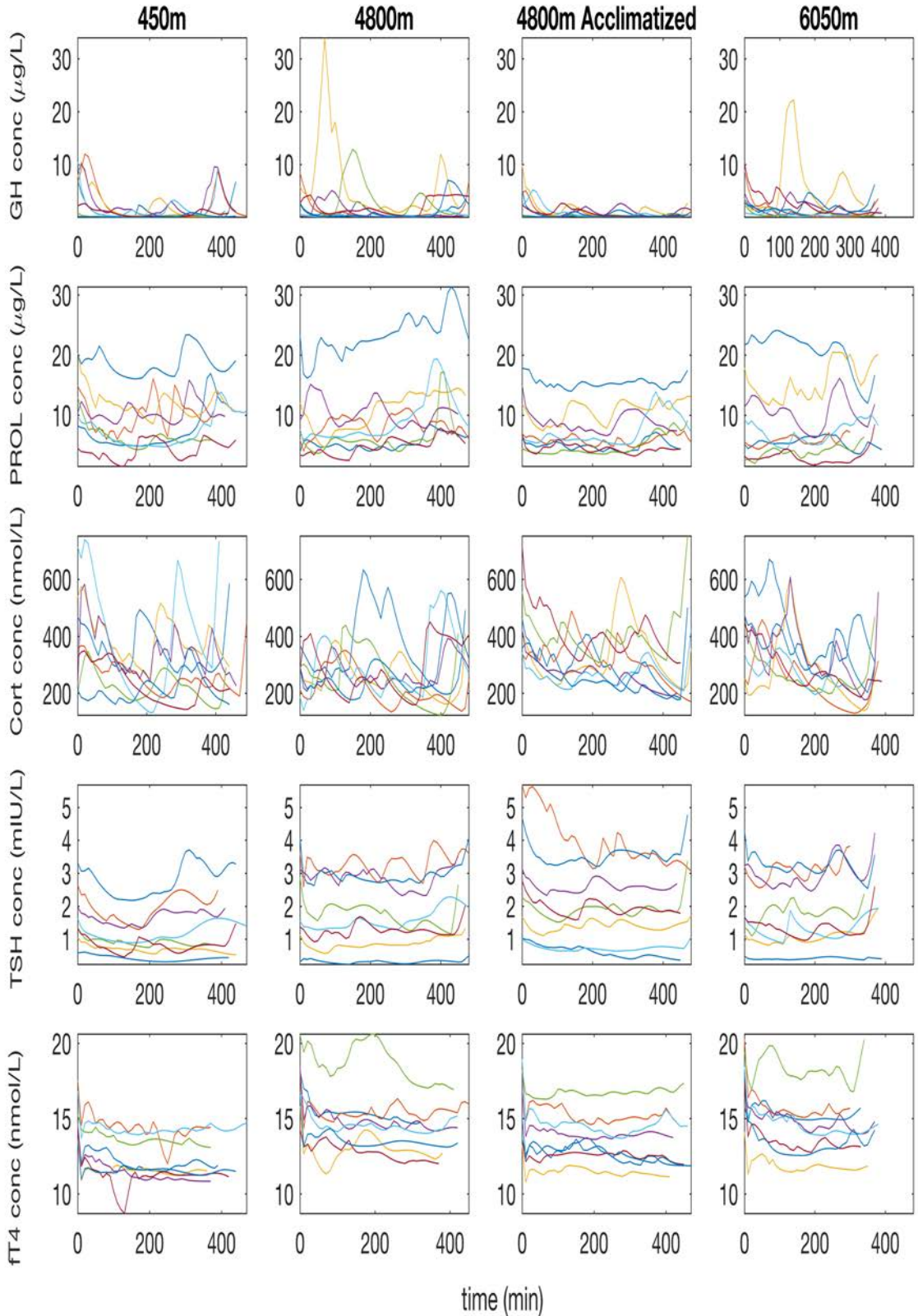
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Concentrations (Rows): GH, PROL, Cortisol, TSH and fT4, Altitudes (Columns)

Fig 1



Recovered Secretion Rates (Rows): GH, PROL, Cortisol, TSH and ft4, Altitudes (Columns)

a.

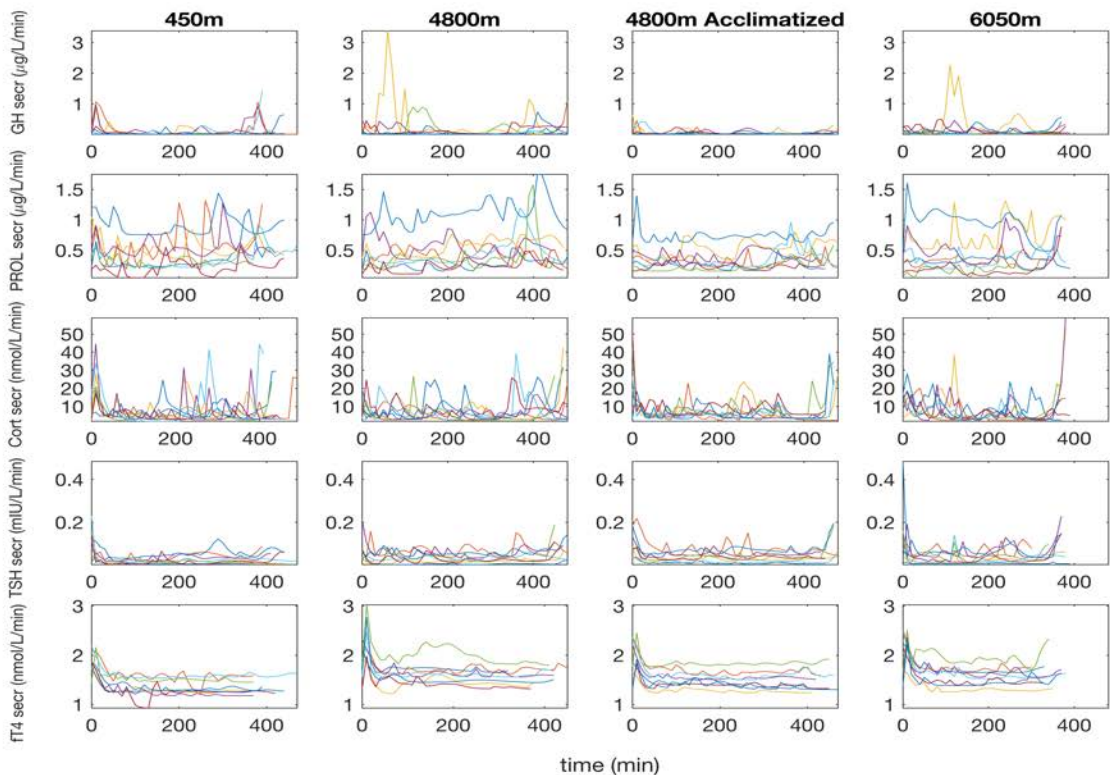
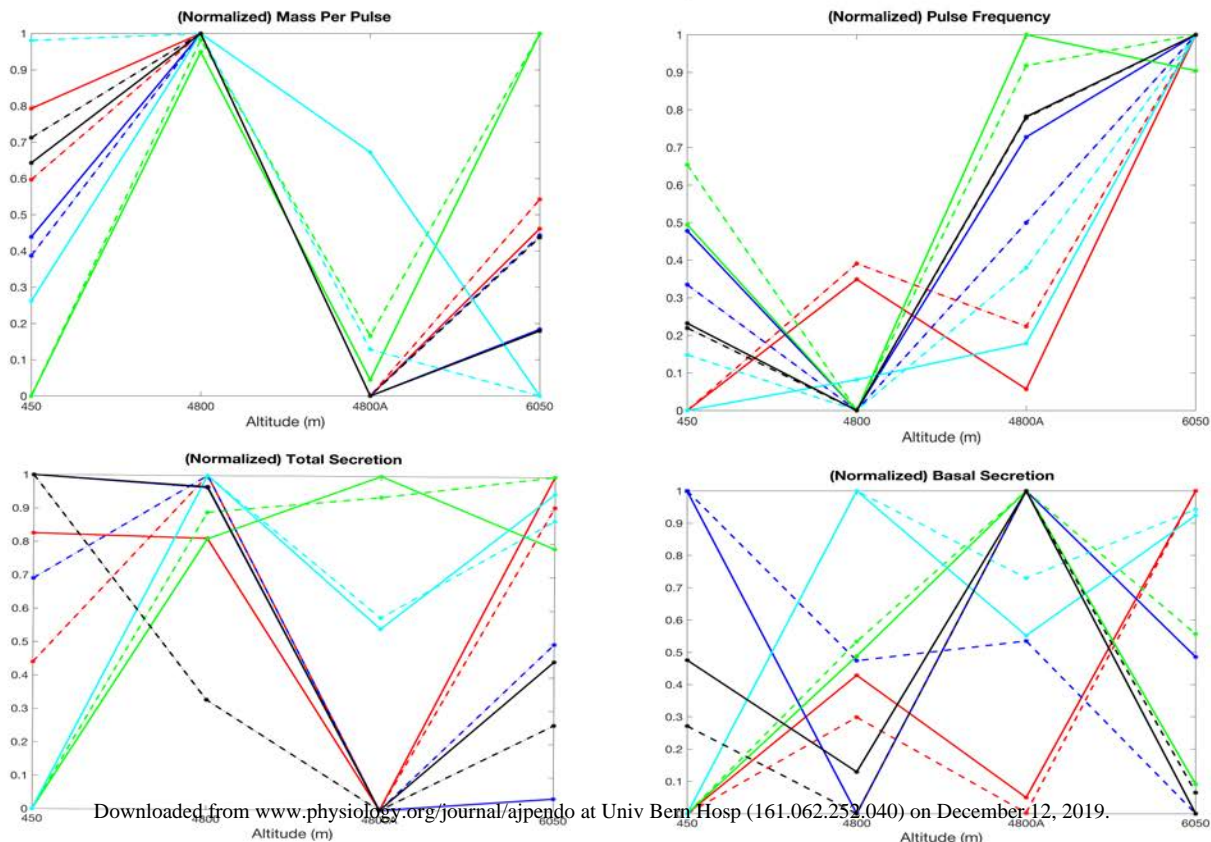


Fig2

b.

General Secretory Patterns in Response to Altitude, Across the Hormones - GH (Red), PROL (Blue), Cortisol (Black), TSH (Green), ft4 (Cyan) Median (Solid), Mean (Dashed)

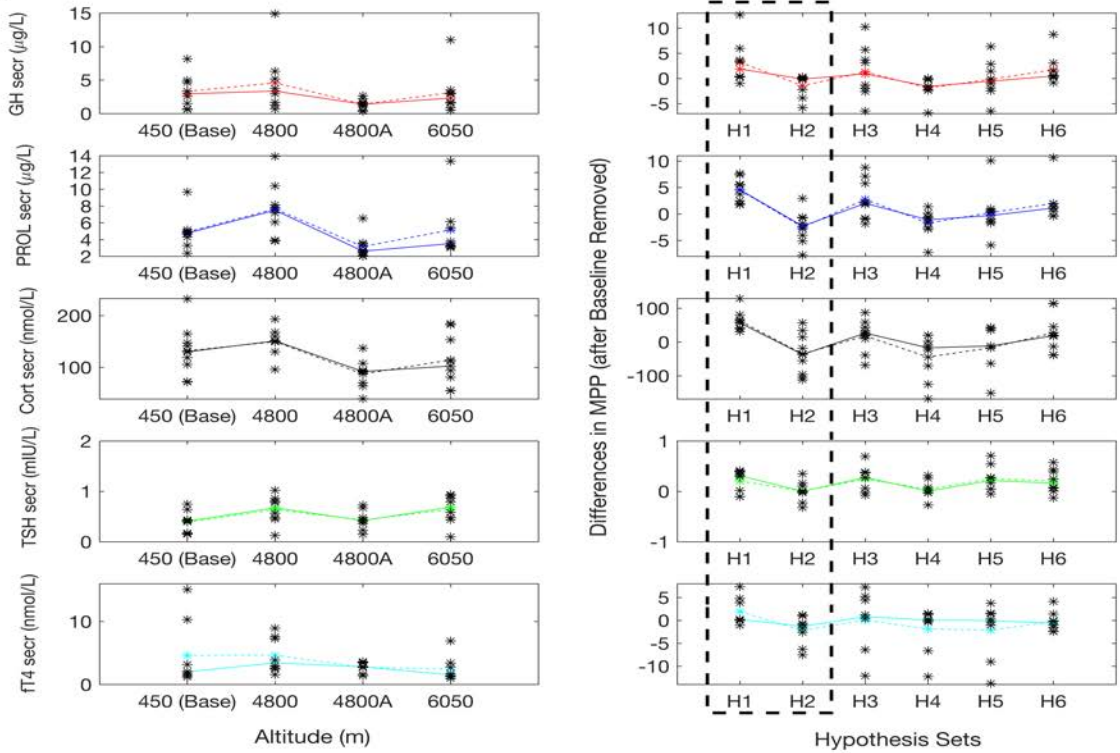


Key Summary Calculations 1-2: MPP, Pulse Freq - Median (Solid), Mean (Dashed) (H1, H2 - Dashed Box)

Fig 3

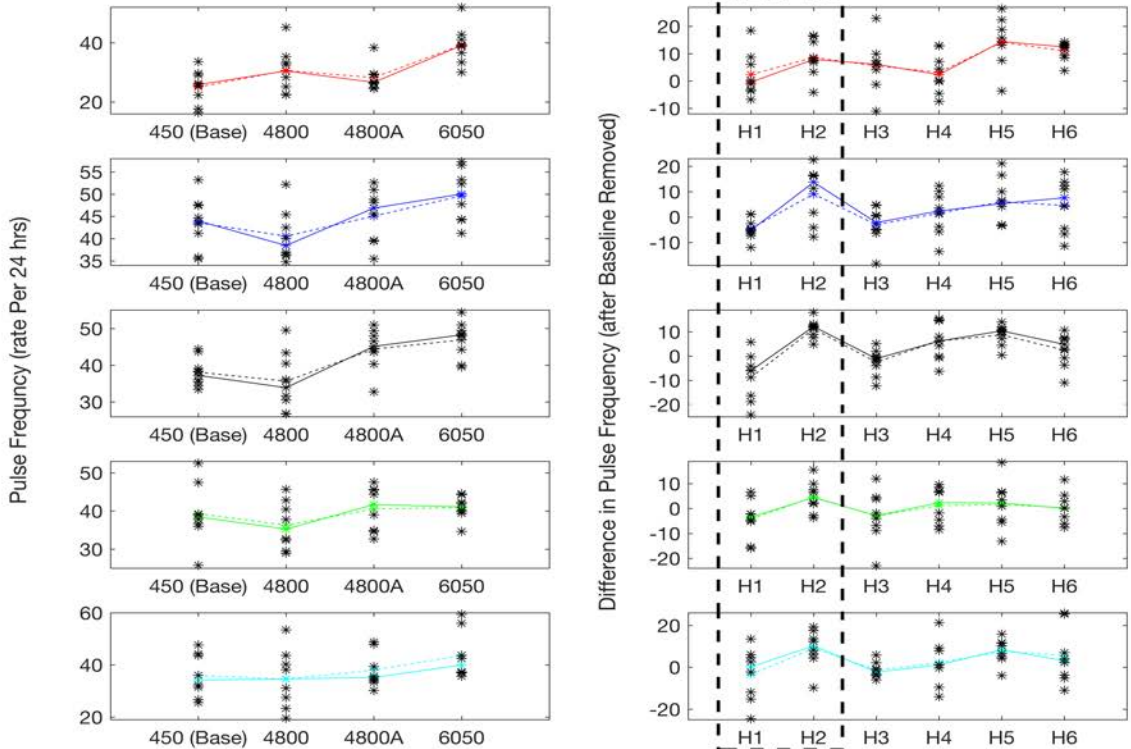
a.

Mass Per Pulse (MPP): GH, PROL, Cort, TSH, ft4 (Rows)

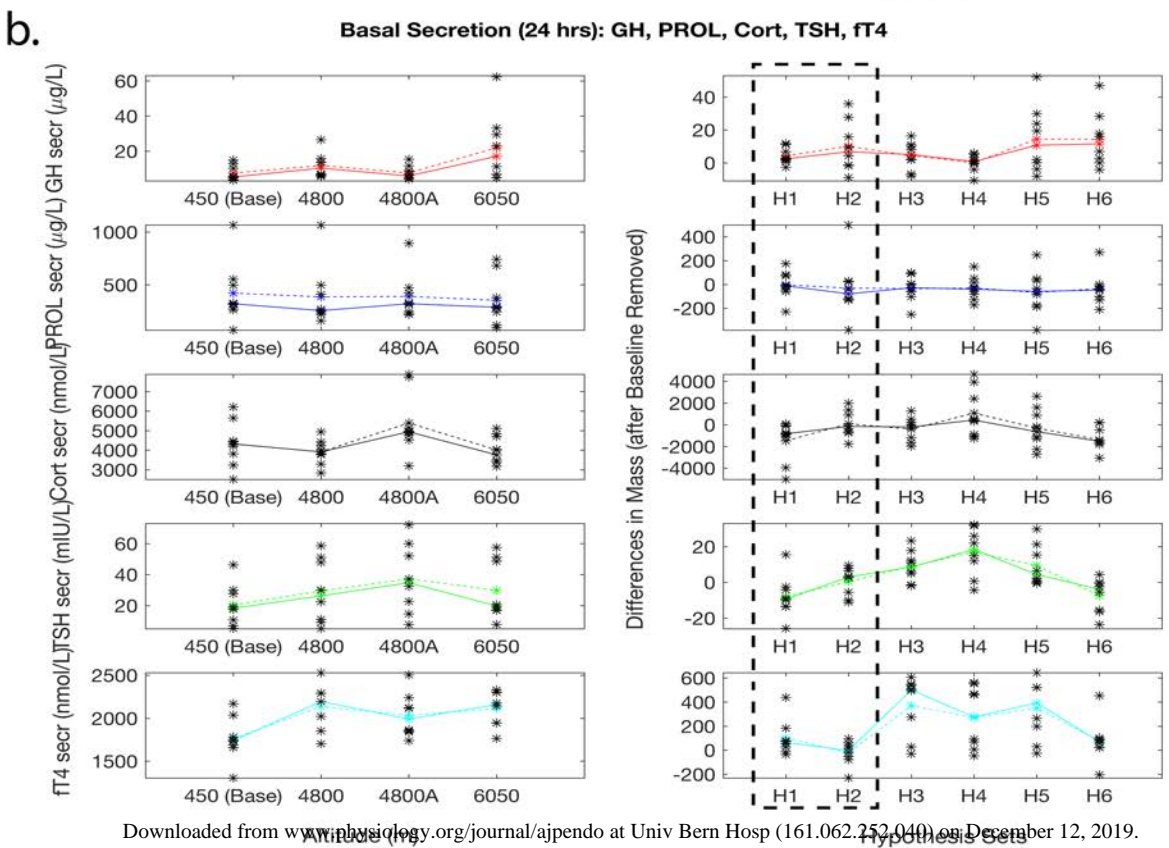
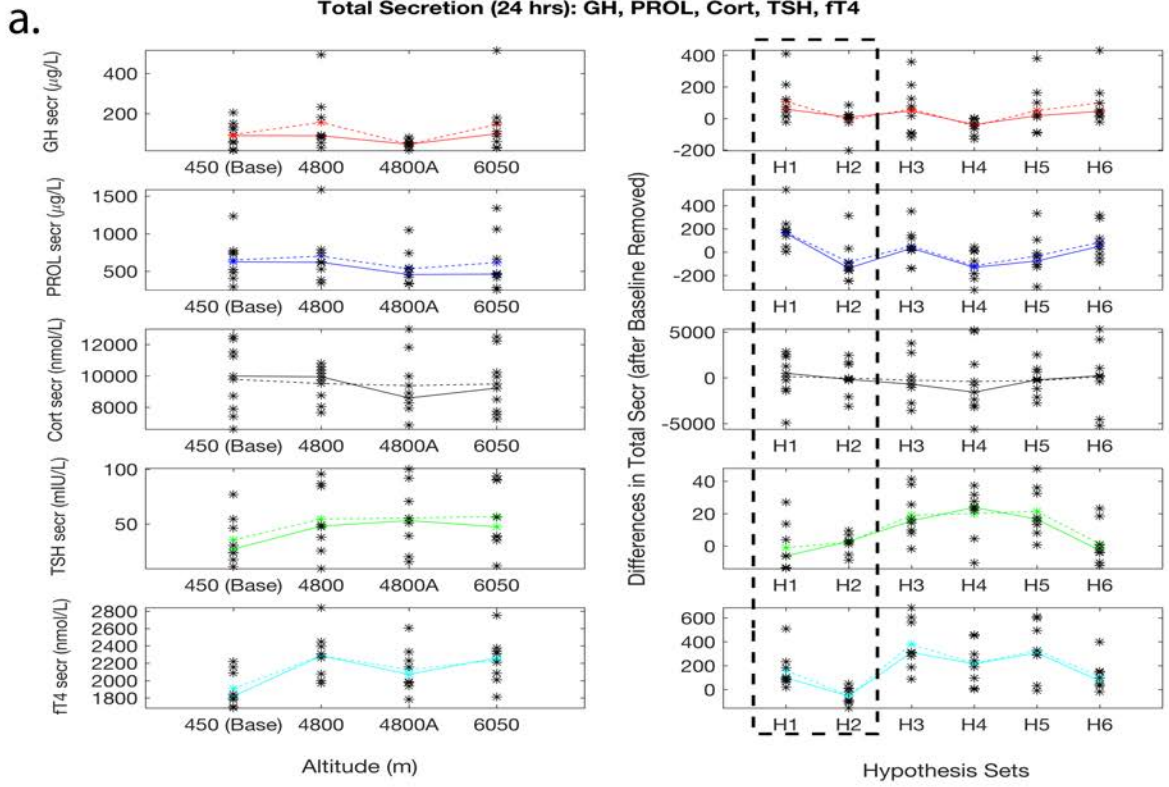


b.

Pulse Frequency (24 hrs): GH, PROL, Cort, TSH, ft4

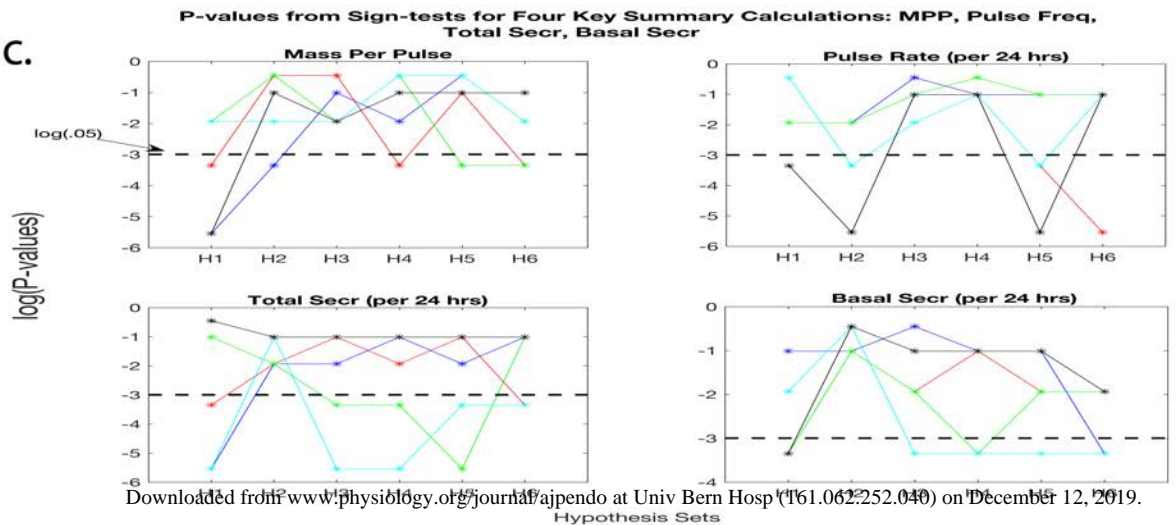
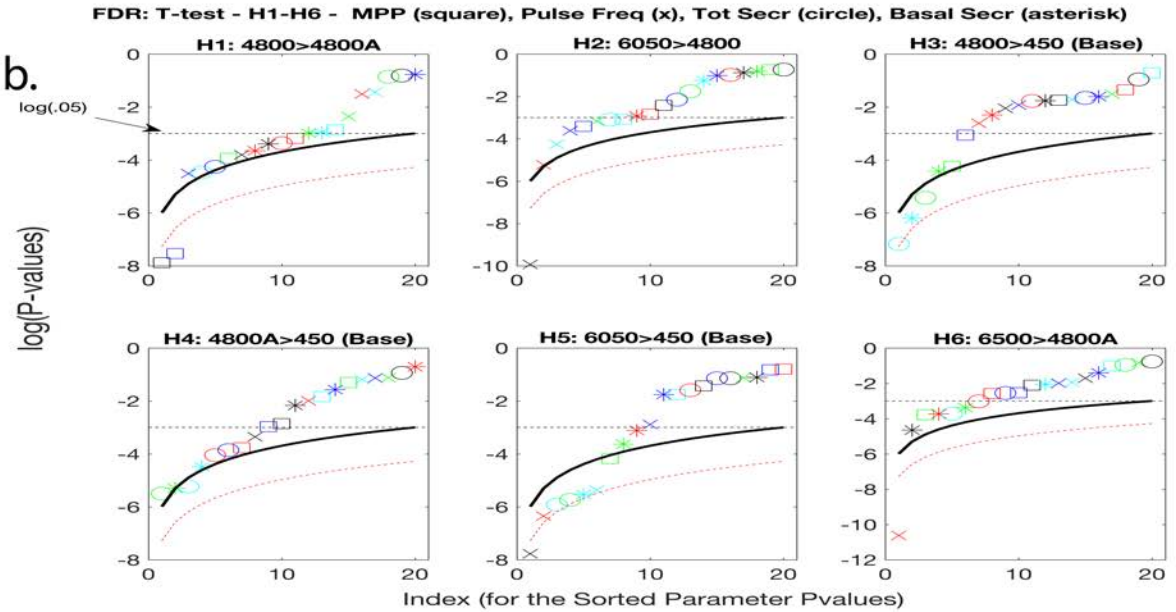
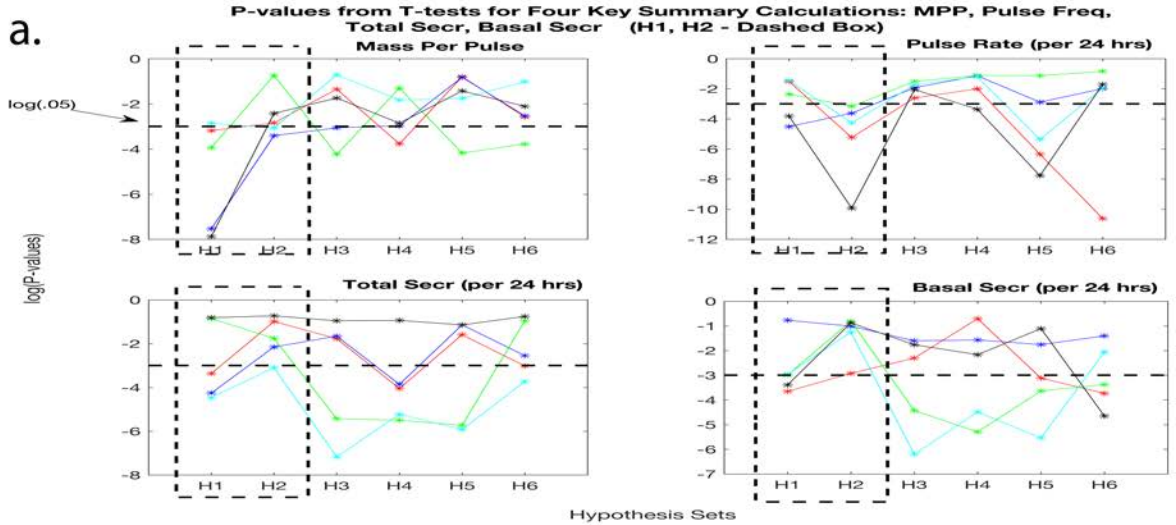


Key Summary Calculations 3-4: Total Secr, Basal Secr - Median (Solid), Mean (Dashed) (H1, H2 - Dashed Box) Fig 4



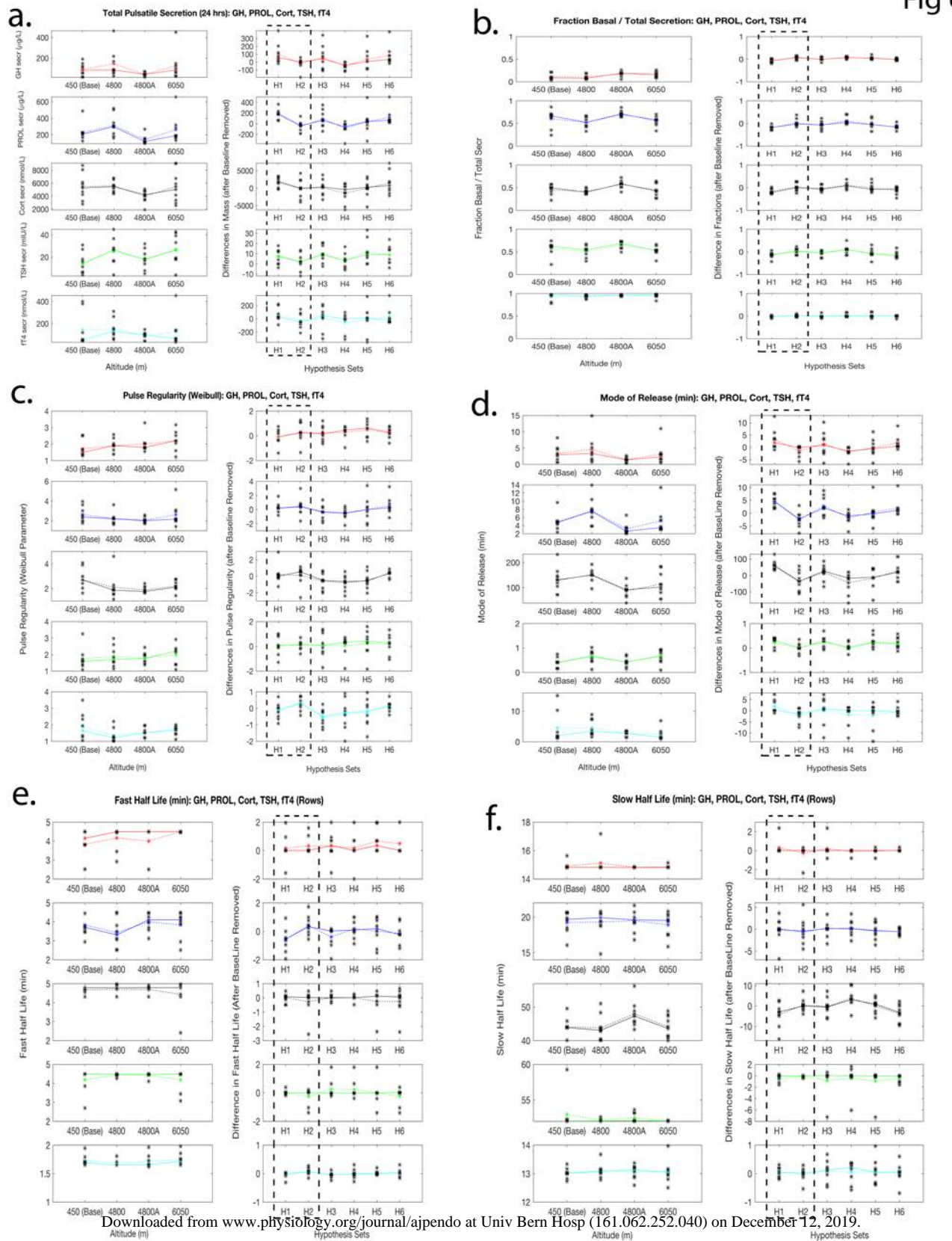
GH (Red), PROL (Blue), Cort (Black), TSH (Green), fT4 (Cyan)

Fig 5



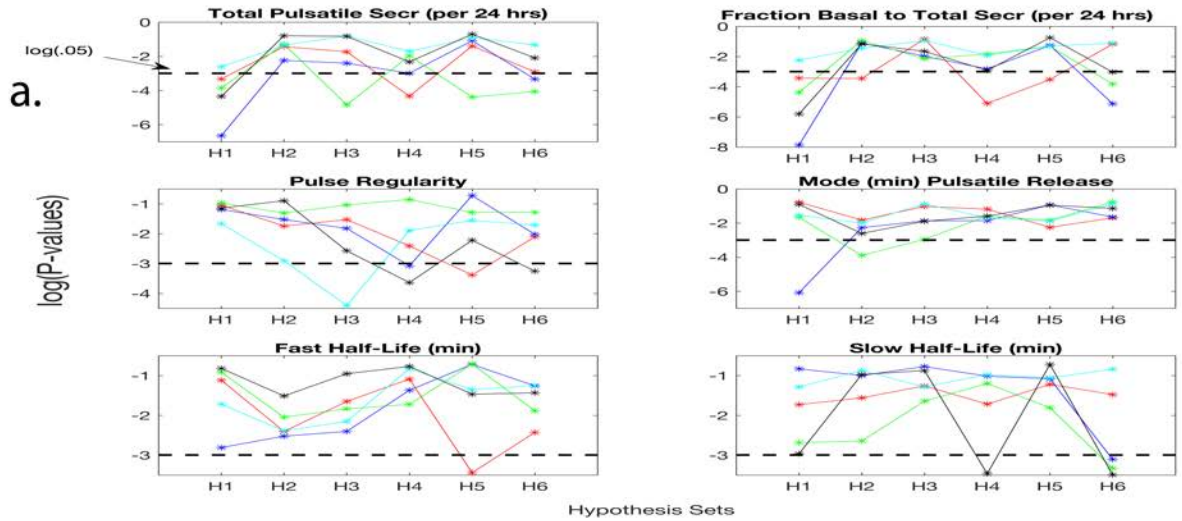
Six Additional Summary Calculations: Total Pul, Frac Basal/Tot Secr, Pulse Regularity, Mode of Release, Halfives - Median (Solid), Mean (Dashed) (H1, H2 - Dashed Box)

Fig 6

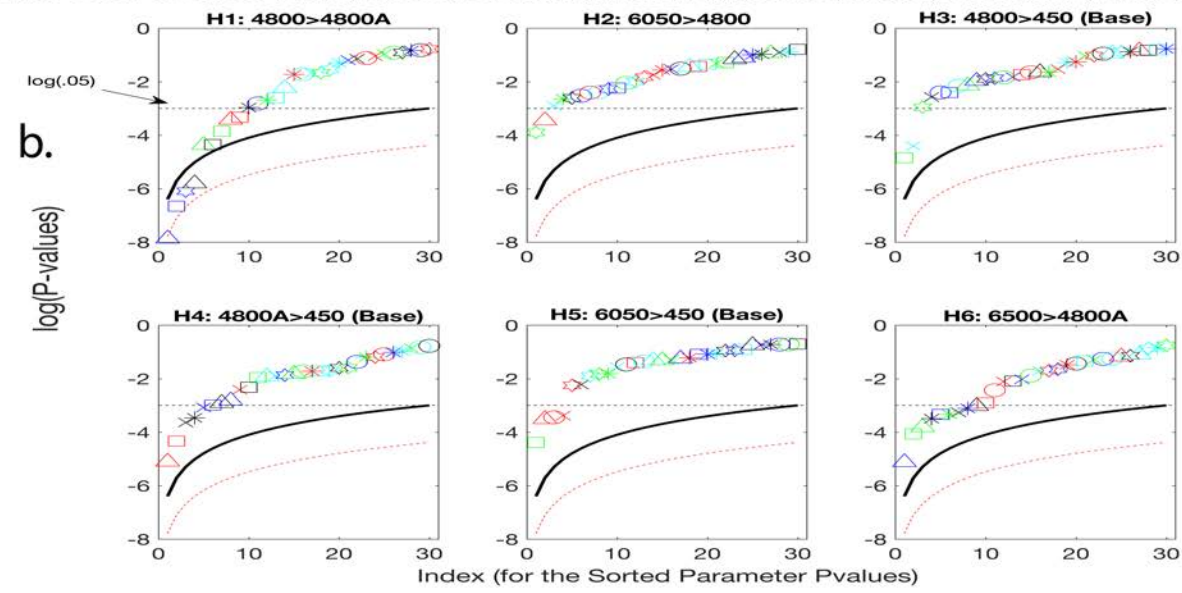


GH(Red), PROL(Blue), Cort(Black), TSH(Green), ft4(Cyan) Fig 7

P-values, T-tests for Six Additional Summary Calculations: Total Pul Secr, FracBasal/Total Secr, Pulse Regularity, Mode of Release, Halfives



FDR: T-test-H1-H-TPulSecr (square), Frac Bas/TSecr (triangle), PulRgrl (x), Mode Rel (hexagon), FastHL (circle), SlowHL (asterisk)



P-values, Sign-tests, Six Additional Summary Calculations: Total Pul Secr, Frac Basal/Total Secr, Pulse Regularity, Mode of Release, Halfives

