

1 **Somatostatin agonist pasireotide promotes a physiological state resembling short-day**
2 **acclimation in the photoperiodic male Siberian hamster (*Phodopus sungorus*)**

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23 **Abstract**

24 The timing of growth in seasonal mammals is inextricably linked to food availability. This is
25 exemplified in the Siberian hamster (*Phodopus sungorus*) which uses the annual cycle of
26 photoperiod to optimally programme energy expenditure in anticipation of seasonal
27 fluctuations in food resources. During the autumn, energy expenditure is progressively
28 minimized by physiological adaptations including a 30% reduction in body mass, comprising
29 of a reduction in both fat and lean tissues. However, the mechanistic basis of this adaptation
30 is still unexplained. We hypothesised that growth hormone (GH) was a likely candidate to
31 underpin these reversible changes in body mass.

32 Administration of pasireotide, a long acting somatostatin receptor agonist developed for the
33 treatment of acromegaly, to male hamsters in long-day photoperiod (LD) produced a body
34 weight loss. This comprised of a reduction in lean and fat mass, including kidneys, testes, and
35 brown adipose tissue typically found in short-day (SD) housed hamsters. Furthermore, when
36 administered to hamsters switched from SD to LD pasireotide retarded the body weight
37 increase compared to vehicle treated hamsters. Pasireotide did not alter photoperiod-mediated
38 changes in hypothalamic energy balance gene expression, but altered expression of *Srif*
39 mRNA expression in the periventricular nucleus and *Ghrh* mRNA expression in the arcuate
40 nucleus consistent with a reduction in GH feedback and concurrent with reduced serum IGF-
41 1. Conversely, GH treatment of SD hamsters increased body mass which included increased
42 mass of liver and kidneys. Together these data point to a role for the GH axis in the
43 determination of seasonal body mass of the Siberian hamster.

44 **Introduction**

45 Maintenance of endothermy during winter months is a physiological challenge for seasonal
46 mammals as food resources are diminished when energy requirements are increased by
47 seasonal environmental changes. Consequently many seasonal mammals have adapted
48 physiology and behaviours to minimize energy expenditure including a cessation of
49 reproduction and slowing of growth, but some small mammals can actively reduce body
50 mass, a change comprised of a reduction in lean, fat, organ and skeletal masses (Dehnel's
51 phenomenon) (1, 2). In temperate climates photoperiod is the predominant seasonal cue that
52 anticipates the forthcoming food and climatic environment. The Siberian hamster is an
53 exemplar of a photoperiodic mammal using seasonal changes in photoperiod to anticipate
54 food availability (3-5). As autumn approaches, the Siberian hamster reduces body mass
55 which includes a reduction in the mass of internal organs such as the kidney, liver, adrenal
56 gland, white adipose tissue, brown adipose tissue, an involution of the reproductive organs
57 and a reduction in skeletal mass may also occur (6, 7). The winter reduction in body mass of
58 the Siberian hamster is initiated by a shortening of day length following the summer solstice
59 and can be simulated in the laboratory by switching hamsters from long day length (LD) to
60 short day length (SD). Conversely during spring the body mass of the Siberian hamster
61 increases (8), reversing the reductions of fat mass and lean mass and can be enabled in the
62 laboratory by switching hamsters from SD to LD (9, 10).

63 The seasonal physiological adaptations are largely determined by a central mechanism
64 governed by thyroid hormone (T3) availability to the hypothalamus (11), which in turn
65 regulates a cohort of genes which contribute to physiological adaptations appropriate for the
66 season (9, 12-15). Conceptually, the change in body mass during the transition from winter to
67 summer (SD to LD) could be considered as a growth response. This is supported by evidence
68 from studies on growth in red deer showing a correlation between growth hormone (GH),

69 IGF-1 and seasonal growth (16, 17). Furthermore, mean levels of circulating growth hormone
70 over a 24h period correlate with photoperiod appropriate body weight in the seasonal rodent,
71 the golden hamster (*Mesocricetus auratus*, 18), which might point to a general role for the
72 GH axis in the regulation of seasonal body size. We therefore hypothesised that hypothalamic
73 neuroendocrine regulation of GH would be involved in the seasonal body mass cycle of the
74 Siberian hamster. GH is produced in the pituitary with secretion promoted by growth
75 hormone releasing hormone (GHRH) from neurons in the arcuate nucleus (ARC) and
76 inhibited by somatostatin (referred to as somatotropin release-inhibiting factor - SRIF)
77 produced by neurons of the periventricular nucleus (PeVN). Circulating GH concentration is
78 regulated by a negative feedback pathway to the hypothalamus, acting to suppress GHRH
79 stimulated synthesis and secretion. As GH falls, GHRH secretion increases and SRIF
80 decreases. Through this feedback mechanism, GH is released in a pulsatile manner (19-21).
81 Interestingly, in the Siberian hamster we and others have shown that *Srif* expressed in the
82 ARC is strongly influenced by photoperiod and downstream of central thyroid hormone
83 balance (10, 14, 15), and so may play a role in the seasonal regulation of the GH axis and
84 thus seasonal body mass.

85 Pasireotide is a somatostatin analogue developed for the treatment of acromegaly, which
86 target somatostatin receptors (SSTRs) at the pituitary to reduce GH secretion (22, 23, 24).
87 Pasireotide has a high affinity for four of the five somatostatin receptor subtypes with highest
88 affinity for SSTR₅ (SSTR₅>₂>₃>₁, 39-fold greater than octreotide, 23). We have utilized
89 pasireotide to suppress pituitary secretion of GH and assessed the effect on seasonal body
90 mass and photoperiod regulated hypothalamic gene expression that underpins the
91 physiological responses of the Siberian hamster. Our data show that GH is a likely proponent
92 of seasonal body mass regulation, but also provide evidence that somatostatin may play a
93 broad role in the seasonal regulation of the hypothalamic neuroendocrine axis.

94 **Materials and Methods**

95 *Animals*

96 Animal husbandry and all experiments were in accordance with the German Animal Welfare
97 Act and approved by the Lower Saxony State Office for Consumer Protection and Food
98 Safety (ref: 13/1246, 14/1453). Adult male Siberian hamsters were taken from a colony
99 maintained at the University of Veterinary Medicine, Hannover, having been bred under
100 natural photoperiod (at 52°N latitude) and ambient temperatures before the summer solstice.
101 Hamsters were then transferred to artificial long days (LD: 16:8h light:dark; lights on: 04:00-
102 20:00 CET; 20-22°C) after weaning and were a minimum of 3 months old at the start of
103 experiments. Food (hamster breeding diet, Altromin 7014, Lage, Germany) and water were
104 available ad libitum, supplemented weekly by a slice of apple. Overhead lighting was
105 provided by fluorescent tubes (Lumilux LF11, Osram, Germany) resulting in a light intensity
106 of ca. 200–350 Lux at cage level. During the dark phase, illumination was limited to dim red
107 light of <5 Lux (Osram, Darkroom red, 15 W). Experimental hamsters were singly housed
108 indoors throughout all experiments. Hamsters were sacrificed by overdose of CO₂ followed
109 by cervical dislocation. The brain and trunk blood, from which serum was isolated, were
110 collected and stored at -70°C before use. Organs, epididymal white adipose tissue (EWAT)
111 and, where applicable, interscapular brown adipose tissue (IBAT) were dissected out and
112 immediately weighed; testes, kidneys and EWAT were returned to carcasses which were
113 stored at -70°C and later thawed for body composition analysis by MRI (EchoMRI Whole
114 Body Composition Analyser, Echo Medical Systems, Houston, Texas).

115

116 *LD Experiment*

117 Sixteen age and weight matched hamsters (3-4 months of age) were maintained in LD, 8
118 hamsters received subcutaneously a dose of long acting release (LAR) pasireotide (Novartis,
119 Basel Switzerland; 160mg/kg based on weight on day of administration) and 8 hamsters
120 received vehicle (25) only (8 hamsters per day; 4 from each group per day). Twenty-eight
121 days following the first injection, pasireotide was re-administered and hamsters were
122 sacrificed at 49 days following first administration.

123 *Switchback Experiment*

124 Thirty-two Siberian hamsters were acclimated to short day photoperiod (SD; 8:16h
125 light:dark; lights on: 09:00-17:00 CET; $20 \pm 1^\circ\text{C}$) for a period of 10 weeks (69-72 days). One
126 hamster did not respond to SD and was excluded from the experiment. The hamsters were
127 divided into four weight matched groups (three groups of 8 and one group of 7). Two groups
128 (n=8 per group) received a subcutaneous dose of LAR pasireotide (160mg/kg based on
129 weight on day of administration) or received vehicle only (1 group n=8 and 1 group n= 7).
130 Injections were carried out over the course of 4 days (8 hamsters per day; 2 from each group
131 per day) to accommodate consistent killing and retrieval of tissues between 4-5h after lights
132 off over a 4 day period. One group of hamsters which had received vehicle (SD-vehicle n=7)
133 and one group which had received pasireotide (SD-pasireotide, n=8) remained in SD. The
134 remaining vehicle treated (SWB-vehicle, n=8) and pasireotide treated (SWB-pasireotide,
135 n=8) groups were returned to LD (16:8h light dark). Twenty-eight days following the first
136 injection, hamsters were re-administered pasireotide or vehicle as appropriate. One SWB-
137 pasireotide hamster had one testis and epididymal fat pad removed due to exposure outside
138 the body cavity during radiotelemetry implant surgery (data to be reported separately), thus
139 reducing the sample size to n=7 for EWAT and paired testes mass analyses. Hamsters were
140 sacrificed at 49 days following first administration.

141

142 *GH Experiment*

143 Siberian hamsters that had been held in SD for 12 weeks were either switched back to LD
144 (n=6) or administered bovine GH ip. (1mg/kg bovine growth hormone – NIDDK, Bethesda,
145 MD, USA, catalogue no: AFP-1032SC; in phosphate buffered saline (PBS) or PBS alone in
146 200µl doses in the final hour of the light phase, daily for 28d (n=12 per group). GH was
147 administered at the end of the light phase to extend a possible circadian elevation in GH over
148 the dark phase, in order to increase the duration of proposed elevated GH which has been
149 shown to exist in other rodents (18) and be important to interpretation of the GH in LD
150 Siberian hamsters. A stock preparation of GH was made at 1 mg/ml in PBS under aseptic
151 conditions, and stored at -20°C in 250µl aliquots. The stock solution was diluted daily at 1
152 µl/g hamster body mass made up to 200µl with PBS just prior to use.

153

154 *Serum glucose and hormone determination*

155 Serum glucose was measured with a glucose monitor and test strips (Accu-Chek, Roche) in
156 duplicate. Serum IGF-1 (established as a biomarker reflecting circulating GH levels, 26) and
157 insulin were determined by ELISA (Mercodia, Uppsala, Sweden and R&D Systems, MN,
158 USA, respectively), following kit instructions for mouse samples. For the insulin ELISA,
159 samples were diluted 2-fold in sample buffer.

160

161 *Riboprobe synthesis*

162 Riboprobes complementary to gene fragments were generated as previously described (11,
163 27-29). To ascertain possible effects on the sensitivity of the GH-axis by pasireotide

164 treatment, additional riboprobes were synthesised for growth hormone receptor (*Gh-r*) and
165 growth hormone releasing hormone (*Ghrh*); For *Gh-r*; primers were F: 5'-
166 AAGTGCCKGTGAGATCCAGACAAC-3' and R: 5'-
167 GGTACGYCCAGAATCRTCATCCT-3', based on a consensus between mouse and rat
168 sequences (mouse Genbank NM_010284 bases 1066-1089 forward primer and bases 1534-
169 1556 reverse primer). For *Ghrh*, primers were based on the partial sequence of *Mesocricetus*
170 *auratus* (golden hamster, Genbank NM_001281590) 1. F: 5'-
171 GATGCCACTCTGGGTGTTCTTTG-3' (bases 26-48), R: 5'-
172 ATCAGGATGGGGGTTTTATTGTAT-3' (bases 464-441). These primers amplified several
173 products and therefore a secondary amplification was performed with the original forward
174 primer and a reverse primer located 3 nucleotides within the target sequence R: 5'-
175 AGGATGGGGGTTTTATTGTATTTA-3' (bases 461-438). This resulted in the
176 amplification of a single product of the correct sequence. Templates for riboprobe synthesis
177 were generated as described previously (10)

178 *In situ hybridisation*

179 Frozen coronal hypothalamic sections were cut at 14µm and mounted on poly-L-lysine
180 coated slides (ThermoScientific, Rockford, IL, USA). Radiolabelled *in situ* hybridisation was
181 carried out as previously described (10). Due to poor tissue preservation ARC region SWB-
182 vehicle sample size was reduced to n=7 and in the *pomc* experiment SWB-Pasireotide sample
183 size was reduced to n=7.

184 *Autoradiographic image analysis*

185 Exposed autoradiographic films were scanned at 600 dpi on an Epson scanner linked to a
186 computer running Image-Pro PLUS analysis software (v4.1.0.0, Media Cybernetics,
187 Wokingham, USA). Integrated optical density was obtained by reference to a ¹⁴C microscale

188 and was determined for three to five sections per slide for each probe, depending on the gene
189 considered, and the accumulated count taken. Relative integrated optical density (IOD), an
190 indirect measure of mRNA expression, was expressed relative to the control group (typically
191 LD-Veh or SD-Veh unless otherwise stated) whose value was defined as 1, and statistical
192 analysis was carried out on raw IOD values.

193 *Statistical Analysis*

194 All data are expressed as mean \pm SEM. Statistical analyses were carried out using Minitab
195 v.15.0 (Minitab, PA, USA) unless stated otherwise. Data were compared by t-test, assuming
196 equal variances, one-way or two-way ANOVA as appropriate, with Tukey *post hoc*
197 comparisons. Where data did not conform to the assumptions of the test, it was transformed
198 by log₁₀ or square root, and statistics performed on transformed data (LD experiment: log₁₀:
199 serum insulin; SWB experiment: log₁₀: serum IGF-1, kidney mass, serum glucose, ARC *Gh-*
200 *r*, ARC *Srif*; square root: ARC *Ghrh*, ARC *Npy*; GH experiment: log₁₀: IBAT of 28 day GH
201 treated hamsters). When data could not be transformed to fit assumptions of the parametric
202 test, Kruskal-Wallis (KW) and Mann-Whitney (MW) tests were performed as appropriate
203 (SWB experiment: paired testes mass, *Dio3* and ARC *Gh-r* mRNA expression; GH
204 experiment: paired testes mass). P-values less than 0.05 were considered statistically
205 significant. When insulin was measured in the SWB experiment, some samples exceeded the
206 limit of the assay and so the data were bound from above, with only the upper limit of the
207 assay being observed rather than the actual value. Therefore a Cox regression was carried out
208 with Bonferroni correction for multiple testing using the statistical software R and p<0.05
209 after this correction was considered statistically significant.

210

211 **Results**

212 **Short-day-like weight loss in long-day hamsters is induced by somatostatin agonists**

213 To test our hypothesis that the adaptation of SD body mass is a growth related phenomenon,
214 pasireotide a long-acting somatostatin analogue was administered subcutaneously to hamsters
215 held in long-day photoperiod (LD) in a 28d slow release particle formula releasing over a
216 period of 7 weeks. In hamsters treated with pasireotide there was a progressive loss of body
217 mass ($15.0 \pm 3.2\%$, $P < 0.001$ vs vehicle; Fig 1A). The weight loss trajectory in these LD
218 hamsters was similar to the weight loss trajectory experienced by hamsters transferred from
219 LD to SD (9,11). Weight loss induced by pasireotide was accompanied by a reduction in lean
220 mass ($P = 0.020$) and a trend for fat mass reduction ($P = 0.091$) (Fig 1B,C). Analysis of serum
221 parameters showed pasireotide decreased circulating insulin-like growth factor-1 (IGF-1;
222 $P = 0.022$, Fig 1D), increased blood glucose concentration ($p = 0.009$, Fig 1F), but had no effect
223 on serum insulin concentration (Fig 1E). In comparison to vehicle treated hamsters, weight
224 loss by pasireotide was manifest in tissue mass reductions which included a decrease in
225 kidney mass ($P = 0.004$), epididymal white adipose tissue mass (EWAT, $P = 0.010$),
226 interscapular brown adipose tissue mass (IBAT, $P = 0.006$) and paired testes mass ($74.7 \pm 5.6\%$,
227 $P < 0.001$), but liver mass was unchanged (table 1).

228 **Hypothalamic gene expression**

229 At the level of the hypothalamus, there was no effect of pasireotide on mRNA expression of
230 *Dio2*, *Srif*, *Pomc*, and *Npy* expression the ARC or GH-r in either the ARC or PVN (Fig 2A,B;
231 Supplementary Fig 1A-D). However, *Ghrh* expression in the ARC was increased by
232 2.10 ± 0.22 fold by pasireotide treatment ($p < 0.001$) while *Srif* expression the PeVN was

233 decreased to approximately one-fifth of vehicle expression (0.22 ± 0.07 fold, $p<0.001$, Figure
234 2C, D).

235 **Pasireotide impairs photoperiod induced weight gain**

236 Hamsters previously acclimated to SD for 10 weeks with a loss of 21% body mass, were
237 administered pasireotide or vehicle and then switched back (SWB) to LD for 7 weeks or
238 maintained in SD. Analysis of change in body mass shows there was an effect of photoperiod
239 and pasireotide with interaction between photoperiod and treatment (Photoperiod:
240 $F(1,27)=83.45$ $p<0.001$; Pasireotide: $F(1,27)=21.85$, $p<0.001$; Interaction: $F(1,27)=4.75$,
241 $p=0.038$, Fig 3A), so that after 7 weeks administration, SWB-vehicle hamsters had increased
242 body mass more than all other groups. More specifically, SWB-vehicle administered
243 hamsters had regained an average 11.5 ± 1.0 g body mass while hamsters administered
244 pasireotide, growth was restricted to 4.1 ± 1.5 g. Overall SWB hamsters gained lean mass, but
245 this was significantly less in pasireotide hamsters and there was no interaction between
246 photoperiod and treatment (Photoperiod: $F(1,27)=23.82$ $p<0.001$; Pasireotide: $F(1,27)=7.01$,
247 $p=0.013$; Interaction: $F(1,27)=1.18$, $p=0.287$, Fig 3B). Similarly, fat mass was increased in
248 SWB hamsters and suppressed by pasireotide treatment with no interaction (Photoperiod:
249 $F(1,27)=28.06$ $p<0.001$; Pasireotide: $F(1,27)=4.83$, $p=0.037$; Interaction: $F(1,27)=2.26$,
250 $p=0.144$, Fig 3C). Serum IGF-1 concentration was increased in SWB hamsters and
251 suppressed by pasireotide, with SD-pasireotide having significantly lower and SWB-vehicle
252 having significantly higher concentration compared to all other treatment groups ($P<0.05$ all
253 comparisons after Bonferroni correction; Fig 3D). As noted in LD hamsters, there was an
254 effect of pasireotide to significantly increase serum glucose concentration, independent of
255 photoperiod (Photoperiod: $F(1,27)=1.18$ $p=0.286$; Pasireotide: $F(1,27)=4.84$, $p=0.036$;
256 Interaction: $F(1,27)=0.32$, $p=0.578$, Fig 3F). Serum insulin concentration was increased
257 significantly by SWB to LD (comparisons where $p<0.05$ after Bonferroni correction: SD-

258 Vehicle vs. SWB-Vehicle; SD-Pasireotide vs. SWB-Pasireotide; SD-Pasireotide vs. SWB-
259 Vehicle, Fig 3E).

260 Liver mass was significantly increased in SWB hamsters, but there was no effect of
261 pasireotide or interaction between photoperiod and treatment (Photoperiod: $F(1,27)=29.62$
262 $p<0.001$; Pasireotide: $F(1,27)=2.01$, $p=0.168$; Interaction: $F(1,27)=0.80$, $p=0.378$, table 2).

263 Kidney mass was significantly increased in SWB hamsters, and suppressed by pasireotide
264 treatment, but there was no interaction between photoperiod and treatment (Photoperiod:
265 $F(1,27)=27.88$ $p<0.001$; Pasireotide: $F(1,27)=16.94$, $p<0.001$; Interaction: $F(1,27)=0.82$,
266 $p=0.374$, table 2). EWAT was significantly increased in SWB hamsters with no effect of
267 pasireotide and no interaction (Photoperiod: $F(1,27)=32.45$ $p<0.001$; Pasireotide:
268 $F(1,27)=1.03$, $p=0.319$; Interaction: $F(1,27)=1.00$, $p=0.327$, table 2). Similarly, IBAT was
269 significantly increased in SWB hamsters, with a tendency of pasireotide to decrease IBAT
270 mass approaching significance, but no interaction between photoperiod and treatment
271 (Photoperiod: $F(1,27)=20.27$ $p<0.001$; Pasireotide: $F(1,27)=4.10$, $p=0.053$; Interaction:
272 $F(1,27)=1.81$, $p=0.189$, table 2). Paired testes mass was significantly increased in SWB
273 hamsters, but there was no overall effect of pasireotide (KW; Photoperiod: $p<0.001$;
274 Pasireotide: $p=0.115$). Within SD there was no effect of pasireotide (MW; $p=0.643$), but
275 within SWB hamsters, pasireotide significantly suppressed testes mass (MW: $p=0.003$ table
276 2).

277 **Hypothalamic gene expression**

278 Expression of *Dio2* was significantly increased by SWB from SD to LD but remained
279 unaffected by pasireotide treatment with no interaction (Photoperiod: $F(1,26)=46.74$,
280 $p<0.001$; Pasireotide: $F(1,26)=0.86$ $p=0.362$; Interaction: $F(1,26)=0.51$, $p=0.482$, Fig 4A).

281 Expression of *Dio3* was very low in in the ventricular ependymal layer of SD hamsters and

282 was undetectable in any of the SWB hamsters, with no effect of pasireotide (KW;
283 Photoperiod: $p=0.001$; Pasireotide: $p=0.915$. MW within SD $p=1.000$, Fig 4B)

284 Expression of *Srif* in the ARC was significantly reduced in SWB hamsters with no effect of
285 pasireotide or interaction between photoperiod and treatment (Photoperiod: $F(1,26)=17.30$,
286 $p<0.001$; Pasireotide: $F(1,26)=0.35$ $p=0.558$; Interaction: $F(1,26)=0.16$, $p=0.694$, Fig 4C), but
287 expression of *Srif* in the PeVN was significantly reduced by pasireotide treatment, with a
288 trend towards an effect of short day photoperiod to reduce expression, but no interaction
289 between photoperiod and treatment (Photoperiod: $F(1,27)=2.93$, $p=0.098$; Pasireotide:
290 $F(1,27)=8.37$ $p=0.007$; Interaction: $F(1,27)=0.42$, $p=0.524$. Fig 4D).

291

292 Pasireotide significantly increased ARC *Ghrh* independently of photoperiod (SD-Pasireotide
293 1.95 ± 0.31 fold change; SWB-Vehicle: 1.12 ± 0.31 fold change; SWB-Pasireotide: 2.98 ± 0.34
294 fold change. Photoperiod: $F(1,26)=2.75$, $p=0.109$; Pasireotide: $F(1,26)=19.62$ $p<0.001$;
295 Interaction: $F(1,26)=0.76$, $p=0.391$; Fig 4E).

296

297 Expression of *Pomc* was significantly raised in SWB hamsters but was unchanged by
298 pasireotide treatment (Photoperiod: $F(1,25)=9.87$, $p=0.004$; Pasireotide: $F(1,25)=1.45$
299 $p=0.239$; Interaction: $F(1,26)=1.32$, $p=0.261$, Supplemental Fig 2A). *Npy* expression was not
300 significantly altered by photoperiod or pasireotide treatment (Photoperiod: $F(1,26)=0.01$,
301 $p=0.905$; Pasireotide: $F(1,26)=0.04$ $p=0.851$; Interaction: $F(1,26)=0.28$, $p=0.603$,
302 Supplemental Fig 2B).

303

304 Expression of *Gh-r* in the ARC or PVN was not significantly altered by photoperiod or
305 pasireotide (ARC: Photoperiod: $F(1,26)=2.83$, $p=0.104$; Pasireotide: $F(1,26)=0.03$ $p=0.860$;

306 Interaction: $F(1,26)=1.14$, $p=0.296$, Supplemental Fig 2C; PVN: KW; Photoperiod: $p=0.665$;
307 Pasireotide: $p=0.604$, Supplemental Fig 2D).

308

309 ***Growth hormone induces body weight increase in SD hamsters***

310 Daily intraperitoneal injection of GH for 28 days in hamsters previously acclimated to SD for
311 12 weeks with an average 25% (10g) loss of body mass showed a mean increase of
312 4.15 ± 0.15 g. This increase paralleled that observed in a separate cohort of hamsters switched
313 from SD to LD (SWB, 4.76 ± 0.76 g) over the same period of time (SWB and GH $P<0.001$ vs
314 vehicle; Fig 5A). Lean mass was significantly increased for GH treated hamsters only
315 ($P=0.023$, Fig 5B), whereas fat mass increased only in SWB hamsters ($P=0.002$, Fig 5C).
316 Serum IGF-1 concentrations were compared by Cox regression. This analysis showed SWB
317 hamsters had greater concentrations of circulating IGF-1 compared to both vehicle and GH
318 treated hamsters (Fig 5D, $P<0.05$ after Bonferroni correction). The small increase in IGF-1 in
319 GH treated hamsters was not significant. Serum insulin was significantly raised by GH
320 treatment ($P=0.011$) and this was also the case in SWB compared to vehicle hamsters
321 ($P=0.008$, Fig 5E).

322 In GH treated hamsters, growth was accompanied by increased liver mass ($P=0.021$) and
323 kidney mass ($P=0.026$). There was a significant increase in EWAT in SWB hamsters
324 compared to vehicle and GH ($P<0.001$ and $P=0.014$ respectively, table 3), but GH did not
325 increase EWAT mass relative to vehicle hamsters. IBAT after 4 weeks in LD or following
326 GH treatment was not significantly different from vehicle hamsters (table 3). As expected,
327 testes mass was significantly increased in SWB ($P<0.001$), but in GH treated hamsters the
328 small increase in testis mass did not reach statistical significance (table 3).

329

330 **Discussion**

331 The Siberian hamster is one of a number of small seasonal mammals that reduce body mass
332 during winter. Even though this weight loss has been extensively described, the mechanisms
333 are not understood. Here we provide evidence to support the view that the GH axis is a
334 potential proponent of the seasonal neuroendocrine axis underpinning this body mass change.

335 The seasonal adaptation of reproductive organ regression and recrudescence is well
336 established in the Siberian hamster, but less well known are the photoperiod-mediated
337 changes in the mass of internal tissues. Seasonal changes in organ mass were first described
338 in the common shrew (*Sorex araneus*) by Dehnel (referred to as Dehnel's phenomenon; 1,2).
339 Although several previous studies on body mass regulation of the Siberian hamster have
340 demonstrated reduction in muscle and organ mass in short photoperiod (6, 30, 31), with the
341 exception of adipose tissue and testes mass (32), this is the first report showing that increase
342 in body mass during increased day length involves increase in mass of those organs which
343 were reduced during SD exposure. We would therefore consider the Siberian hamster as a
344 member of a group of mammals that exhibit Dehnel's phenomenon.

345 We initiated these studies with the hypothesis that GH underpins the seasonal body weight
346 changes in the Siberian hamster. Although photoperiod-regulated effects on frequency and
347 amplitude of pulsatile GH secretion have been previously demonstrated in the golden hamster
348 (18), due to the pulsatile nature of GH secretion and small size of the Siberian hamster, direct
349 measurement of GH was not feasible. In the absence of a GH receptor antagonist for use in
350 rodents, we approached our hypothesis by utilizing pasireotide, a somatostatin receptor
351 agonist that was developed for the treatment of excess GH production by pituitary
352 tumours/adenomas (acromegaly) and inhibition of tumour growth (25, 33, 34). This long

353 acting somatostatin receptor agonist was delivered in an encapsulated form which steadily
354 releases the agonist over a period of 28 days.

355 Pasireotide, when administered to male hamsters in LD, caused a gradual body mass loss
356 similar to that which can be observed in hamsters transferred from LD to SD photoperiod.
357 MRI analysis of body composition showed a loss of lean mass which was reflected in a loss
358 of kidney mass and testes mass both of which occur in response to SD (6, 30, 31). However, a
359 reduction in liver mass observed in SD hamsters was not obtained.

360 In this LD paradigm, fat mass determined by MRI showed a trend toward a reduction by
361 pasireotide. This trend is supported by significantly lower EWAT and IBAT measurement in
362 pasireotide treated hamsters and is characteristic of hamsters held in SD (7, 35). This may
363 reflect an involvement of GH or another neuroendocrine pathway in EWAT regulation, but
364 interestingly pasireotide reveals a neuroendocrine basis for regulating IBAT mass, an
365 important fat depot in thermogenesis and energy expenditure (36).

366 As expected, pasireotide treatment resulted in a reduction of circulating IGF-1, which as a
367 biomarker of endogenous GH is indicative of a suppression of GH secretion (26), a
368 conclusion which is supported by increased *Ghrh* expression in the ARC and reduced *Srif*
369 expression in the PeVN as a result of reduced GH feedback to the hypothalamus (19-21).
370 Furthermore in a preliminary experiment with the somatostatin agonist octreotide, a similar
371 trajectory of body mass loss occurred with terminal liver, kidney and EWAT masses similar
372 to pasireotide treated hamsters, but only a small effect on paired testis weight (data not
373 shown).

374 Pasireotide has highest affinity for SSTR₅ (22, 23), thus the effect of pasireotide to reduce
375 growth would be consistent with data for SSTR₅ subtype distribution in the rat pituitary
376 where SSTR₅ is present on 72% of somatotrophic cells. A reduction in GH may contribute to

377 loss of testicular mass through an action on testicular Leydig cells (37), but the effect of the
378 somatostatin analogues could also be due in part to antagonism of FSH at testicular Sertoli
379 cells (38). However, this seems unlikely as the effect on sertoli cells appears to be mediated
380 by SSTR₂ (39). A further possibility is a direct effect of pasireotide on hypothalamic *Kiss-1*
381 or *Rfrp3* expression, but inaccessibility of somatostatin analogues to the brain renders this
382 unlikely (see below). A more likely explanation is the inhibition of pituitary LH and FSH
383 secretion by pasireotide (38), where in the rat 21% of gonadotropes express SSTR₅ (40). With
384 pasireotide treatment, we did not systematically observe for an effect on pelage, but no
385 striking change in pelage was noted by the end of 7 weeks of treatment. Although in the rat
386 36% of lactotropes express SSTR₅, no effect of pasireotide on prolactin secretion from rat
387 pituicyte primary cell cultures has been found (41), suggesting prolactin secretion may not be
388 subject to regulation by somatostatin. However, further analysis of SSTR₅ distribution and
389 other somatostatin receptor subtypes in the hamster pituitary is required to understand the
390 mechanistic basis for the action of somatostatin and related analogues.

391 Pasireotide also impaired body mass accretion in hamsters switched from SD (at or close to
392 the body weight nadir) to LD photoperiod (SWB hamsters). This was reflected in an overall
393 reduction in lean mass and fat mass in pasireotide treated hamsters. Although measurement of
394 tissue mass showed no significant effect of pasireotide to suppress tissue regrowth in SWB
395 hamsters, the 7.4g difference between vehicle and pasireotide treated hamsters is likely to be
396 due to the sum of differences of tissues which individually have not reached statistical
397 significance. The exception to this was testes mass. Paired testes mass was increased in SWB
398 hamsters, but this increase was significantly albeit not completely retarded by pasireotide.

399 Circulating IGF-1 was increased in SWB-vehicle hamsters but was decreased in both SD and
400 SWB hamsters treated with pasireotide compared to vehicle treated hamsters indicative of
401 suppression of pituitary GH secretion. A reduction in GH secretion and reduced GH feedback

402 to the hypothalamus by pasireotide is also supported by evidence of increased *Ghrh* mRNA
403 expression in the ARC and reduced *Srif* expression in the PeVN (19,20). The inability of
404 pasireotide to completely suppress LD physiology in SWB hamsters is likely to be due to
405 restoration of stimulatory hormones from the hypothalamus, driven by the substantial
406 increase in *Dio2* expression in the hypothalamus (10) competing with the inhibitory action of
407 pasireotide via SSTR signalling on appropriate neuroendocrine cells of the pituitary.

408 Pasireotide in both experiments resulted in a small hyperglycaemic response as observed in
409 humans and rodents (42,43). In hamsters switched from SD to LD, increased insulin was
410 consistent with previous observation of elevated levels in LD (27,29), but no significant
411 suppression of serum insulin was found in either experiment after pasireotide treatment, as
412 reported in human and rodent studies (42,43). However, a suppressive effect on insulin may
413 have been masked as the hamsters were not fasted prior to taking the terminal blood samples.

414

415 In this study, we found no evidence to suggest pasireotide has an effect on photoperiod gene
416 expression or central homeostatic mechanisms of energy balance to account for the observed
417 effect on body and organ masses (31, 44-46). Therefore the most parsimonious explanation
418 for the observed effect of pasireotide is an inhibition of GH secretion from the pituitary
419 gland. This is supported by an observed increase following pasireotide treatment in *Ghrh*
420 expression in the ARC and decrease in *Srif* expression in the PeVN which is consistent with
421 an inhibition of GH feedback to the homeostatic control of GH output from the pituitary
422 gland. Additionally, somatostatin and somatostatin analogues are known to have little or poor
423 accessibility to the brain and may actively be transported from CNS to blood (47-49).
424 Therefore a direct effect of the analogues in the brain is unlikely and the observed effects are

425 more likely to be due to the presence of SSTRs on relevant hormone secreting cells of the
426 pituitary.

427 We and Klosen et al (10, 14, 15, 31) have recently demonstrated a robust induction of *Srif*
428 expression in the ARC of SD hamsters and we have hypothesised that these neurons may be
429 important to seasonal growth (10, 14, 31). Klosen et al also showed that in SD hamsters, TSH
430 reduces *Srif* expression to the LD state suggesting expression is downstream of photoperiod
431 mediated change in hypothalamic T3 concentration (15). Unlike *Srif* expression in the PeVN,
432 ARC expression was not affected by pasireotide. Consequently, if SRIF neurons in the ARC
433 are important for seasonal physiological responses including growth, these neurons are
434 resistant to GH feedback, which might be anticipated for a regulatory system that must reflect
435 the prevailing photoperiod.

436 In the context of a broad role for somatostatin in suppressing the seasonal neuroendocrine
437 axis, it is interesting to note that *Srif* expression is one of only a small number of genes
438 showing increased expression in the ARC in SD photoperiod (10). We would hypothesize
439 that ARC expressing *Srif* neurons project to the pituitary enabling direct regulation and
440 possibly setting the tone of GH secretion upon which circadian regulation mediated by SRIF
441 neurons of the PeVN is imposed. Some support for this hypothesis may be evident in the
442 reported SD increase in somatostatin content of median eminence of the golden hamster and
443 the observation of limited projection of SRIF containing ARC neurons to the median
444 eminence in the dog (50, 51).

445 Daily GH injections resulted in increased growth of SD adapted Siberian hamsters, reaching
446 significance compared to SD vehicle control hamsters within 14 days. This growth was in
447 advance of growth in hamsters switched to LD, the latter not attaining significant difference
448 until 21 days after SWB. However, both groups finished with the same body mass increase
449 28 days following application of GH treatment or exposure to LD. Analysis of serum IGF-1

450 showed a significant increase in SWB hamsters with several samples exceeding the top
451 standard in the ELISA assay. This limited the statistical analysis to a Cox regression and
452 consequently the small increase in IGF-1 as a result of GH injection was not detected as
453 significant. The response of IGF-1 to GH injection was poor, but it has previously been noted
454 that IGF-1 may not be a reliable pharmacodynamic marker of exogenous GH treatment in
455 rodents (52). The increase in body mass was contributed by an increase in both liver and
456 kidney mass for GH treated hamsters. Changes in these tissues approached significance in
457 SWB hamsters, and had the hamsters remained in LD for a further 2-4 weeks to recover to
458 their starting body weight, changes in this group would likely have achieved significance.
459 The SWB hamsters, however, had significant increases in EWAT, IBAT and testes masses,
460 none of which were increased by GH treatment suggesting that GH by itself is not involved in
461 the seasonal regulation of these tissues.

462 Interestingly, liver mass was unresponsive to pasireotide in either the LD or SWB hamsters,
463 but did respond to GH treatment in SD hamsters. This may indicate that while a
464 pharmacological dose of GH is capable of inducing liver growth, regulation of liver mass in
465 the Siberian hamster is not dependent on endogenous GH or IGF-1. A possible mediator of
466 liver mass could be seasonal changes in prolactin (31, 53), the latter being regulated by
467 tuberulin released by the pars tuberalis during summer and suppressed by melatonin during
468 winter (54, 55) and known to promote normal liver growth, survival and regeneration in
469 rodents (56).

470 **Summary**

471 Taken together, these data support a view of an involvement of the GH axis in photoperiod
472 mediated body weight regulation of the Siberian hamster. A previous study in the rat had
473 shown pasireotide reduced circulating IGF-1 and completely suppressed growth (32).

474 Although a direct action of pasireotide on peripheral tissues cannot be ruled out, we would
475 reason that a reduction in GH underpins the response observed; firstly GH increases lean
476 mass of SD hamsters, secondly somatostatin would have to act at several different tissues to
477 reduce or suppress their mass simultaneously in a manner which mimicked the gradual
478 decline seen SD or increase in LD respectively. A seasonal regulation of the GH axis is
479 supported by studies in red deer showing a correlation between GH, IGF-1 and seasonal
480 growth (16, 17) and photoperiod dependent GH secretion in the golden hamster (18).
481 Therefore our data would extend the concept of a seasonal regulation of the GH axis to both
482 large and small seasonal mammals. Intriguingly, the effect of pasireotide on multiple
483 physiological parameters indicates somatostatin may have a broad role in suppressing
484 multiple neuroendocrine pathways and provides evidence for an involvement of the
485 neuroendocrine axes in regulating seasonal physiological adaptations that have not hitherto
486 been considered to be under neuroendocrine regulation (white and brown adipose tissue).
487 Although further work is required to demonstrate an involvement of GH in other seasonal
488 mammals that exhibit Dehnel's phenomenon, (1, 2) our data may explain in part, the
489 biological basis of this phenomenon.

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499

500 **Conflict of interest**

501 Herbert Schmid is an employee of Norvatis AG

502

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660 Table 1

Tissue (g)	Vehicle	Pasireotide
Liver	1.32 ± 0.07	1.25 ± 0.02
Kidneys	0.41 ± 0.02	0.34 ± 0.01***
EWAT	0.69 ± 0.07	0.45 ± 0.04*
IBAT	0.20 ± 0.01	0.15 ± 0.01**
Paired Testes	0.76 ± 0.04	0.19 ± 0.04***

661

662

663 Table 2

Tissue (g)	SD		SWB		Result of statistical comparison	
	Vehicle	Pasireotide	Vehicle	Pasireotide		
Liver	1.23 ± 0.05	1.19 ± 0.05	1.77 ± 1.11	1.57 ± 0.08	Photoperiod	P < 0.001
					Drug	NS
					Interaction	NS
Kidneys	0.38 ± 0.02	0.32 ± 0.01	0.48 ± 0.02	0.40 ± 0.01	Photoperiod	P < 0.001
					Drug	P < 0.001
					Interaction	NS
EWAT	0.37 ± 0.10	0.21 ± 0.03	0.75 ± 0.08	0.75 ± 0.10	Photoperiod	P < 0.001
					Drug	NS
					Interaction	NS
IBAT	0.11 ± 0.02	0.10 ± 0.01	0.24 ± 0.04	0.15 ± 0.01	Photoperiod	P < 0.001
					Drug	NS
					Interaction	NS
Paired Testes	0.13 ± 0.04	0.08 ± 0.02	0.88 ± 0.06	0.51 ± 0.07**	Photoperiod (KW)	P < 0.001
					Drug (KW)	NS

664

665

666 Table 3

667

Tissue (g)	Vehicle	GH	SWB
Liver	1.11 ± 0.04	1.36 ± 0.07*	1.37 ± 0.11
Kidneys	0.32 ± 0.01	0.38 ± 0.01*	0.37 ± 0.02
EWAT	0.18 ± 0.02	0.26 ± 0.03 [†]	0.41 ± 0.05***
IBAT	0.11 ± 0.01	0.12 ± 0.01	0.19 ± 0.04
Paired Testes	0.06 ± 0.01	0.10 ± 0.02 ^{†††}	0.51 ± 0.01***

668

669

670 **Table legends**

671 Table 1: Terminal tissue masses of LD-Siberian hamsters treated with vehicle or pasireotide
672 for 49 d. Data are expressed as mean \pm SEM. Significant differences signify the effect of
673 pasireotide on organ mass, compared by paired t-test. *P < 0.05, ** P<0.01, ***P <0.001.
674 IBAT, interscapular brown adipose tissue; EWAT, epididymal white adipose tissue. All
675 groups n = 8.

676

677 Table 2: Terminal tissue masses of Siberian hamsters acclimated to SD before switch back to
678 LD (SWB) or remaining in SD and treatment with pasireotide or vehicle for 49 d. Data are
679 expressed as mean \pm SEM. Statistical comparisons are by 2-way ANOVA or Kruskal-Wallis
680 tests (KW) as indicated. ** P<0.01 effect of pasireotide within SWB, Mann-Whitney test.
681 IBAT, interscapular brown adipose tissue; EWAT, epididymal white adipose tissue. SD-
682 Vehicle n = 7, all other groups n = 8.

683

684 Table 3: Terminal tissue mass of SD Siberian hamsters treated with vehicle, pasireotide or
685 switched back to LD (SWB) for 28 d. Data are expressed as mean \pm SEM. Statistical
686 comparisons are by 1 – way ANOVA with the exception of paired testes mass, compared by
687 Mann-Whitney tests. [†] P < 0.05, ^{†††} P < 0.001 compared to SWB. * P < 0.05, *** P < 0.001
688 compared to Vehicle. IBAT, interscapular brown adipose tissue; EWAT, epididymal white
689 adipose tissue; SD, short day photoperiod (8 : 16 h light : dark). Vehicle and GH both n = 12,
690 SWB n = 6.

691

692 **Figure legends**

693 Figure 1. Pasireotide reduces body mass in LD-acclimated male Siberian hamsters. Change in
694 body mass (A) of hamsters housed in LD treated with vehicle or pasireotide for 49 days,
695 statistical differences indicated are between vehicle and pasireotide groups. Overall fat mass
696 (B) and lean mass (C) of vehicle and pasireotide treated hamsters post dissection, measured
697 by MRI. Terminal serum IGF-1 (D) and insulin (E) measured by ELISA and serum glucose
698 (F). Statistical comparison carried out by paired t-test, *P<0.05, **P<0.01, ***P<0.001, n.s.:
699 not significant. All groups n = 8. Data are all expressed as mean ± SEM.

700

701 Figure 2. Pasireotide does not directly alter photoperiod programmed hypothalamic gene
702 expression, but does alter mRNA expression stimulated by GH-axis feedback to the
703 hypothalamus in LD acclimated Siberian hamsters. Relative mRNA expression of *Dio2* (A),
704 *Srif* (B), and *Ghrh* (D) in the arcuate nucleus (ARC) and periventricular nucleus (PeVN - D).
705 Statistical comparisons are by paired t-test; ***p<0.001, n.s.: no significant differences;
706 example images showing mRNA expression pattern are shown for vehicle and pasireotide
707 treatments. All groups n = 8. Data are all expressed as mean ± SEM relative to vehicle group
708 data. Shown is a representative section of the named mRNA expression in the measured
709 region of the hypothalamus.

710

711 Figure 3. Pasireotide inhibits LD photoperiod driven body mass accretion. Change in body
712 mass of hamsters treated with vehicle or pasireotide and returned to LD or remaining in SD
713 photoperiod (A) **p<0.01, ***p<0.001 and different to all other groups, a: p<0.05 SWB-
714 Pasireotide vs SD-Pasireotide; b: p<0.05 SWB-Pasireotide vs both SD groups. Lean (B) and

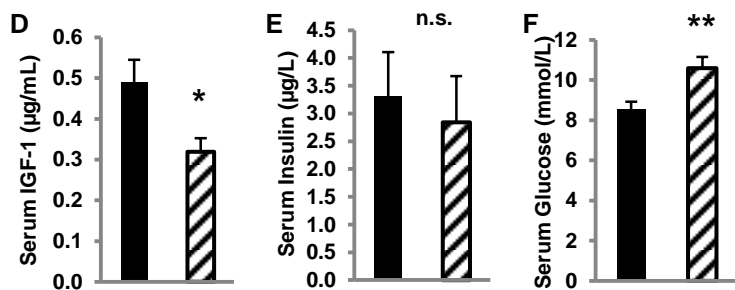
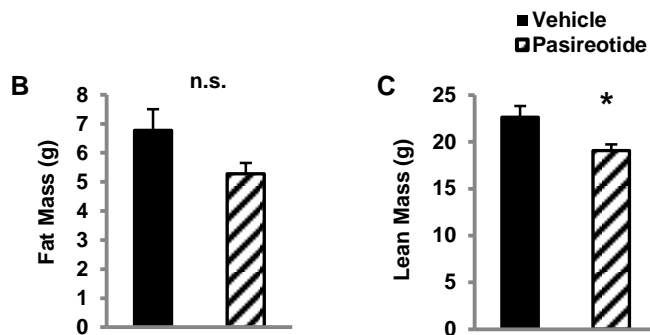
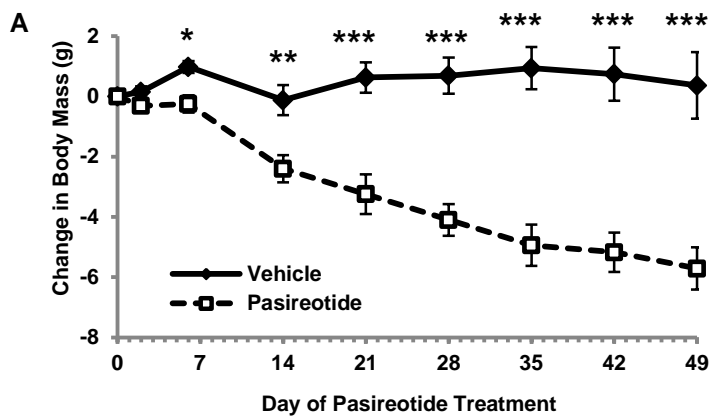
715 fat (C) mass measured by MRI * $p < 0.05$, *** $p < 0.001$. Terminal serum IGF-1(D); * $p < 0.05$
716 compared to all groups. Terminal insulin (E); * $p < 0.05$. Serum glucose (F); * $p < 0.05$.
717 Statistical comparisons are by 2-way ANOVA with the exception of E, compared by Cox
718 regression. SD-Vehicle $n = 7$, SD-Pasireotide, SWB-Vehicle and SWB-Pasireotide all $n = 8$;
719 data are all expressed as mean \pm SEM.

720

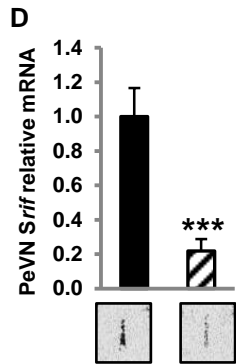
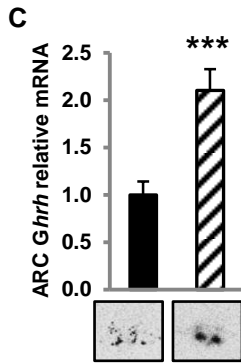
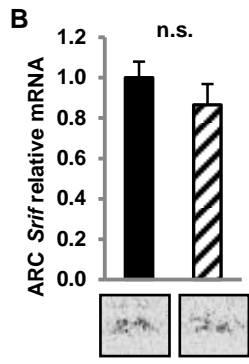
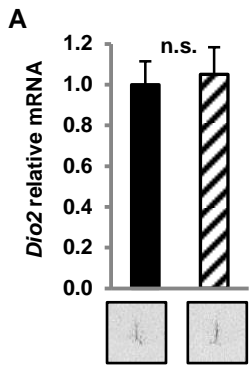
721 Figure 4. Pasireotide does not directly alter photoperiod programmed hypothalamic gene
722 expression in SD and SWB hamsters, but does alter mRNA expression stimulated by GH-axis
723 feedback to the hypothalamus. Relative mRNA expression of *Dio 2* (A), *Dio 3* (B), *Srif* (C) in
724 the arcuate nucleus (ARC), *Srif* (D) in the Periventricular nucleus (PeVN) and of *Ghrh* (E)
725 in the ARC. Statistical comparison is by 2-way ANOVA or Kruskal-Wallis and Mann
726 Whitney tests (B), ** $p < 0.01$, *** $p < 0.001$, n.s.: no significant differences; example images
727 showing mRNA expression pattern are shown for SD and SWB treatments (A-C) or Vehicle
728 and Pasireotide treatments (D,E). All groups $n=8$ except for panels A and B: SD-Vehicle $n =$
729 7; Panels C, D and E: ARC *Srif* and *Ghrh*, SD-Vehicle $n = 7$, SWB-Vehicle $n = 7$. Data are
730 expressed as mean \pm SEM, relative to SD-Vehicle.

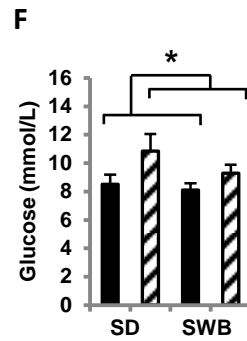
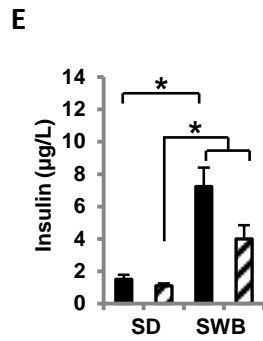
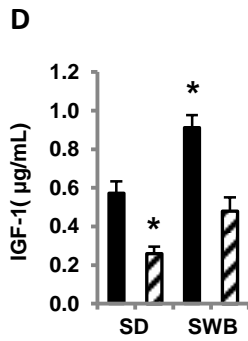
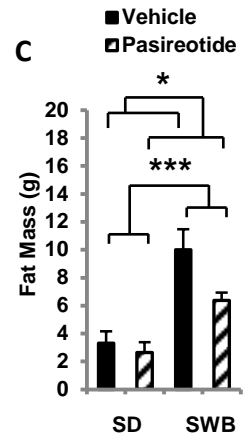
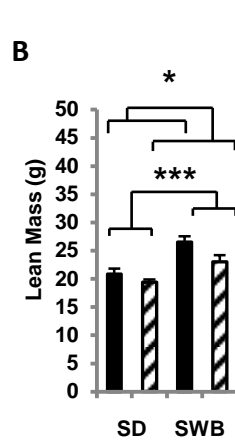
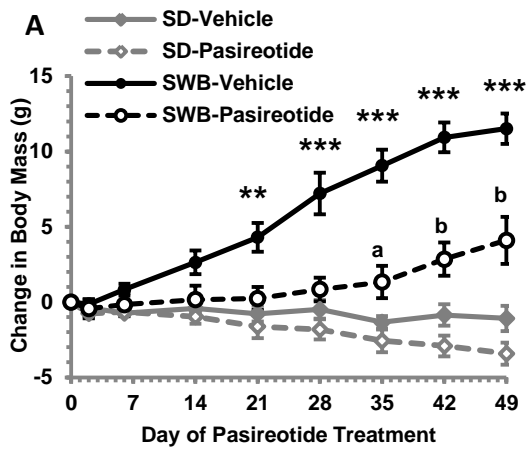
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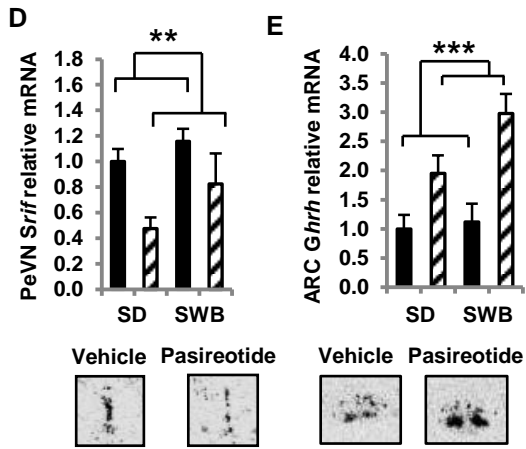
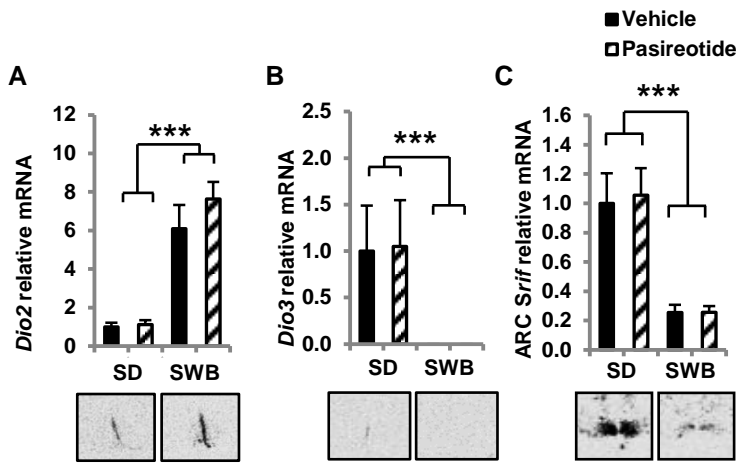
732 Figure 5. Comparison of effect of exogenous bovine GH and LD photoperiod (SWB) on body
733 mass, and circulating IGF-1 and insulin concentrations in SD Siberian hamsters. Change in
734 body mass of hamsters previously acclimated to SD before daily administration of GH or
735 vehicle or switched to LD for 28d; a: $P < 0.05$ Vehicle vs. GH; b: $P < 0.05$ SWB vs. Vehicle (A);
736 effect on lean mass (B); fat mass (C); terminal serum IGF-1(D) and insulin (E) concentration.
737 Comparisons are by 1-way ANOVA; * $P < 0.05$, *** $P < 0.001$; Vehicle and GH both $n = 12$,
738 SWB $n = 6$. Data are all expressed as mean \pm SEM.

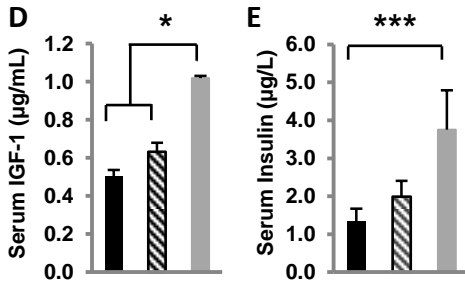
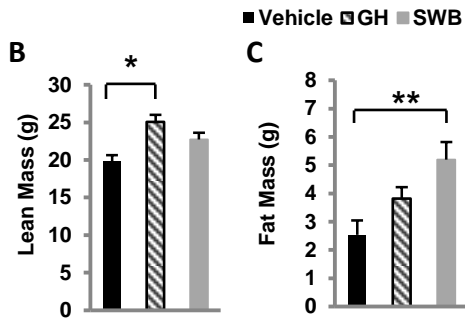
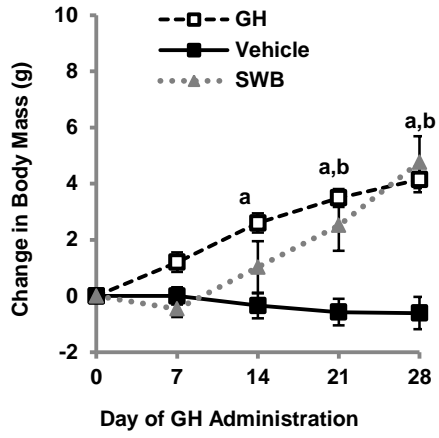


■ Vehicle
▨ Pasireotide







A

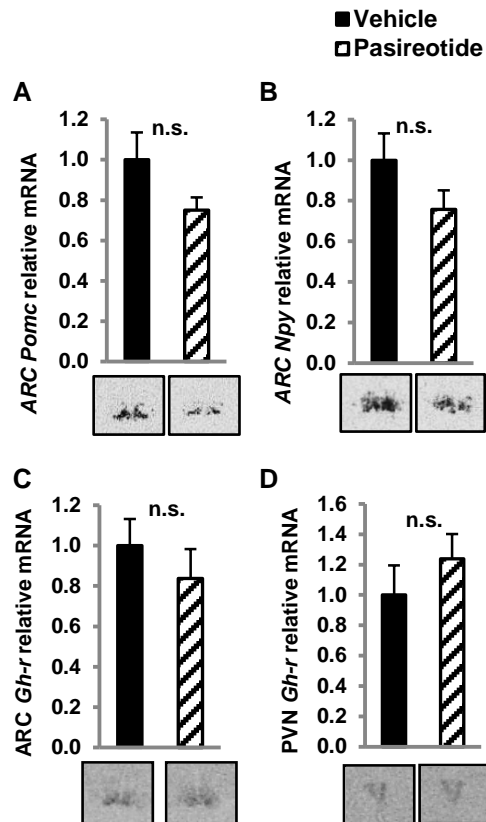
Somatostatin agonist pasireotide promotes a physiological state resembling short-day acclimatization in the photoperiodic male Siberian hamster (*Phodopus sungorus*)

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Stephan Steinlechner^{2,5} and Perry Barrett^{1,5,6}**

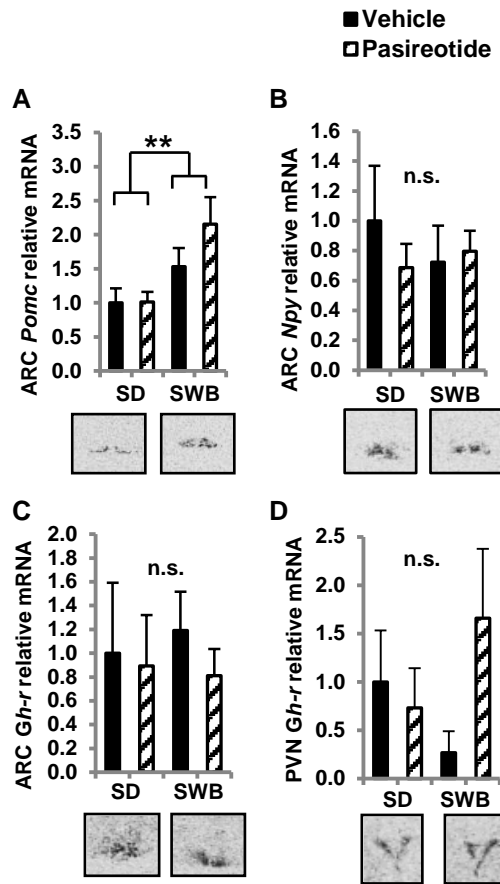
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³Novartis Pharma AG, WSJ-103.5.10.1, CH-4002 Basel, Switzerland



Supplementary figure 1. Relative mRNA expression in the hypothalamus of LD hamsters vehicle or pasireotide treated of Pomc (A), Npy (B), Gh-r (C) in the arcuate nucleus (ARC) and Gh-r (D) in the periventricular nucleus (PeVN). No significant differences (n.s.) were found, n=8 for both groups.



Supplementary figure 2. Relative mRNA expression in the hypothalamus of SD acclimated hamsters remaining in SD or to LD (SWB) following administration of either vehicle or pasireotide. *Pomc* (A), *Npy* (B), *Gh-r* (C) in the arcuate nucleus (ARC) and *Gh-r* (D) in the periventricular nucleus. ** $p < 0.01$, n.s. no significant differences; $n = 8$ all groups except SD-vehicle, $n = 7$.