# Endogenous Somatostatin Is Critical in Regulating the Acute Effects of L-Arginine on Growth Hormone and Insulin Release in Mice

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L-arginine (L-Arg) rapidly stimulates GH and insulin release in vivo. It has been hypothesized that L-Arg stimulates GH release by lowering hypothalamic somatostatin (SST) tone. L-Arg may also act directly at the pituitary to stimulate GH release. Moreover, L-Arg has a direct stimulatory effect on $\beta$-cells, which is thought to be blunted by the release of SST from pancreatic $\delta$-cells. To confirm the role of endogenous SST on L-Arg-induced GH and insulin release, wild-type (WT) and SST-knockout (SST-KO) mice were injected with L-Arg (ip; 0.8 g/kg), and pre-/post-injection GH, insulin, and glucose levels were measured. In WT mice, L-Arg evoked a 6-fold increase in circulating GH. However, there was only a modest increase in GH levels in WT pituitary cell cultures treated with L-Arg. In contrast, L-Arg failed to increase GH in SST-KO beyond their already elevated levels. These results further support the hypothesis that the primary mechanism by which L-Arg acutely increases GH in vivo is by lowering hypothalamic SST input to the pituitary and not via direct pituitary effects. Additionally, L-Arg induced a clear first-phase insulin secretion in WT mice, but not in SST-KO. However, SST-KO, but not WT mice, displayed a robust and sustained second-phase insulin release. These results further support a role for endogenous SST in regulating L-Arg-mediated insulin release. **(*Endocrinology* 154: 2393–2398, 2013)**

It is well documented that injection of the amino acid, L–arginine (L-Arg), rapidly increases circulating GH levels in a variety of species (1–6). This stimulatory action of L-Arg is thought to be primarily mediated by suppressing hypothalamic somatostatin (SST) input to the pituitary somatotropes (1, 7). However, there is also evidence that L-Arg can act directly at the pituitary level to stimulate GH synthesis/release (8–10). In addition to the stimulatory actions of L-Arg on GH release, L-Arg has also been shown to directly stimulate insulin release from pancreatic $\beta$-cells. However, in this context, L-Arg also increases SST release from pancreatic $\delta$-cells, which in turn is thought to suppress the rise in insulin (11–15).

In order to confirm the role of endogenous SST in regulating the stimulatory actions of L-Arg on GH and insulin release in vivo, the current study examined the acute effects of L-Arg on circulating GH and insulin levels in male and female SST-knockout (SST-KO) mice and their wild-type (WT) littermate controls.

## Materials and Methods

### Animals

Male/female SST-KO and their WT littermate controls were bred in house and maintained on a C57Bl/6J background. The

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Abbreviations: L-Arg, L-arginine; GSIS, glucose-stimulated insulin secretion; SST, somatostatin; SST-KO, somatostatin knockout; WT, wild type.

SST-KO strain was originally developed and validated by Zeyda et al. (16). WT C57Bl/6J mice, (Charles River, Barcelona, Spain), were bred in house and used to prepare primary pituitary cultures to test the direct effects of L-Arg, as described below. All mice were maintained under standard conditions of light (12-h light/12-h dark cycle) and temperature (22–24°C), with free access to tap water and food (SAFE-diets, Barcelona, Spain). All experimental procedures were approved by the Institutional Animal Care and Use Committees (IACUC) of the University of Cordoba. Mice were handled daily at least 1 week before any experimental procedure to acclimate them to personnel and handling procedures.

### Primary mouse pituitary cell cultures

Primary cultures were prepared from 12- to 13 week-old male and female WT-mice as previously reported (17). Cells were plated in 48 wells/plate (30 000 cells per well) and incubated in 95% $O_2$:5% $CO_2$ at 37°C for 24 hours in serum containing media [DMEM containing glucose [1 g/liter], 10% fetal bovine serum, 0.15% BSA, 6 mM HEPES, 2 mM L-glutamine, 1% penicillin/streptomycin [Sigma-Aldrich, St. Louis, Missouri]. Then, cells were washed in serum-free media and preincubated for 1 hour. Media were removed and replaced with medium with or without L-Arg (100 $\mu$M, Sigma-Aldrich). After 1 or 24 hours, medium was removed, centrifuged to discard any detached cells, and then stored at −20°C for subsequent GH analysis.

### L-arginine stimulation tests

Ad libitum fed male/female SST-KO and WT (7 mo-old), were treated with L-Arg [0.8 g/kg, ip, PBS pH 7.4; dose chosen according to previous studies (18)]. A blood sample was collected from tail vein nicks just before L-Arg injection (t0) and 30 and 60 minutes after injection, for determination of circulating GH levels. One month later, the mice were fasted overnight (16 h) and again treated with L-Arg, and blood was collected at t0, 30, and 60 minutes postinjection for insulin and glucose determinations. Based on the differences observed in L-Arg-induced insulin secretion between SST-KO and WT at 30 and 60 minutes postinjection, a separate set of male mice (4 mo of age), was fasted overnight and treated with L-Arg, and blood was collected at 0, 2, 5, 15, 30, and 60 minutes postinjection for insulin/glucose determinations to analyze first-phase insulin release. In all experiments, an anesthetic cream (Emla, 2.5% lidocaine: 2.5% prilocaine) was applied 10 minutes before blood collection. Blood was mixed with 1 $\mu$L 0.32 M $K_3$EDTA immediately after collection, centrifuged and plasma stored at −20°C for subsequent analysis of GH or insulin by ELISA.

### Glucose, GH, and insulin measurement

Glucose levels were determined from fresh tail-vein blood samples using the Glucocard-glucometer (Arkray; Amstelveen, The Netherlands). Hormones were assessed using commercial ELISA kits for mouse GH and insulin (Millipore; Madrid, Spain).

### Statistical analysis

Student's *t* tests were used to evaluate the impact of lack of SST on basal GH, glucose (fed/fasted) and insulin within gender, the area under the curve of the glucose response, and to compare the in vitro GH response to L-Arg treatment in WT primary pituitary cultures. One-way ANOVA, followed by Newman-Keuls post hoc test, was used to evaluate the time-dependent impact of L-Arg on GH/insulin/glucose levels. Two-way ANOVA, followed by Bonferroni post hoc test for multiple comparisons, was used to evaluate the impact of lack of SST and L-Arg treatment on GH/insulin/glucose levels.

## Results

Male and female SST-KO mice have elevated GH levels, compared with WT controls (Table 1). In WT male and female mice, acute injection of L-Arg resulted in an increase in GH levels that reached 6 times the baseline values at 60 minutes after injection (Figure 1A). However, L-Arg failed to further augment circulating GH levels in SST-KO mice.

In order to determine whether the L-Arg-induced GH release, observed in WT-mice, is in part due to a direct effect on the pituitary, primary pituitary cultures from WT male and female mice were treated with L-Arg (100 $\mu$M) or vehicle for 1 or 24 hours. L-Arg stimulated GH secretion in primary pituitary cultures from male (145% of vehicle-treated cultures) but failed to modify GH secretion in cultures prepared from female mice (Figure 1B).

Under fasted conditions, 4-month-old SST-KO mice were euglycemic, although their insulin levels were greater

**Table 1.** GH, Glucose, and Insulin Levels in WT and SST-KO Mice before L-Arg Injection

| | Males | | Females | |
|---|---|---|---|---|
| | **WT** | **SST-KO** | **WT** | **SST-KO** |
| Age (months) | Mean SEM | Mean SEM | Mean SEM | Mean SEM |
| 7 GH ng/mL (fed) | 2.40 ± 0.98 | 9.07[a] ± 2.10 | 2.85 ± 1.78 | 15.99[b] ± 3.16 |
| 7 Glucose mg/dL (fed) | 127.5 ± 5.5 | 142.0 ± 6.8 | 120.7 ± 5.1 | 131.0 ± 6.0 |
| 8 Glucose mg/dL (fasted) | 119.0 ± 8.0 | 157.2 ± 22.8 | 98.3 ± 8.1 | 111.5 ± 22.3 |
| 8 Insulin ng/mL (fasted) | 0.546 ± 0.11 | 0.532 ± 0.07 | 0.220 ± 0.03 | 0.271 ± 0.03 |
| 4 Glucose mg/dL (fasted) | 65.9 ± 4.5 | 67.1 ± 5.3 | nd | nd |
| 4 Insulin ng/mL(fasted) | 1.222 ± 0.09 | 1.55[c] ± 0.09 | nd | nd |

Values represent the mean ± SEM of 5–13 mice per genotype per gender. Superscipt letters indicate differences between WT and SST-KO within gender: [a] $P < .01$; [b] $P < .001$; [c] $P < .05$. nd, Not determined.

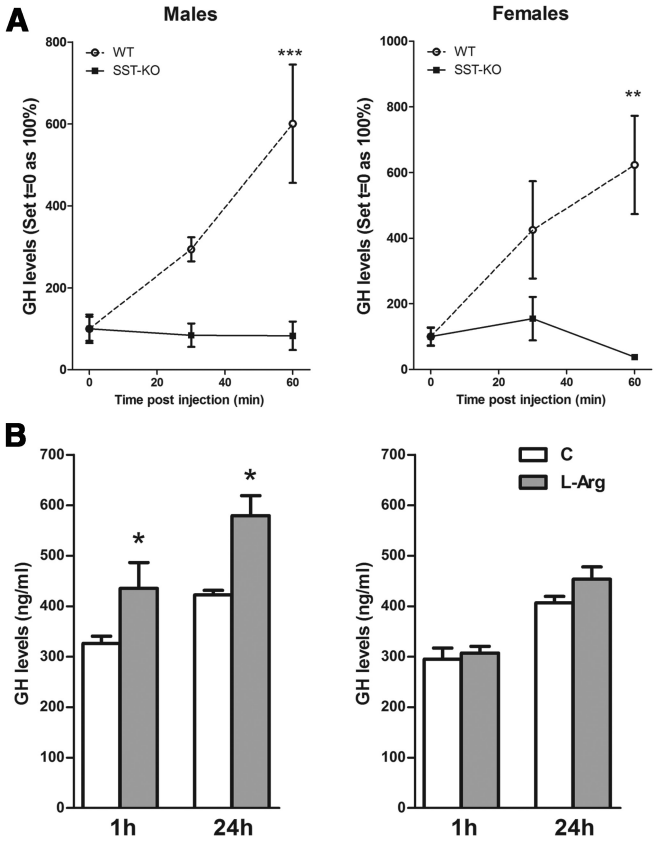


**Figure 1.** Acute in Vivo L-Arg-Induced GH Secretion in WT and SST-KO Fed Male and Female Mice and in Vitro L-Arg-Induced GH Secretion in Male and Female WT Pituitary Cells in Vitro A, Plasma GH levels in male (left) and female (right) WT and SST-KO mice in response to L-Arg (ip; 0.8 g/kg). Values are shown as means ± SEM of 5–7 mice per genotype per gender. B, In vitro GH levels in male (left) and female (right) WT primary pituitary cell cultures treated with 100 $\mu$M of L-Arg for 1 hour or 24 hours. Values are means ± SEM of 3 independent experiments (3–4 individual wells per treatment per experiment per gender). Asterisks indicate differences between WT and SST-KO (A) or L-Arg treated against vehicle-treated controls (B). *, $P < .05$; **, $P < .01$; ***, $P < .001$.

than in WT controls (Table 1). In these same WT mice, L-Arg evoked a rapid rise in insulin, which fell to baseline at 30 minutes postinjection (Figure 2A). In contrast, a clear first-phase insulin release (0–5 min) was not observed in SST-KO mice (Figure 2A). Interestingly, L-Arg did evoke a significant rise in insulin 15 minutes after injection, which peaked at 30 minutes and declined by 60 minutes, but remained elevated above preinjection values. Despite the L-Arg-induced rise in insulin, glucose levels were also increased after L-Arg injection (5–15 min, Figure 2B), and this increase was more pronounced in SST-KO mice (area under the curve, 0–15 min), but returned to control levels by 60 minutes. Consistent with these observations, in an older group of mice (8 mo of age), L-Arg stimulated insulin levels 30 minutes postinjection (earliest time point examined) in SST-KO mice but not WT controls (Figure 2C). In contrast to the younger mice, the L-Arg-induced rise in insulin in 7 month-old SST-KO mice was associated with hypoglycemia at 30 and 60 minutes postinjection (Figure 2D).

## Discussion

### Role of endogenous SST in basal and L-Arg-stimulated GH release

L-Arg stimulates GH release in humans, pigs, dogs, rats (1–6), and in mice (Figure 1A). However, limited studies have been conducted regarding the mechanism by which L-Arg mediates this effect. Earlier work, in humans, demonstrated that L-Arg could potentiate GH-releasing hormone-induced GH secretion (1, 7) but fails to stimulate GH after pretreatment with exogenous SST analogs (7). In addition, L-Arg was shown to be an effective stimulator of GH release in pigs, whereas this effect was abolished after hypothalamic-pituitary stalk transection (2, 6). These findings led to the conclusion that the primary mechanism by which L-Arg stimulates GH release is through reducing endogenous hypothalamic SST tone on the pituitary. Therefore, L-Arg alone, or in combination with GHRH, has been routinely used as a diagnostic tool to assess the capacity of the pituitary somatotrope to release GH (for review see Ref. 19). The GH-releasing effect of L-Arg was later shown to require GHRH, based on studies showing the GH response to L-Arg was severely blunted in humans pretreated with GHRH antagonists (20–22). This finding could be interpreted to mean L-Arg may also stimulate hypothalamic GHRH output, perhaps directly or indirectly, by removing SST inhibition of GHRH-producing neurons (23, 24). However, the fact that high-dose GHRH effects are enhanced in combination with L-Arg (19) and that SST directly blocks GHRH-stimulated GH release in vitro (25) indicates that L-Arg could rapidly reduce hypothalamic SST tone, allowing for the unimpeded stimulatory actions of endogenous GHRH on GH release.

In order to confirm the role of endogenous SST in mediating the in vivo actions of L-Arg, circulating GH levels were compared in WT and SST-KO mice under basal conditions and after L-Arg challenge. In both male/female WT mice, but not SST-KO mice, a bolus injection of L-Arg dramatically increased circulating GH levels. A direct pituitary effect of L-Arg on in vivo GH release in WT mice is unlikely, based on the modest increase in GH levels observed in primary pituitary cell cultures treated with L-Arg. In line with this, it has been previously reported in a different strain of SST-KO mice (26) that pituitary GH content is reduced and, therefore, it is possible that the pituitary somatotrope of SST-KO mice may not have sufficient GH stores to respond to secretagogue challenge.

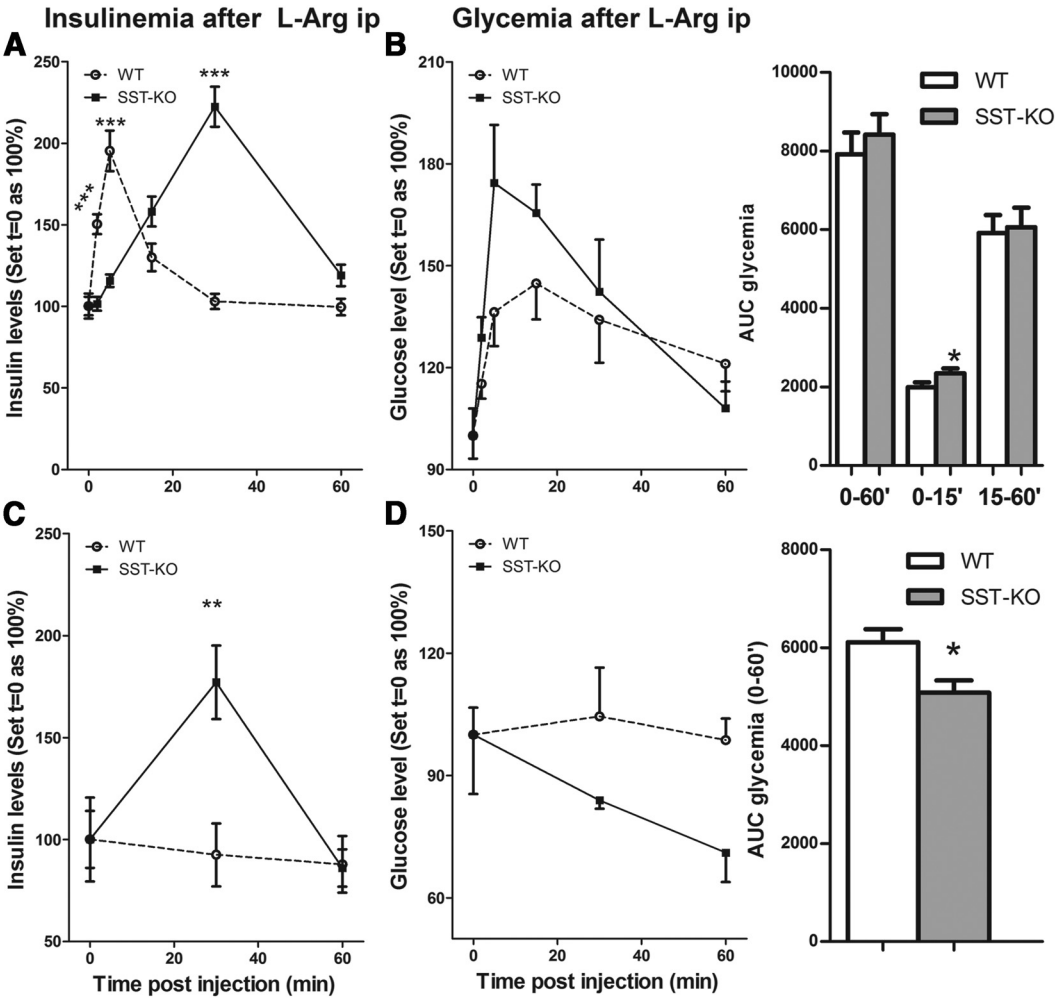


**Figure 2.** Relative Insulin (A and C) and Glucose (B and D) Response to L-Arg (ip; 0.8 g/kg) in Fasted 4 (A and B) and 7 (C and D) Month-Old WT and SST-KO Male Mice Values are shown as means ± SEM of 5–8 mice per genotype. Asterisks indicate differences between WT and SST-KO. *, $P < .05$; **, $P < .01$; ***, $P < .001$. AUC, area under the curve.

However, this possibility is lessened by the fact that pulse release is maintained in SST-KO mice (27) and the fact that primary pituitary cell cultures from SST-KO mice respond normally to GHRH challenge (Supplemental Figure 1 published on The Endocrine Society's Journals Online web site at http://endo.endojournals.org). Therefore, our current observation, together with previous studies (1, 2, 6, 7), supports the hypothesis that the primary mechanism by which L-Arg acutely increases GH in vivo is by lowering endogenous hypothalamic SST input to the pituitary and not via direct pituitary effects.

## Role of endogenous SST in basal and L-Arg-stimulated insulin release

The postprandial rise in circulating glucose stimulates pancreatic β-cells to release insulin and blocks the ability of α-cells to release glucagon. Insulin, in turn, is thought to stimulate δ-cells to release SST, which suppresses both insulin/glucagon release. However, recent data indicate that factors other than insulin may be involved in the glucose-induced rise in SST (28). Nevertheless, the critical role that SST plays in the paracrine regulation of islet function is supported by studies showing enhanced glucose-stimulated insulin secretion (GSIS) in islets from SST-KO mice (13). Enhanced GSIS is also observed in vivo in mice lacking SST or SST receptors (29–31). However, the increase in GSIS in SST/SST receptor-modified mice is age- and sex dependent and is not always associated with an increase in basal insulin levels. In the current report, we did observe a modest but significant increase in fasted insulin levels in male SST-KO, whereas no change in glucose levels was observed (similar to a previous report) (32). This may be due to the fact that SST-KO mice display enhanced glucagon release (13), which might counteract peripheral insulin actions. However, we cannot discount the possibility that the elevated GH, ghrelin, and glucocorticoids observed in SST-KO mice (17, 33) may play a role in glucose homeostasis.

L-Arg, like glucose, is a well-known direct stimulator of insulin release, an effect that could be mediated through activation of the GPRC6A receptor, although this remains controversial (34, 35). L-Arg also rapidly stimulates the release of glucagon and SST (13, 14). In the current report, we show that the insulin response to L-Arg is dramatically altered in SST-KO. Specifically, they lack the first-phase insulin release observed in WT mice. Given that L-Arg has been shown to act directly on β-cells to stimulate insulin release, these data suggest that the SST-KO environment leads to β-cell dysfunction. One factor that may contribute to this dysfunction in SST-KO mice is elevated ghrelin (32), in which ghrelin has been shown to attenuate the glucose-mediated rise in intracellular [$Ca^{2+}$] in β-cells and to reduce in vivo/in vitro insulin release (36–38). Despite the lack of first-phase insulin release, second-phase insulin release was dramatically enhanced in SST-KO. It is unlikely that this effect is directly due to L-Arg-stimulated insulin release, because L-Arg would be rapidly cleared from the system after ip injection. However, one possible secondary effector could be glucagon, because SST-KO has been shown to have enhanced L-Arg-induced glucagon release (13), and glucagon is known to directly stimulate insulin secretion (39). Although the importance of locally produced SST in regulating islet homeostasis is clear, we cannot rule out the possibility that loss of hypothalamic SST also impacts islet homeostasis. This possibility is supported by early studies showing that central infusion of SST suppresses insulin, leading to hyperglycemia, and ventral medial hypothalamic lesion results in hyperinsulinemia (40).

Despite the L-Arg-induced rise in insulin, L-Arg also induced an acute rise in glucose levels, an effect that was more pronounced in SST-KO. This differential response may also be due to enhanced glucagon release (13, 14, 41). It should be noted that in the younger SST-KO mice (4 mo of age) glucose remained elevated 30 minutes after L-Arg injection, whereas in 8 month-old SST-KO mice, mild hypoglycemia was observed at 30 and 60 minutes after L-Arg injection, consistent with the elevated insulin levels observed in these mice. These differences might be simply due to the age of the mice. They also could be due to experimental processes, in which older mice were only briefly restrained at each time point (0, 30, and 60 min) for blood collection, whereas younger mice were restrained from 0–15 minutes after L-Arg injection, in order to obtain more frequent blood samples, and prolonged restraint stress has been shown to increase glucose levels in rodents (42).

Taken together, this work demonstrates the critical role of endogenous SST in mediating the effects of L-Arg on GH and insulin secretion in vivo.

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## References

1. **Alba-Roth J, Müller OA, Schopohl J, von Werder K .** Arginine stimulates growth hormone secretion by suppressing endogenous somatostatin secretion. *J Clin Endocrinol Metab*. 1988;67(6):1186–1189.
2. **Anderson LL, Ford JJ, Klindt J, Molina JR, Vale WW, Rivier J.** Growth hormone and prolactin secretion in hypophysial stalk-transected pigs as affected by growth hormone and prolactin-releasing and inhibiting factors. *Proc Soc Exp Biol Med*. 1991; 196(2):194–202.
3. **Kimura S, Kameyama K, Hosoi E, Saito S .** [The effects of PGE2, 2-DG and L-arginine on hypothalamic GHRH and SRIF releases in conscious rats, with respect to GH secretion]. *Nihon Naibunpi Gakkai Zasshi*. 1989;65(2):113–127.
4. **Nakano T, Harano Y, Emura J, Kimura T, Sakakibara S, Shigeta Y .** Development of specific and non-specific somatostatin analogs. *Horm Metab Res*. 1986;18(2):98–102.
5. **Valverde I, Peñalva A, Ghigo E, Casanueva FF, Dieguez C.** Involvement of nitric oxide in the regulation of growth hormone secretion in dogs. *Neuroendocrinology*. 2001;74(4):213–219.
6. **Mori T, Inoue S, Egawa M, Takamura Y, Minami S, Wakabayashi I.** Impaired growth hormone secretion in VMH lesioned rats. *Int J Obes Relat Metab Disord*. 1993;17(6):349–353.
7. **Masuda A, Shibasaki T, Hotta M, et al.** Insulin-induced hypoglycemia, L-dopa and arginine stimulate GH secretion through different mechanisms in man. *Regul Pept*. 1990;31(1):53–64.
8. **Rubinek T, Rubinfeld H, Hadani M, Barkai G, Shimon I.** Nitric oxide stimulates growth hormone secretion from human fetal pituitaries and cultured pituitary adenomas. *Endocrine*. 2005;28(2):209–216.
9. **Adrião M, Chrisman CJ, Bielavsky M, Olinto SC, Shiraishi EM, Nunes MT .** Arginine increases growth hormone gene expression in rat pituitary and GH3 cells. *Neuroendocrinology*. 2004;79(1):26–33.
10. **Villalobos C, Núñez L, Garcia-Sancho J.** Mechanisms for stimulation of rat anterior pituitary cells by arginine and other amino acids. *J Physiol*. 1997;502(Pt 2):421–431.
11. **Cejvan K, Coy DH, Holst JJ, Cerasi E, Efendic S .** Gliclazide directly inhibits arginine-induced glucagon release. *Diabetes*. 2002; 51(Suppl 3):S381–S384.
12. **Guenifi A, Ahrén B, Abdel-Halim SM.** Differential effects of glucagon-like peptide-1 (7–36)amide versus cholecystokinin on arginine-

induced islet hormone release in vivo and in vitro. *Pancreas*. 2001; 22(1):58–64.
13. **Hauge-Evans AC, King AJ, Carmignac D, et al.** Somatostatin secreted by islet δ-cells fulfills multiple roles as a paracrine regulator of islet function. *Diabetes*. 2009;58(2):403–411.
14. **Hauge-Evans AC, King AJ, Fairhall K, Persaud SJ, Jones PM .** A role for islet somatostatin in mediating sympathetic regulation of glucagon secretion. *Islets*. 2010;2(6):341–344.
15. **Sako Y, Wasada T, Umeda F, Ibayashi H .** Effect of glibenclamide on pancreatic hormone release from isolated perifused islets of normal and cysteamine-treated rats. *Metabolism*. 1986;35(10):944–949.
16. **Zeyda T, Diehl N, Paylor R, Brennan MB, Hochgeschwender U.** Impairment in motor learning of somatostatin null mutant mice. *Brain Res*. 2001;906(1–2):107–114.
17. **Luque RM, Gahete MD, Hochgeschwender U, Kineman RD.** 2006 Evidence that endogenous SST inhibits ACTH and ghrelin expression by independent pathways. *Am J Physiol Endocrinol Metab*. 291(2):E395–E403.
18. **Buchmann I, Milakofsky L, Harris N, Hofford JM, Vogel WH.** Effect of arginine administration on plasma and brain levels of arginine and various related amino compounds in the rat. *Pharmacology*. 1996;53(3):133–142.
19. **Gasco V, Corneli G, Rovere S, et al.** Diagnosis of adult GH deficiency. *Pituitary*. 2008; 11(2):121–128.
20. **Ocampo-Lim B, Guo W, DeMott-Friberg R, Barkan AL, Jaffe CA.** Nocturnal growth hormone (GH) secretion is eliminated by infusion of GH-releasing hormone antagonist. *J Clin Endocrinol Metab*. 1996;81(12):4396–4399.
21. **Hanew K, Utsumi A .** The role of endogenous GHRH in arginine-, insulin-, clonidine- and l-dopa-induced GH release in normal subjects. *Eur J Endocrinol*. 2002;146(2):197–202.
22. **Nass R, Pezzoli SS, Chapman IM, et al.** IGF-I does not affect the net increase in GH release in response to arginine. *Am J Physiol Endocrinol Metab*. 2002;283(4):E702–E710.
23. **Luque RM, Park S, Kineman RD.** Role of endogenous somatostatin in regulating GH output under basal conditions and in response to metabolic extremes. *Mol Cell Endocrinol*. 2008;286(1–2):155–168.
24. **Mori K, Kim J, Sasaki K .** Electrophysiological effect of ghrelin and somatostatin on rat hypothalamic arcuate neurons in vitro. *Peptides*. 2010;31(6):1139–1145.
25. **Córdoba-Chacón J, Gahete MD, Culler MD, Castãno JP, Kineman RD, Luque RM.** Somatostatin dramatically stimulates growth hormone release from primate somatotrophs acting at low doses via somatostatin receptor 5 and cyclic AMP. *J Neuroendocrinol*. 2012; 24(3):453–463.
26. **Low MJ, Otero-Corchon V, Parlow AF, Ramirez JL, et al.** Somatostatin is required for masculinization of growth hormone-regulated hepatic gene expression but not of somatic growth. *J Clin Invest*. 2001;107(12):1571–1580.
27. **Low MJ, Rubinstein M, Otero-Corchon V.** Altered patterns of growth hormone secretion in somatostatin knockout mice. In: Srikant CB, ed. *Somatostatin*. Norwell, MA: Kluwer Academic Publishers; 2004:65–80.
28. **Hauge-Evans AC, Anderson RL, Persaud SJ, Jones PM** δ-Cell secretory responses to insulin secretagogues are not mediated indirectly by insulin. *Diabetologia*. 2012;55(7):1995–2004.
29. **Wang XP, Norman M, Yang J, et al.** The effect of global SSTR5 gene ablation on the endocrine pancreas and glucose regulation in aging mice. *J Surg Res*. 2005;129(1):64–72.
30. **Wang XP, Norman M, Yang J, et al.** Alterations in glucose homeostasis in SSTR1 gene-ablated mice. *Mol Cell Endocrinol*. 2006; 247(1–2):82–90.
31. **Wang XP, Norman MA, Yang J, et al.** Double-gene ablation of SSTR1 and SSTR5 results in hyperinsulinemia and improved glucose tolerance in mice. *Surgery*. 2004;136(3):585–592.
32. **Córdoba-Chacon J, Gahete MD, Pozo-Salas AI, et al.** Cortistatin is not a somatostatin analogue but stimulates prolactin release and inhibits GH and ACTH in a gender-dependent fashion: potential role of ghrelin. *Endocrinology*. 2011;152(12):4800–4812.
33. **Luque RM, Kineman RD.** Gender-dependent role of endogenous somatostatin in regulating growth hormone-axis function in mice. *Endocrinology* 2007;148(12):5998–6006.
34. **Pi M, Wu Y, Lenchik NI, Gerling I, Quarles LD.** GPRC6A mediates the effects of L-arginine on insulin secretion in mouse pancreatic islets. *Endocrinology* 2012;153(10):4608–4615.
35. **Smajilovic S, Clemmensen C, Johansen LD, et al.** The L-α-amino acid receptor GPRC6A is expressed in the islets of Langerhans but is not involved in L-arginine-induced insulin release. *Amino Acids*. 2013;44(2):383–390.
36. **Dezaki K, Hosoda H, Kakei M, et al.** Endogenous ghrelin in pancreatic islets restricts insulin release by attenuating Ca2+ signaling in β-cells: implication in the glycemic control in rodents. *Diabetes*. 2004;53(12):3142–3151.
37. **Egido EM, Rodriguez-Gallardo J, Silvestre RA, Marco J.** Inhibitory effect of ghrelin on insulin and pancreatic somatostatin secretion. *Eur J Endocrinol*. 2002;146(2):241–244.
38. **Park S, Jiang H, Zhang H, Smith RG.** Modification of ghrelin receptor signaling by somatostatin receptor-5 regulates insulin release. *Proc Natl Acad Sci USA*. 2012;109(46):19003–19008.
39. **Wang Q, Liang X, Wang S** Intra-islet glucagon secretion and action in the regulation of glucose homeostasis. *Front Physiol*. 2012;3:485
40. **Frohman LA.** CNS peptides and glucoregulation. *Annu Rev Physiol*. 1983;45;95–107.
41. **Strowski MZ, Parmar RM, Blake AD, Schaeffer JM.** Somatostatin inhibits insulin and glucagon secretion via two receptors subtypes: an in vitro study of pancreatic islets from somatostatin receptor 2 knockout mice. *Endocrinology*. 2000;141(1):111–117.
42. **Tabata H, Kitamura T, Nagamatsu N.** Comparison of effects of restraint, cage transportation, anaesthesia and repeated bleeding on plasma glucose levels between mice and rats. *Lab Anim*. 1998;32(2): 143–148.