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Effects of L-arginine on growth hormone and insulin-like growth factor 1

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Abstract L-Arginine has been reported to promote cellular and organismal growth. In this study, the effects of L-arginine on the expression of growth hormone (GH) and insulin-like growth factor 1 (IGF-1), the two key growth factors, are investigated in cultured GH3 pituitary epithelium and HepG2 cells, respectively. L-Arginine significantly induced the gene expression of GH and IGF-1 in GH3 pituitary epithelium and HepG2 hepatocytes respectively, and reduced IGF binding protein-1 gene expression in HepG2 cells assessed via quantitative polymerase chain reaction analysis. L-Arginine also significantly induced GH and IGF-1 hormone secretion from GH3 and HepG2 cells, respectively. In addition, the multi-target ELISA analysis conducted revealed that phosphorylation of p-38 MAPK, MEK, and JNK were significantly increased in HepG2 cells, suggesting L-arginine-induced activation of the MAPK signaling pathway. These results suggest that L-arginine promotes the synthesis and secretion of GH and IGF-1 in vitro and induces the MAPK signaling cascade in cultured hepatocytes.

Keywords L-Arginine · Growth hormone · Insulin-like growth factor 1 · Mitogen-activated protein kinase

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Introduction

Cellular growth is a complex biological event that includes regulation of cell proliferation, differentiation, and metabolism. Growth hormone (GH) and insulin-like growth factor 1 (IGF-1) are key growth stimulating hormones in mammals [1]. GH is a master regulator to stimulate cell proliferation, growth, reproduction, and regeneration and is synthesized and secreted from somatotropic cells within the anterior pituitary gland [2]. The binding of GH to GH-receptors in the hepatocytes stimulates the Janus kinase/signal transducer and activator of transcription signaling pathway [3] and then induces the synthesis and secretion of IGF-1, a proinsulin, in the liver [4]. IGF-1 is targeted to various tissues and it promotes organismal growth by inducing cell proliferation and regulation of energy metabolism [5]. Accordingly, injection of GH and IGF-1 in animals has demonstrated organismal growth in vivo. IGF binding protein 1 (IGFBP-1) plays a role in regulation of IGF-1 activity; its binding with IGF-1 in the hepatocytes represses secretion of IGF-1 into the circulation [6]. Several cellular signaling pathways regulate cell proliferation. The PI3K/Akt/mTOR signaling axis [7] and MAPK pathway have been reported as major cell proliferation signaling pathways [8]. In particular, the MAPK pathway can be regulated by several signaling molecules including Src homology and collagen; Ras and Raf; and transcription factors such as c-Jun [9], c-Fos [10], Elk [11], and c-Myc [12] to induce the target gene expressions required for cell proliferation.

Cellular growth is stimulated by several amino acids. L-Arginine is a semi-essential amino acid required for infants and child growth that promotes GH secretion by inhibiting somatostatin, which suppresses the GH-releasing hormone [13]. L-Arginine has been reported to stimulate protein

synthesis by activating the mTORC1 signaling pathway via activation of the PI3K pathway [14, 15]; however, alternative mechanisms of L-arginine for promoting cell proliferation and growth are elusive. In this study, we examined the effect of L-arginine on the expression and secretion of GH and IGF-1, two key growth hormones, on GH3 pituitary epithelium and HepG2 hepatocytes, respectively. Gene expression and hormone secretion were quantified and the primary cellular signaling pathways regulated by L-arginine were studied in cultured hepatocytes.

Materials and methods

Cell culture and treatment

GH3 and HepG2 cells were obtained from Korean Cell Line Bank (Seoul, Korea) and were cultured in the Dulbecco's Modified Eagle Medium (DMEM; Hyclone, Logan, UT, USA) containing 10% heat-inactivated fetal bovine serum (FBS; Hyclone) and 1% penicillin/streptomycin (PEST; Welgene Inc., Seoul, Korea). Cells were maintained at 37 °C in a humidified atmosphere of 5% CO_2. L-Arginine and β-estradiol (a positive control) were purchased from Sigma-Aldrich (St. Louis, MO, USA). L-Arginine (1 M) and β-estradiol (100 μM) were dissolved in doubly distilled water and dimethyl sulfoxide (DMSO), respectively, as stock solutions and were stored at − 20 °C before treatment. GH3 and HepG2 cells were cultured in a six-well plate and incubated in serum-free media with L-arginine (5, 10, and 25 mM), β-estradiol (100 nM), or vehicle (water) for 24 h.

Quantitative polymerase chain reaction (qPCR) analysis

Total RNA was extracted from GH3 and HepG2 cells using RNAiso Plus, a total RNA extraction solution (Takara, Otsu, Japan), according to a previously defined method [16]. The cDNA was synthesized from 2 μg of total RNA using M-MLV reverse transcriptase (Mbiotech, Seoul, Korea) and oligo (dT). Gene expression levels were measured using a Bio-Rad iQ5 iCycler system with RealMasterMix SYBR ROX (5 Prime, Hamburg, Germany). The primer sequences were as follows: rat GH forward 5′-AGGGCATCCAGGCTCTGAT-3′ and reverse 5′-GCATGTTGGCGTCAAACTTG-3′; rat β-actin forward 5′-CATGTACGTTGCTATCCAGGC-3′ and reverse 5′-CTCCTTAATGTCACGCACGAT-3′; human IGF-1 forward 5′-GCTCTTCAGTTCGTGTGTG-3′ and reverse 5′-GACTTGGCAGGCTTGAGG-3′; human IGFBP-1 forward 5′-TATGATGGCTCGAAGGCTCT-3′ and reverse 5′-TAGACGCACCAGCAGAGTC-3′; and human β-actin forward 5′-CCTTCTTGGGTATGGAATCC-3′ and reverse 5′-TCTGCATCCTGTCAGCAATG-3′. The reaction conditions for rat primers were 95 °C for 4.5 min, followed by 40 cycles of 95 °C for 10 s, 55 °C for 30 s, and 68 °C for 20 s, and those for human primers were 95 °C for 10 min, followed by 40 cycles of 95 °C for 15 s, 60 °C for 1 min, and 68 °C for 20 s. Gene expression levels were calculated using iQ5 Optical System software (version 2; Bio-Rad), employing the expression levels of β-actin as reference.

Quantification of GH and IGF-1 secretion

The concentrations of GH (Invitrogen, Camarillo, CA, USA) and IGF-1 (Koma Biotech, Seoul, Korea) were quantified using enzyme-linked immunosorbent assays. In brief, cells were cultured in a six-well plate with 1 mL of culture medium and stimulated with L-arginine (5, 10, and 25 mM), β-estradiol (100 nM), or vehicle for 24 h. GH and IGF-1 secretion was then quantified in the culture media of GH3 and HepG2 cells, respectively. The hormone levels in the sample were calculated according to the standard curve produced with recombinant hormones supplied by the manufacturers. The absorbance of samples was measured using a microplate reader (Bio-Rad Laboratories Inc., Hercules, CA, USA) at 450 nm.

MAPK signaling analysis

Effects of L-arginine on MAPK pathways were assessed using a PathScan multi-target ELISA kit (Cell Signaling Technology, Beverly, MA, USA). Multi-target ELISA quantifies protein expression and phosphorylation of multiple proteins in the MAPK pathway including MEK, ERK, p38 and JNK. In the conducted experiment, HepG2 cells were incubated in serum-free media containing L-arginine (100 μM) for 15 min. Cell lysates were harvested from vehicle (distilled water)-treated controls and L-arginine-treated cells. Measurements of the total- and phosphoforms of proteins in the MAPK pathway were quantified using an ELISA-based method according to the protocol of the manufacturer. The absorbance of samples was read using a Model 680 microplate reader (Bio-Rad) at 450 nm.

MEK, p38 MAPK, and JNK inhibitor treatment

HepG2 cells were seeded in a six-well plate overnight and incubated using a 20-μM MEK inhibitor PD98059 (Cayman, MI, USA), 20-μM p38 MAPK inhibitor SB203580 (Cayman), or 20-μM JNK inhibitor SP600125 (Cayman). After 6 h, cells were treated with L-arginine (10 mM) or vehicle (water) for 24 h in the presence of inhibitors. Total RNA was extracted from cells and used for qPCR analysis.

Statistical analysis

Data are presented as the mean ± standard error of the mean (SEM). ANOVA and *t* test were used for multiple ($n > 2$) and two-group comparison, respectively. $P < 0.05$ was considered statistically significant.

Results and discussion

The GH and IGF-1 are major hormones for regulating growth such as cell proliferation, development, differentiation in multiple tissues including bone and muscle [17–20], thereby promoting growth performance.

The effect of L-arginine on the gene expressions of GH and IGF-1 was examined in cultured cells. The mRNA expressions of GH and IGF-1 were quantified via qPCR in GH3 and HepG2 cells, respectively, after stimulation with L-arginine for 24 h. In GH3 cells, GH mRNA expression levels significantly increased 2.11 and 4.88 fold when using 10 and 25 mM L-arginine compared with those of the control, respectively (Fig. 1A). In HepG2 cells, IGF-1 mRNA expression levels increased by 2.79 and 3.65 fold when using 10 and 25 mM L-arginine compared with those of the control, respectively (Fig. 1B). However, in HepG2 cells, the IGFBP-1 mRNA expression levels reduced 0.40 and 0.51 fold when using 10 and 25 mM L-arginine, respectively, compared with those of the control (Fig. 1C). β-estradiol, a positive control, increased GH and IGF-1 gene expression levels 4.65 and 2.94 fold, respectively, and reduced IGFBP-1 gene expression levels 0.54 fold when using 100 nM L-arginine compared with those of the control (Fig. 1A, C). These results suggest that L-arginine induces the expression of GH and IGF-1 genes and inhibits IGFBP-1 gene expression in vitro.

Next, we investigate the effect of L-arginine on the secretion of GH and IGF-1 hormones from GH3 and HepG2 cells, respectively. Hormone levels were quantified in the culture media after stimulating cells with L-arginine for 24 h. Stimulation with 10 and 25 mM of L-arginine significantly increased GH secretion 1.08 and 1.58 fold, respectively. β-Estradiol, a positive control, also improved protein level (1.66 fold) in the medium at 100 nM compared with that of the control (Fig. 2A). In HepG2 cells, IGF-1 secretion increased 1.61 and 2.09 fold via L-arginine stimulation at 10 mM and 25 mM, respectively, and β-estradiol increased IGF-1 gene expression levels 4.33 fold at 100 nM (Fig. 2B). These results suggest that L-arginine induces GH and IGF-1 hormone secretion in cultured cells after induction of their gene expressions.

Inducing mRNA expressions of subsequent secretion of IGF-1 could stimulate cellular grown of hepatocytes activated via MAPK signaling pathways. We next investigate whether L-arginine could stimulate MAPK pathways in HepG2 cells. Since several signaling options are known in MAPK pathways on cellular growth, we performed MAPK

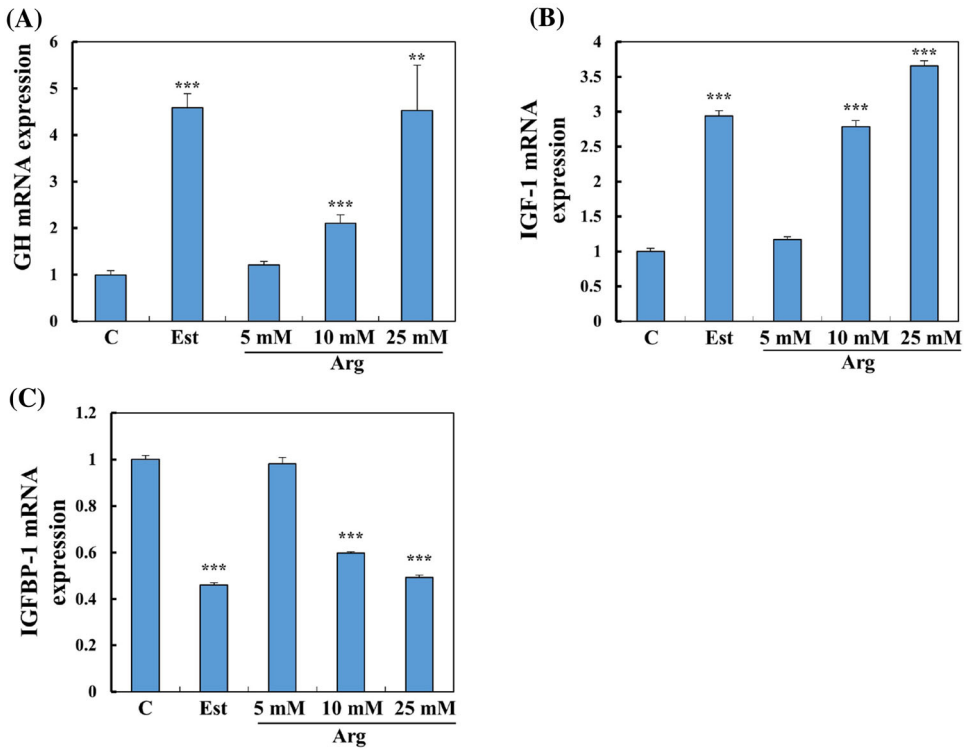

Fig. 1 The gene expression of GH, IGF-1, and IGFBP-1 in cells stimulated with L-arginine. (**A**) The mRNA expression levels of GH were quantified in GH3, (**B**, **C**) the IGF-1 and IGFBP-1 expression levels in HepG2 cells, respectively, after treatment with L-arginine for 24 h. C, vehicle-treated (doubly distilled water) negative control; Est, 100 nM estradiol (positive control); Arg, L-arginine. $^{**}P < 0.01$; $^{***}P < 0.001$ versus the control group for the ANOVA test

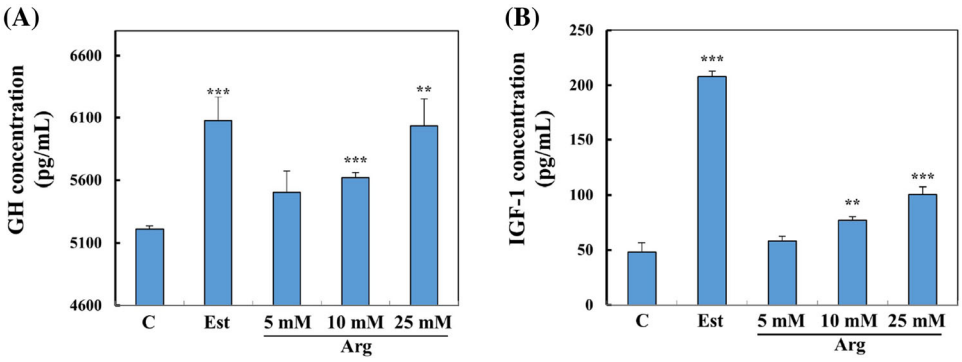

Fig. 2 The effects of L-arginine on GH and IGF-1 secretion. (**A**) GH hormone concentration in GH3 cells, (**B**) IGF-1 concentration in HepG2 cells. Vehicle-treated (doubly distilled water) negative control; Est, 100 nM estradiol (positive control); Arg, L-arginine. $^{**}P < 0.01$; $^{***}P < 0.001$ versus the control group for the ANOVA test

multi-target ELISA to simultaneously assess the levels of total proteins and phosphorylation status of the MAPK signaling cascade. Results revealed that phosphorylation of MEK1/2 (Ser217/221), which is upstream kinase of MAPKs, significantly increased (2.15 fold) via 100 μM L-arginine stimulation at 15 min compared with the levels at 0 min (Fig. 3A). Phosphorylation of p38 MAPK (Thr180/Tyr182) and JNK (Thr183/Tyr185) also significantly increased (1.45 and 6.15 fold, respectively) after 15 min of L-arginine stimulation compared with the levels at the baseline (Fig. 3B). The presence of unphosphorylated forms of MEK1 decreased with an increase in phosphorylated forms (Fig. 3A). To evaluate the effects of L-arginine on GH and IGF-1 mediated via MEK, p38 MAPK, and JNK signaling, we performed MEK, p38 MAPK, and JNK inhibitor experiments in HepG2 cells. The effects of L-arginine on IGF-1 induction and IGFBP-1 reduction were completely abrogated in HepG2 cells treated with MEK, p38 MAPK, and JNK inhibitors (Fig. 3C). These results suggest that L-arginine triggers MEK, p38 MAPK, and JNK signaling pathway to promote cell proliferation in HepG2 cells.

GH and IGF-1 regulate energy metabolism to provide energy molecules required for ATP synthesis in cellular and organismal growth [21]. For example, GH induces triglyceride lipolysis in visceral adipose tissue and subcutaneous adipose tissue and increases the release of circulatory free fatty acids [22]. Therefore, blood free fatty acids are shuttled to be consumed tissues on growth. These direct actions of GH are regulated by the MAPK cascade [23].

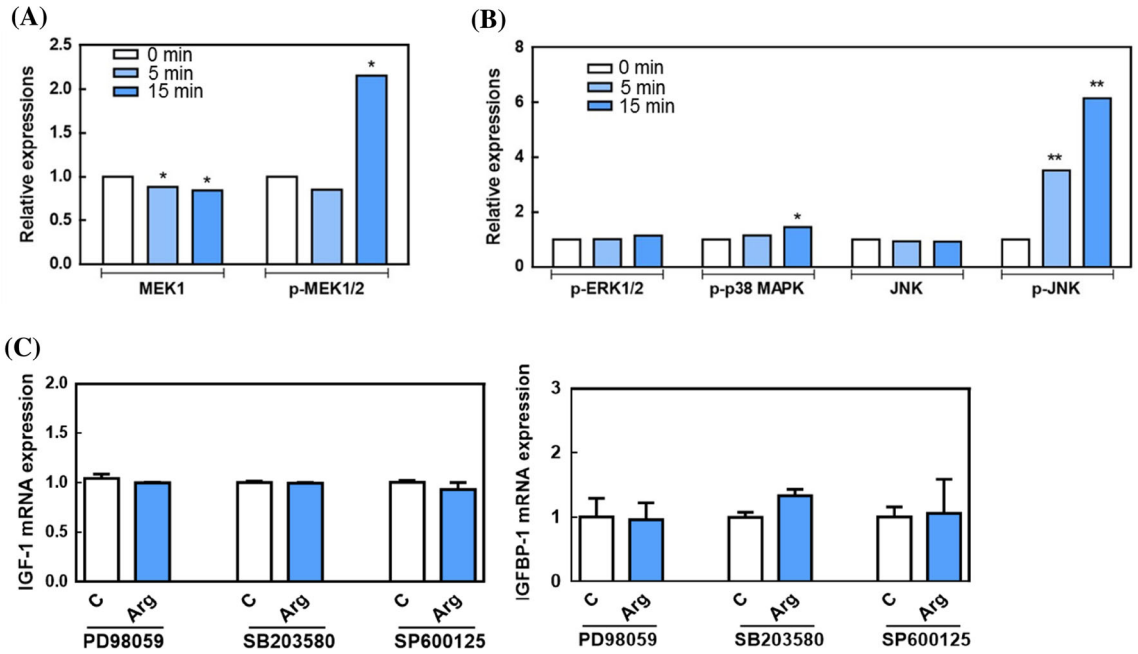

Fig. 3 Activation of the MEK, p38 MAPK, and JNK signaling pathways by L-arginine in HepG2 cells. (**A**) MEK1 and p-MEK1/2 expression levels. (**B**) p-ERK1/2, p-p38 MAPK, JNK, and p-JNK expression levels. (**C**) The mRNA expression levels of IGF-1 and IGFBP-1 expressions in HepG2 cells treated using a 20-μM MEK inhibitor PD98059, 20-μM p38 MAPK inhibitor SB203580, or 20-μM JNK inhibitor SP600125. Arg, 10 mM L-arginine. $^{*}P < 0.05$; $^{**}P < 0.01$ versus the control group for the ANOVA test. The expression levels of IGF-1 and IGFBP-1 were tested using the *t*-test

Initiation of growth stimulation, however, is mainly caused by IGF-1, which is induced by GH. GH activates the JAK-STAT signaling axis by binding to a GH receptor in hepatocytes and subsequently induces IGF-1 synthesis [24]. The secreted IGF-1 promotes cellular growth of the target tissues including cartilage, muscle, bone, kidneys, lungs, skin, and nerves via activation of PI3K/Akt/mTOR signaling axis [25]. As such, GH and IGF-1 are major growth-promoting hormones in organisms.

The results of our study show that L-arginine significantly increased GH gene expression levels in GH3 cells and induced IGF-1 while suppressing the IGFBP-1 mRNA expression levels in HepG2 cells (Fig. 1). These in turn induced the secretion of GH and IGF-1 hormones from GH3 and HepG2 cells, respectively, suggesting that L-arginine is a positive regulator for GH and IGF-1 synthesis and secretion. These effects of L-arginine are similar to those of β-estradiol, although the potency of former is weaker (Fig. 2).

In mammals, L-arginine is known as a precursor of nitric oxide production [26] of which nitric oxide is an important cellular signaling molecule and neurotransmitter that modulates cGMP-dependent signaling cascades to perform various physiological functions including vasodilation and protection of ischemic damage of the liver [27, 28]. In addition to nitric-oxide production, L-arginine stimulates cellular and organismal growth via activation of the mTOR pathway [15]. This study examines the role of L-arginine on growth, which stimulates mRNA expression and secretion of GH and IGF-1 from pituitary epithelium and hepatocytes, respectively, and activates the MEK, p38 MAPK, and JNK signaling pathways in HepG2 cells.

This study suggests that L-arginine may have dual functions in cell proliferation and tissue growth. First, L-arginine directly activated the MEK, p38 MAPK, and JNK signaling cascades as a short-term effect, as shown through 15-min stimulation. Second, L-arginine induced GH and IGF-1 mRNA expressions and subsequent secretion, as seen via a long-term treatment (24 h).

First, short-term stimulation of L-arginine induced phosphorylation of MEK (MAPK kinase), JNK, and p38 MAPK (MAPK super family). Phosphorylation of ERK (p44/42 MAPK), a kinase in the MAPK family, was also increased; however, it was nonsignificant. ERK is a MAPK protein that when phosphorylated by MEK1/2 promotes genes expression related to proliferation and differentiation by activating transcription factors such as Elk-1. JNK and p38 are stimulated by both stress response and growth factors; however, different signaling pathways share some target proteins. For example, JNK is activated by MEK4/7 and phosphorylates c-Jun, ATF2, and Elk-1, while p38 is phosphorylated by MEK3/6 and targets MK2, MK3, and ATF2. Therefore, the activation of ERK, JNK, and p38 by L-arginine found in this study indicates that L-arginine activates three major MAPK pathways, which collectively contributes to the induction of cell proliferation and tissue growth in hepatocytes. It is possible that L-arginine may directly interact with a growth hormone receptor or an upstream kinase in the MEK, p38 MAPK, and JNK signaling pathways as an allosteric regulator. The mechanism of L-arginine in the activation of multiple pathways in MEK, p38 MAPK, and JNK signaling should be further investigated in the future.

Second, long-term stimulation of GH3 and HepG2 cells with L-arginine induced mRNA expressions and hormone secretion [13]. In these cells, we confirmed the effect of L-arginine on GH expression reported by Adriao et al. [13], and we report a novel effect of L-arginine on IGF-1 gene expression and hormone secretion.

In summary, the results of this study suggest two novel functions of L-arginine of cellular growth. Treatment with L-arginine significantly increased GH and IGF-1 expression at both gene and protein levels, whereas that of IGFBP-1 decreased significantly in vitro. In addition, the analysis of the MAPK pathway demonstrated that these growth-promoting effects were linked to increased activation of the MEK, p38 MAPK, and JNK signaling cascade. Therefore, intake of L-arginine may be important for tissue and organismal growth.

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Compliance with ethical standards

Conflict of interest The authors declare that there is no conflict of interest.

References

1. Root AW. Neurophysiological regulation of the secretion of growth hormone. J. Endocrinol. Invest. 12: 3–7 (1989).
2. Ross RJ. GH, IGF-I and binding proteins in altered nutritional states. Int. J. Obes. Relat. Metab. Disord. 24 Suppl 2: S92–95 (2000).
3. Yamauchi T, Ueki K, Tobe K, Tamemoto H, Sekine N, Wada M, Honjo M, Takahashi M, Takahashi T, Hirai H, Tsushima T, Akanuma Y, Fujita T, Komuro I, Yazaki Y, Kadowaki T. Growth hormone-induced tyrosine phosphorylation of EGF receptor as an essential element leading to MAP kinase activation and gene expression. Endocr. J. 45: S27–31 (1998).
4. Nilsson A, Ohlsson C, Isaksson OG, Lindahl A, Isgaard J. Hormonal regulation of longitudinal bone growth. Eur. J. Clin. Nutr. 48: 150–160 (1994).
5. Butler AA, LeRoith D. Minireview: tissue-specific versus generalized gene targeting of the igf1 and igf1r genes and their roles in insulin-like growth factor physiology. Endocrinology 142: 1685–1688 (2001).

6. Kelley KM, Oh Y, Gargosky SE, Gucev Z, Matsumoto T, Hwa V, Ng L, Simpson DM, Rosenfeld RG. Insulin-like growth factor-binding proteins (IGFBPs) and their regulatory dynamics. Int. J. Biochem. Cell Biol. 28: 619–637 (1996).
7. Levine AJ, Feng Z, Mak TW, You H, Jin S. Coordination and communication between the p53 and IGF-1-AKT-TOR signal transduction pathways. Genes Dev. 20: 267–275 (2006).
8. Werner H, Le Roith D. The insulin-like growth factor-I receptor signaling pathways are important for tumorigenesis and inhibition of apoptosis. Crit. Rev. Oncog. 8: 71–92 (1997).
9. Lagarrigue S, Heberden C, Martel P, Gaillard-Sanchez I. The transformation of c-jun-overexpressing cells is correlated with IGFS-induced c-jun phosphorylation. Biochem. Biophys. Res. Commun. 217: 501–508 (1995).
10. Ong J, Yamashita S, Melmed S. Insulin-like growth factor I induces c-fos messenger ribonucleic acid in L6 rat skeletal muscle cells. Endocrinology 120: 353–357 (1987).
11. Delahunty KM, Shultz KL, Gronowicz GA, Koczon-Jaremko B, Adamo ML, Horton LG, Lorenzo J, Donahue LR, Ackert-Bicknell C, Kream BE, Beamer WG, Rosen CJ. Congenic mice provide in vivo evidence for a genetic locus that modulates serum insulin-like growth factor-I and bone acquisition. Endocrinology 147: 3915–3923 (2006).
12. Murphy LJ, Bell GI, Friesen HG. Growth hormone stimulates sequential induction of c-myc and insulin-like growth factor I expression in vivo. Endocrinology 120: 1806–1812 (1987).
13. Adriao M, Chrisman CJ, Bielavsky M, Olinto SC, Shiraishi EM, Nunes MT. Arginine increases growth hormone gene expression in rat pituitary and GH3 cells. Neuroendocrinology 79: 26–33 (2004).
14. Kong X, Tan B, Yin Y, Gao H, Li X, Jaeger LA, Bazer FW, Wu G. L-Arginine stimulates the mTOR signaling pathway and protein synthesis in porcine trophectoderm cells. J. Nutr. Biochem. 23: 1178–1183 (2012).
15. Yao K, Yin YL, Chu W, Liu Z, Deng D, Li T, Huang R, Zhang J, Tan B, Wang W, Wu G. Dietary arginine supplementation increases mTOR signaling activity in skeletal muscle of neonatal pigs. J. Nutr. 138: 867–872 (2008).
16. Jia Y, Kim JY, Jun HJ, Kim SJ, Lee JH, Hoang MH, Hwang KY, Um SJ, Chang HI, Lee SJ. The natural carotenoid astaxanthin, a PPAR-alpha agonist and PPAR-gamma antagonist, reduces hepatic lipid accumulation by rewiring the transcriptome in lipid-loaded hepatocytes. Mol. Nutr. Food Res. 56: 878–888 (2012).
17. Bakker NE, Kuppens RJ, Siemensma EP, Tummers-de Lind van Wijngaarden RF, Festen DA, Bindels-de Heus GC, Bocca G, Haring DA, Hoorweg-Nijman JJ, Houdijk EC, Jira PE, Lunshof L, Odink RJ, Oostdijk W, Rotteveel J, Van Alfen AA, Van Leeuwen M, Van Wieringen H, Wegdam-den Boer ME, Zwaveling-Soonawala N, Hokken-Koelega AC. Bone mineral density in children and adolescents with prader-willi syndrome: a longitudinal study during puberty and 9 years of growth hormone treatment. J. Clin. Endocrinol. Metab. 100: 1609–1618 (2015).
18. Gourmelen M, Le Bouc Y, Girard F, Binoux M. Serum levels of insulin-like growth factor (IGF) and IGF binding protein in constitutionally tall children and adolescents. J. Clin. Endocrinol. Metab. 59: 1197–1203 (1984).
19. Rubeck KZ, Bertelsen S, Vestergaard P, Jorgensen JO. Impact of GH substitution on exercise capacity and muscle strength in GH-deficient adults: a meta-analysis of blinded, placebo-controlled trials. Clin. Endocrinol. (Oxf). 71: 860–866 (2009).
20. Widdowson WM, Gibney J. The effect of growth hormone (GH) replacement on muscle strength in patients with GH-deficiency: a meta-analysis. Clin. Endocrinol. (Oxf). 72: 787–792 (2010).
21. Brown-Borg HM, Bartke A. GH and IGF1: roles in energy metabolism of long-living GH mutant mice. J. Gerontol. A Biol. Sci. Med. Sci. 67: 652–660 (2012).
22. Samra JS, Clark ML, Humphreys SM, MacDonald IA, Bannister PA, Matthews DR, Frayn KN. Suppression of the nocturnal rise in growth hormone reduces subsequent lipolysis in subcutaneous adipose tissue. Eur. J. Clin. Invest. 29: 1045–1052 (1999).
23. Lanning NJ, Carter-Su C. Recent advances in growth hormone signaling. Rev. Endocr. Metab. Disord. 7: 225–235 (2006).
24. Herrington J, Smit LS, Schwartz J, Carter-Su C. The role of STAT proteins in growth hormone signaling. Oncogene 19: 2585–2597 (2000).
25. Feng Z, Levine AJ. The regulation of energy metabolism and the IGF-1/mTOR pathways by the p53 protein. Trends Cell Biol. 20: 427–434 (2010).
26. Boger RH. The pharmacodynamics of L-arginine. Altern. Ther. Health Med. 20: 48–54 (2014).
27. Chausse AA, Nivet-Antoine V, Martin C, Clot JP, Galen FX. Protective effect of nitric oxide on isolated rat hepatocytes submitted to an oxidative stress. Metabolism 51: 175–179 (2002).
28. Luscher TF. Endothelium-derived nitric oxide: the endogenous nitrovasodilator in the human cardiovascular system. Eur. Heart J. 12 Suppl E: 2–11 (1991).