Egr1 mediates the effect of insulin on leptin transcription in adipocytes

Omar Mohtar, Cafer Ozdemir, Debashish Roy, Dharti Shantaram, Andrew Emili, and Konstantin V. Kandror

From the Department of Biochemistry, Boston University School of Medicine, Boston, Massachusetts 02118

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In mammals, leptin production in adipocytes is up-regulated by feeding and insulin. Although this regulatory connection is central to all physiological effects of leptin, its molecular mechanism remains unknown. Here, we show that the transcription factor early growth response 1, Egr1, is rapidly but transiently induced by insulin in adipose cells both in vitro and in vivo, and its induction is followed by an increase in leptin transcription. ChIP and luciferase assays demonstrate that Egr1 directly binds to and activates the leptin promoter. Interestingly, the lipid droplet protein FSP27 may work as a co-factor for Egr1 in regulating leptin expression. By using siRNA-mediated knockout of Egr1 along with its overexpression in adipocytes, we demonstrate that Egr1 is both necessary and sufficient for the stimulatory effect of insulin on leptin transcription.

Leptin, a 16-kDa product of the ob gene (1), is synthesized predominantly in adipocytes and targets the central nervous system. It has been established as a major metabolic regulator that controls food intake, energy expenditure, neuroendocrine functions, carbohydrate and lipid metabolism, and several other important physiological functions of the mammalian organism (2–5). Regardless of how leptin exerts its biological activity, it is essential that its production in adipocytes is coupled to nutrient uptake and energy status of the body as circulating leptin levels increase within hours after feeding and decrease shortly after food deprivation (3, 6–8). Although in humans, this effect may not be as fast and robust as in rodents, all mammals studied thus far demonstrate a direct link between food intake and circulating leptin (3). Based on these results, the predominant hypothesis in the field has been that leptin expression is controlled by nutrients and/or insulin. Indeed, multiple studies have shown that insulin increases leptin production by adipose cells both in vivo and in vitro (3). Although this regulatory connection is central to all proposed mechanisms of leptin action, its mechanism remains unknown. Here, we are showing that insulin/mTORC1-inducible transcription factor Egr1 binds to the leptin promoter and activates leptin expression in 3T3-L1 adipocytes.

Results

Treatment of differentiated 3T3-L1 adipocytes with insulin causes a strong but transient induction of Egr1 (see also Ref. 9) followed by an increase in the leptin mRNA (Fig. 1A). By the same token, a single intraperitoneal insulin injection induces Egr1 and leptin mRNA in epididymal fat pads (Fig. 1B) and in circulating leptin in vivo (Fig. 1C).

A luciferase assay performed in HEK 293T cells demonstrates that induction of Egr1 not only precedes leptin expression in adipocytes, but Egr1 can activate the leptin promoter (Fig. 2A). Furthermore, a ChIP assay carried out in differentiated 3T3-L1 adipocytes shows that Egr1 directly binds to the leptin promoter in an insulin-sensitive fashion (Fig. 2B).

Previously, we have found that the lipid droplet protein FSP27 (also known as CIDEC) represents a co-repressor of Egr1 (10). In agreement with these results, we now show that FSP27 blocks the effect of Egr1 on the activity of the leptin promoter (Fig. 2C).

To determine whether Egr1 is necessary for the effect of insulin on leptin transcription, we have knocked down Egr1 in 3T3-L1 adipocytes with the help of siRNA. As is shown in Fig. 2D, knockdown of Egr1 not only decreases expression of leptin in basal cells, but also completely blocks up-regulation of leptin mRNA by insulin. Importantly, Egr1 not only is necessary but also is sufficient for the up-regulation of leptin expression in adipocytes; overexpression of Egr1 in these cells with the help of adenoviral infection strongly increases levels of the leptin mRNA (Fig. 2E).

Previously, we have determined that treatment of cultured adipocytes with insulin stimulates expression of both Egr1 mRNA and protein. The MEK inhibitor PD98059 blocks insulin-stimulated increase in the Egr1 mRNA but has only a moderate effect on the Egr1 protein. Expression of the latter is completely suppressed by the mTORC1 inhibitor, PP242 (11), suggesting that the mTORC1-mediated pathway is essential for the activation of Egr1 expression by insulin. Here, we confirm this result and also show that transcription of leptin correlates with the expression of Egr1 in PD98059- and PP242-treated adipocytes (Fig. 3A).

To further prove the role of mTORC1 and Egr1 in the regulation of leptin transcription, we decided to mimic the effect of insulin specifically on the expression of Egr1 by deleting its highly structured 5′-UTR using the CRISPR/Cas9 technique (note the loss of the 2.5 kb band in the Δ5′UTR lane in Fig. 3B).
This procedure does not significantly change levels of the Egr1 mRNA (Fig. 3C), but it dramatically elevates expression of the Egr1 protein (Fig. 3D). Administration of insulin to the “engineered” cells has only a minimal stimulatory effect on the Egr1 protein levels, suggesting that deletion of the 5’-UTR raises its biosynthesis to the maximum. This result supports our previous conclusion that insulin increases translation of the Egr1 mRNA primarily via the mTORC1–4E-BP1/2 regulatory axis (11). As expected, the luciferase assay has demonstrated that the activity of the leptin promoter in these cells is strongly increased (Fig. 3E).

**Discussion**

In this study, we provide evidence that insulin/mTORC1-inducible transcription factor Egr1 mediates the physiologically significant stimulatory effect of insulin on leptin transcription. In analyzing these data, it is essential to keep in mind that leptin production by adipose cells is regulated at several different levels: transcription, translation, secretion, and autophagic degradation (12, 13). Importantly, both transcription (this report) and translation of leptin are positively regulated by insulin via the mTORC1-mediated pathway (14–16) and, therefore, should work in concert to deliver higher leptin amounts in response to nutrients and insulin. As activity of mTORC1 depends not only on insulin levels but also on nutrient and energy availability (17, 18), this model provides an additional physiological dimension to the regulation of leptin production. At the same time, mechanisms of regulated leptin secretion and degradation remain virtually unstudied but may substantially contribute to changes in circulating leptin. For example, Lee and Fried (19) have found that up to 50% of newly synthesized leptin molecules are rapidly degraded in lysosomes instead of being secreted.
The problem of leptin production has another important aspect. In addition to the short-term connection with food intake, circulating leptin levels are known to be steadily elevated in obesity (3, 4). At the cellular level, larger adipocytes contain and secrete more leptin than smaller cells (20, 21). Because the adipocyte size is defined primarily by the volume of the central lipid droplet, this phenomenon may show a cell-autonomous connection between the amount of stored energy (i.e., obesity at the molecular level) and leptin expression. The correlation between the size of the adipocyte and the level of leptin production has been recognized for a long time, but its mechanism remains obscure. It is unlikely that a single act of food intake can change the size of the adipocyte in a significant fashion, so there should be another explanation for this phenomenon.

To this end, it has been established that fat storage in adipocytes is also controlled by mTORC1. In particular, inhibition of mTORC1 signaling suppresses early adipogenesis (22–26) and/or lipogenesis (27–32). In parallel, mTORC1 inhibits lipolysis and promotes triglyceride storage in adipocytes via the Egr1-mediated transcriptional suppression of the rate-limiting lipolytic enzyme, ATGL (9, 31). Importantly, chronic overnutrition and obesity lead to continuous activation of mTORC1 (9, 33–35), which should promote triglyceride storage and increase the size of adipocytes on one hand and stimulate leptin production on the other. In other words, the size of the adipocyte may serve as an indicator of the cumulative mTORC1 activity that may explain the correlation between the adipocyte size and leptin production.

In addition, we and others have found that lipid droplets (LDs)2 can directly “talk” to the cell nucleus via LD proteins CIDEA (36), FSP27 (also known as CIDECA) (10), and perilipin 5 (37). According to our findings (10), FSP27 is not only localized on the surface of LDs, but also present in the cell nucleus, where it binds to and regulates transcriptional activity of Egr1. In agreement with these results, we now show that FSP27 blocks the effect of Egr1 on the activity of the leptin promoter (Fig. 2C). Thus, we hypothesize that FSP27 may link the size of LDs (that account for most of the adipocyte volume) to leptin expression by inhibiting Egr1. In other words, we suggest that increasing size of LDs may “pull” FSP27 out of the nucleus, which should lead to the activation of leptin expression via Egr1. Finally, when our manuscript was in preparation, it was reported that leptin may represent a direct transcriptional target of Egr1 in human breast cancer cells treated with TNFα (38), which is consistent with our results.

**Experimental procedures**

**Materials**

Rabbit monoclonal antibodies against β-actin (8457S), glyceraldehyde-3-phosphate dehydrogenase (14C10), Egr1 (4153S) and anti-rabbit IgG horseradish peroxidase–conjugated secondary antibody used for Western blotting were purchased from Cell Signaling (Beverly, MA). Polyclonal antibody against Egr1 for ChIP was purchased from Novus Biologicals (Centennial, CO) (NPB1-7877S). Anti-mouse IgG, human recombinant insulin (91077C), Nonidet P-40, and BSA were obtained from Sigma-Aldrich. Bovine serum and fetal bovine serum (FBS) were purchased from Atlanta Biologicals (Lawrenceville, GA). Dulbecco’s modified Eagle’s medium (DMEM), 0.25% trypsin-EDTA, Opti-MEM, Lipofectamine 2000, TRIZol® reagent, and sheep anti-mouse Dynabeads m-280 were purchased from Thermo Fisher Scientific. Sterile zirconium oxide beads mea-

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2 The abbreviations used are: LD, lipid droplet; FBS, fetal bovine serum; DMEM, Dulbecco’s modified Eagle’s medium; qPCR, quantitative PCR.
suring 1.0 mm in diameter and bullet homogenizer (Standard BBX24) were purchased from Next Advance (Troy, NY).

**Animals**

12–16-week-old male C57BL6j mice were obtained from Charles River Laboratories (Wilmington, MA) and acclimatized for 2 weeks with a 12-h light/day cycle. Prior to experiments, animals were housed in complete darkness (free-running) for 3 days with access to food and water ad libitum. On the fourth day, animals were fasted for 6 h and injected with insulin (2 units/kg) intraperitoneally. All experimental procedures were approved by the Boston University School of Medicine Animal Care and Use Committee.

**Cell culture and fractionation**

Culturing and differentiation of 3T3-L1 cells were performed as described previously (9). Differentiated 3T3-L1 adipocytes were washed twice with cold PBS and lysed with 200 µl of cold radioimmune precipitation assay buffer (Millipore, Burlington, MA) supplemented with Halt™ Protease and Phosphatase Inhibitor Mixture (Thermo Fisher Scientific) for 10 min on ice. Cell lysates were then centrifuged at 14,000 × g for 15 min, and protein concentration was determined using the Pierce BCA Protein Assay kit (Thermo Fisher Scientific) on a Biotek Synergy™ HT microplate reader (Biotek, Winooski, VT).

**Fractionation of mouse adipose tissue**

Freshly excised adipose tissue (~100 mg) were collected in prechilled microcentrifuge tubes containing 1 scoop (measuring apparatus provided by Next Advance) of sterile 1.0-mm diameter RNase-free zirconium oxide beads and 200 µl of extraction buffer (40 mM HEPES, 120 mM NaCl, and 1 mM EDTA). Tissue was homogenized in a bullet blender homogenizer and for 5 min each at the maximum intensity. Lysates were then centrifuged at 1,000 × g for 10 min at 4 °C with rotation. Samples were centrifuged at 14,000 × g for 10 min on ice. Total RNA was extracted from cultured adipocytes and mouse adipose tissue using TRIzol® reagent followed by extraction through RNasey columns from EPOCH (Sugar Land, TX). Total RNA (1 µg) underwent reverse transcription using random decamers through the RETROscript kit (Ambion, Austin, TX). The following primers were used for RT-qPCR: leptin forward, 5’-CACCACACCCCTCATCAAGAC-3’; leptin reverse, 5’-AGCTTCTTAGAAGGCGACGCA-3’; Egr1 forward, 5’-CCCAAAACACGGGAGGACCT-3’; Egr1 reverse, 5’-ACTGAGTGGCCAAGCTTATAA-3’; 36B4 forward, 5’-TCAGTGTCCAGCTCAGAC-3’; 36B4 reverse, 5’-AATTTCCAATGTGCTGCTTGG-3’. Expression was measured using the ΔΔCt method, including -fold expression of treatments compared with control. Each experiment was done in triplicate and repeated at least three times.

**Adenoviral infection of 3T3-L1 adipocytes**

The adenoviral vector containing human Egr1 cDNA (AdEgr1) was kindly provided by E. Hofer (Medical University of Vienna, Vienna, Austria). GFP containing adenovirus was kindly provided by Andy Greenberg (Tufts Medical Center, Boston, MA). Differentiated 3T3-L1 adipocytes in 24-well plates were serum-starved in Opti-MEM medium for 2 h. AdEGR1 or AdGFP were then added to cells at a multiplicity of infection of 4,000. After 24 h, the medium was replaced with DMEM supplemented with 10% FBS, 100 units/ml penicillin, 100 µg/ml streptomycin, and 2 mM L-glutamine. After 48 h, cells were harvested for protein and RNA analysis.

**siRNA-mediated knockdown of Egr1**

Suspension transfections were performed according to Kilroy et al. (39). Briefly, 3T3-L1 cells were differentiated until day 4. Cells were washed gently with PBS, trypsinized with 1 ml of 0.25% trypsin-EDTA for 3–5 min, resuspended in 10% FBS/DMEM (5 ml), and placed immediately into 24-well plates containing either Egr1 siRNA or scrambled siRNA. To prepare siRNA transfection mix, scrambled and Egr1 siRNA (Dharmacon, Lafayette, CO) were mixed with Opti-MEM medium at a final concentration of 100 nM and allowed to sit for 5 min at room temperature. DharmaFECT Duo (Dharmacon, Lafayette, CO) was added to the previous mixture (1.4 µl/cm² surface area of transfection). The mixture was allowed to sit for 20 min at room temperature, and then 200 µl was added to each well of a 24-well plate. Cells were incubated for 24 h and thereafter replenished with fresh 10% FBS/DMEM and maintained for an additional 48 h before being harvested for the analysis of protein and RNA.

**ChIP**

ChIP was performed with the EZ-ChIP kit from Millipore (Burlington, MA). Briefly, 3T3-L1 adipocytes were cross-linked with 18.5% formaldehyde for 10 min and then quenched with 0.125 M glycine for 5 min at 37 °C. Cells were then lysed in SDS lysis buffer. Chromatin fragments were prepared by sonication in the Bioruptor Sonicator using 1.5 ml of Bioruptor® Plus TPX microtubes (Diagenode, Denville, NJ) on ice for 15 cycles (30 s with 30-s intervals) at the high-intensity setting. Novus Egr1 antibody or nonspecific mouse IgG (10 µg) was added to sonicated samples and incubated overnight at 4 °C with rotation. Protein G-agarose (60 µl) was added to samples and incubated for 1 h at 4 °C with rotation. Samples were centrifuged at 3,000 × g for 1 min to pellet the beads and washed before removing the supernatant fraction. Protein–DNA complexes were eluted, treated with RNase A and proteinase K, and transferred to DNA purification spin columns to be eluted and stored in −20 °C.

**Purified DNA eluates were analyzed by qPCR according to Cardamone et al. (40), using the primers 5’-TCCGGCCCTCCGGCTAGGAGAGCTC-3’ (forward) and 5’-GAGTCTCCAG- TGGTGGGGCAGGA-3’ (reverse) to detect the Egr1 binding site. Dilutions of total lysates (1:10, 1:100, and 1:1,000) were amplified by PCR to form a standard reference curve. Numbers from eluates relative to the total lysate were used to calculate the slope and intercept for the standard reference curve. Ct values of samples were input in the formula, relative input = 10(Ct − intercept)×10/slope.**

**ACCELERATED COMMUNICATION:** Egr1 activates leptin expression

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Construction of leptin promoter reporter

Leptin core promoter spanning −350/+27 was amplified from mouse genomic DNA by PCR with the primers 5′-TAA-GCAAGCTAGCTGTAGCCTCTTGCTCCCTGCG-3′ (forward) and 5′-TGCTTAAAGCTTACCTGGACGTCTGGAGCAGGATCC-3′ (reverse). Underlined sequences contain restriction enzyme sites Nhel for forward and HindIII for reverse primer. Upstream of the restriction enzyme sites are additional base pairs to reduce GC content. pGL4.1 luciferase vector was purchased from Promega (Madison, WI). Amplified PCR segments were purified using a PCR purification kit (Qia-gen, Hilden, Germany). Products were ligated into the Nhel and HindIII sites of pGL4.1 vector ligated following an overnight ligation method, using the Quick Ligation kit (New England Biolabs, Ipswich, MA), yielding the leptin promoter reporter (−350/+27). Ligated plasmid was then transformed into DH5α supercompetent cells (New England Biolabs, Ipswich, MA). Successfully transformed colonies were extracted for plasmid DNA and verified by sequencing.

Luciferase assay

Human embryonic kidney cells (HEK 293T) were grown to 70% confluence in 24-well plates (Corning, Tewksbury, MA) and subsequently transfected with Lipofectamine 2000 (9). To normalize transfection efficiency, samples were co-transfected with 100 ng of pRL-CMV-Renilla (Promega, Madison, WI). After 48 h, cells were washed twice with PBS and harvested with passive lysis buffer (Promega). Luciferase activities were determined by the Dual-Luciferase® Reporter Assay System kit (Promega) using a Biotek Synergy™ HT microplate reader (Biotek, Winooski, VT).

Leptin ELISA

Mouse blood samples (200 μl) were incubated at room temperature for 25 min before being centrifuged at 2,000 × g for 10 min at 4 °C. Serum was then collected, and circulating leptin was measured using the mouse leptin ELISA kit (Thermo Fisher Scientific). Samples were read at 450-nm absorbance by a Biotek Synergy™ HT microplate reader and were fitted by a standard curve to determine leptin concentration.

Deletion of the 5′-UTR of the Egr1 mRNA using CRISPR/Cas9

The DNA region encoding the 5′-UTR of the Egr1 mRNA was deleted using a CRISPR/Cas9 system in 3T3-L1 preadipocytes as described previously (41). Briefly, two single guide RNAs (5′-GGGCTTCCCTCCCCAGTCCCGAG-3′ and 5′-GCCCTCACTAGCTCGCCGCCCAGGC-3′) targeting either the upstream or downstream region of the 5′-UTR encoding DNA were designed using online software (http://chopchop.cbu. uib.no)3 (42).

The lentiviral plasmid pS-PAX2. 3T3-L1 cells transduced with lentivirus were cultured under selection with puromycin (InvivoGen, San Diego, CA) (5 μg/ml) for 4 days. Single colonies of stably transduced cells were grown in 10% FBS/DMEM containing puromycin (5 μg/ml). The genomic region surrounding the single guide RNA target site was amplified by PCR using the primers 5′-TTTAAAACCTTGAGCTCCCG-3′ and 5′-GGGTGTCCCTAGACATCCCCT-3′ and sequenced.

Gel electrophoresis and immunoblotting

Proteins were separated in either 8 or 12% SDS-polyacrylamide gels, and Western blotting was performed as described previously (9). Protein bands were detected with Immobilon Western Chemiluminescent horseradish peroxidase substrate (Millipore) using a Bio-Rad image station.

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References

1. Zhang, Y., Proenca, R., Maffei, M., Barone, M., Leopold, L., and Friedman, J. M. (1994) Positional cloning of the mouse obese gene and its human homologue. Nature 372, 425–432 CrossRef Medline
2. Dalamaga, M., Chou, S. H., Shields, K., Papageorgiou, P., Polyzos, S. A., and Mantzoros, C. S. (2013) Leptin at the intersection of neuroendocrinology and metabolism: current evidence and therapeutic perspectives. Cell Metab. 18, 29–42 CrossRef Medline
3. Ahima, R. S., and Flier, J. S. (2000) Leptin. Annu. Rev. Physiol. 62, 413–437 CrossRef Medline
4. Friedman, J. M. (2009) Leptin at 14 y of age: an ongoing story. Am. J. Clin. Nutr. 89, 973S–979S CrossRef Medline
5. Zeng, W., Pirzgalska, R. M., Pereira, M. M., Kubasova, N., Barateiro, A., Seixas, E., Lu, Y. H., Kozlova, A., Voss, H., Martins, G. G., Friedman, J. M., and Domingos, A. I. (2015) Sympathetic neuro-adipose connections mediate leptin-driven lipolysis. Cell 163, 84–94 CrossRef Medline
6. Levy, J. R., LeGall-Salmon, E., Santos, M., Pandak, W. M., and Stevens, W. (1997) Effect of enteral versus parenteral nutrition on leptin gene expression and release into the circulation. Biochem. Biophys. Res. Commun. 237, 98–102 CrossRef Medline
7. Frederich, R. C., Löffmann, B., Hamann, A., Napolitano-Rosen, A., Kahn, B. B., Lowell, B. B., and Flier, J. S. (1995) Expression of ob mRNA and its encoded protein in rodents. Impact of nutrition and obesity. J. Clin. Invest. 96, 1658–1663 CrossRef Medline
8. Saladin, R., De Vos, P., Guerre-Millo, M., Leturque, A., Girard, J., Staels, B., and Auwerx, J. (1995) Transient increase in obese gene expression after food intake or insulin administration. Nature 377, 527–529 CrossRef Medline
9. Chakrabarti, P., Kim, J. Y., Singh, M., Shin, Y. K., Kim, J., Kumbrink, J., Wu, Y. Y., Lee, M. J., Kirsch, K. H., Fried, S. K., and Kandror, K. V. (2013) Insulin inhibits lipolysis in adipocytes via the evolutionarily conserved mTORC1-Egr1-mediated pathway. Mol. Cell. Biol. 33, 3659–3666 CrossRef Medline
10. Singh, M., Kaur, R., Lee, M. J., Pickering, R. T., Sharma, V. M., Puri, V., and Kandror, K. V. (2014) Fat-specific protein 27 inhibits lipolysis by facilitating the inhibitory effect of transcription factor Egr1 on transcription of adipose triglyceride lipase. J. Biol. Chem. 289, 14481–14487 CrossRef Medline
11. Singh, M., Shin, Y. K., Yang, X., Zehr, B., Chakrabarti, P., and Kandror, K. V. (2015) 4E-BPs control fat storage by regulating the expression of Egr1 and ATG1. J. Biol. Chem. 290, 17331–17338 CrossRef Medline
12. Kandror, K. V. (2016) Translational and post-translational control of leptin production by fat cells. in Post-transcriptional Mechanisms in Endo...
27. Lamming, D. W., and Sabatini, D. M. (2013) A central role for mTOR in lipid homeostasis. *Cell Metab.* **18**, 465–469 CrossRef Medline

28. Ricoult, S. J., and Manning, B. D. (2013) The multifaceted role of mTORC1 in the control of lipid metabolism. *EMBO Rep.* **14**, 242–251 CrossRef Medline

29. Porstmann, T., Santos, C. R., Griffiths, B., Cully, M., Wu, M., Leever, S., Griffiths, I. R., Chung, Y. L., and Schulze, A. (2008) SREBP activity is regulated by mTORC1 and contributes to Akt-dependent cell growth. *Cell Metab.* **8**, 224–236 CrossRef Medline

30. Li, S., Brown, M. S., and Goldstein, J. L. (2010) Bifurcation of insulin signaling pathway in rat liver: mTORC1 required for stimulation of lipogenesis, but not inhibition of gluconeogenesis. *Proc. Natl. Acad. Sci. U.S.A.* **107**, 3441–3446 CrossRef Medline

31. Chakrabarti, P., English, T., Shi, J., Smas, C. M., and Kandror, K. V. (2010) The mTOR complex 1 suppresses lipolysis, stimulates lipogenesis and promotes fat storage. *Diabetes* **59**, 775–781 CrossRef Medline

32. Laplante, M., and Sabatini, D. M. (2009) An emerging role of mTOR in lipid biosynthesis. *Curr. Biol.* **19**, R1046–R1052 CrossRef Medline

33. Khamzina, L., Veilleux, A., Bergeron, S., and Marette, A. (2005) Increased activation of the mammalian target of rapamycin pathway in liver and skeletal muscle of obese rats: possible involvement in obesity-linked insulin resistance. *Endocriology* **146**, 1473–1481 CrossRef Medline

34. Wang, C. Y., Kim, H. H., Hiroi, Y., Sawada, N., Salomone, S., Benjamin, L. E., Walsh, K., Moskwotz, M. A., and Liao, J. K. (2009) Obesity increases vascular senescence and susceptibility to ischemic injury through chronic activation of Akt and mTOR. *Sci. Signal.* 2, ra11 CrossRef Medline

35. Um, S. H., Frigerio, F., Watanabe, M., Picard, F., Joaquin, M., Sticker, M., Fumagalli, S., Allegrini, P. R., Kozma, S. C., Auwerx, J., and Thomas, G. (2004) Absence of S6K1 protects against age- and diet-induced obesity while enhancing insulin sensitivity. *Nature* **431**, 200–205 CrossRef Medline

36. Wang, W., Lv, N., Zhang, S., Shui, G., Qian, H., Zhang, J., Chen, Y., Xie, Y., Shen, Y., Wenk, M. R., and Li, P. (2012) Cidea is an essential transcriptional coactivator regulating mammary gland secretion of milk lipids. *Nat. Med.* **18**, 235–243 CrossRef Medline

37. Gallardo-Montejano, V. I., Saxena, G., Kusminski, C. M., Yang, C., McAfee, J. L., Hahner, L., Hoch, K., Dubinsky, W., Narkar, V. A., and Bickel, P. E. (2016) Nuclear perlipin 5 integrates lipid droplet lipolysis with PGC-1α/SIRT1-dependent transcriptional regulation of mitochondrial function. *Nat. Commun.* **7**, 12723 CrossRef Medline

38. Kim, J., Jung, E., Choi, J., Min, D. Y., Lee, Y. H., and Shin, S. Y. (2018) Leptin is a direct transcriptional target of EGR1 in human breast cancer cells. *Mol. Biol. Rep.* 10.1007/s10533-018-4474-3 CrossRef Medline

39. Kilroy, G., Burk, D. H., and Floyd, Z. E. (2009) High efficiency lipid-based siRNA transfection of adipocytes in suspension. *PLoS One* **4**, e6940 CrossRef Medline

40. Cardamone, M. D., Orofino, J., Labadorf, A., and Perissi, V. (2018) Chromatin immunoprecipitation of murine brown adipose tissue. *J. Vis. Exp.* e58682 CrossRef Medline

41. Sanjana, N. E., Shalem, O., and Zhang, F. (2014) Improved vectors and genome-wide libraries for CRISPR screening. *Nat. Methods* **11**, 783–784 CrossRef Medline

42. Labun, K., Guo, X., Chavez, A., Church, G., Gagnon, J. A., and Valen, E. (2019) Accurate analysis of genuine CRISPR editing events with ampic. *Genome Res.* pi: gr.244293.118 CrossRef Medline