Keywords: retinoic acid, adipogenesis, nuclear receptors, fatty acid-binding protein, cellular retinoic acid-binding protein, RAR, PPAR
Submitted: 12/02/12
Revised: 01/03/13
Accepted: 01/03/13
http://dx.doi.org/10.4161/adip.23489

Correspondence to: Noa Noy; Email: noa.noy@case.edu

Commentary to: Berry DC, DeSantis D, Soltanian H, Croniger CM, Noy N. Retinoic acid upregulates preadipocyte genes to block adipogenesis and suppress diet-induced obesity. Diabetes 2012; 61:1112–21; PMID:22396202; http://dx.doi.org/10.2337/db11-1620

The one-two punch
Retinoic acid suppresses obesity both by promoting energy expenditure and by inhibiting adipogenesis

Noa Noy
Departments of Pharmacology and Nutrition; Case Western Reserve University School of Medicine; Cleveland, OH USA

The vitamin A metabolite retinoic acid (RA) regulates gene transcription by activating the nuclear receptors RAR and PPARβ/δ and their cognate lipid binding proteins CRABP-II, which delivers RA to RAR, and FABP5, which shuttles the hormone to PPARβ/δ. In preadipocytes, RA signals predominantly through CRABP-II and the RAR isotype RARγ to induce the expression of hallmark markers of preadipocytes Pref-1, Sox9, and KLF2. RA thus maintains the preadipocyte phenotype and inhibits adipogenesis. In mature adipocytes, RA activates both of its receptors to upregulate expression of genes that enhance lipid oxidation, energy dissipation, and insulin responses. Consequently, RA potently protects mice from diet-induced obesity and insulin resistance by two distinct mechanisms: by counteracting adipogenesis, thereby moderating the formation of new fat cells, and by promoting energy expenditure, thereby preventing adipocyte hypertrophy.

The primary cells of adipose tissue, adipocytes, coordinate energy homeostasis and serve as endocrine cells, giving rise to signaling cytokines that control multiple cellular functions. The adipose tissue begins to develop in late gestation but adipocyte number dramatically expands after birth and continues to increase through puberty.1 Even in adult adipose tissue, about 10% of adipocytes turn over every year2 and adipogenesis can be induced by environmental cues such as consumption of a high-fat diet.3,5 Proper adipogenesis throughout life is thus of critical importance for maintaining health, and malformation or dysfunction of adipocytes underlie the development of various pathologies, including obesity and type 2 diabetes. Adipocytes are generated from mesenchymal stem cells by a two-step process entailing commitment of stem cells to the adipocyte lineage, followed by terminal differentiation of adipocyte progenitors, preadipocytes, into mature fat cells.6 The second step can be triggered by adipogenic signals including insulin, glucocorticoid receptor agonists and agents that elevate cellular cAMP levels.7,8 These signaling molecules modulate the expression of numerous genes, thereby inducing differentiation and allowing adipogenesis to proceed9,10 (reviewed in ref. 11).

Of special note among regulatory factors involved in adipocyte biology is the transcriptionally active metabolite of vitamin A retinoic acid (RA). The biological activities of this hormone originate from its ability to activate several members of the nuclear receptor family of transcription factors: the classical RA receptors RARα, RARβ and RARγ12 and the peroxisome proliferator activated receptor β/δ (PPARβ/δ).13-17 The partitioning of RA between its receptors is regulated by two intracellular lipid-binding proteins that deliver it from sites of synthesis in the cytosol to cognate receptors in the nucleus, cellular RA binding protein II (CRABP-II) transports RA to RARs and fatty acid binding protein type 5 (FABP5) shuttles it to PPARβ/δ. The spectrum of genes whose expression is regulated by RA and, accordingly, cellular responses to the hormone are thus determined by
the relative expression levels of these binding proteins in specific cells; RA controls expression of RAR target genes in cells that display a high CRABP-II/FABP5 ratio, but it regulates PPARβ/δ target genes in cells in which this ratio is low. In preadipocytes, RA signals predominantly through the CRABP-II/RAR path. However, adipocyte differentiation is accompanied by downregulation of CRABP-II and RARs and by upregulation of FABP5 and PPARβ/δ, and consequently, the alternative pathway is enabled and RA can activate both of its receptors in mature adipocytes.

Various observations indicate that vitamin A is closely involved in regulation of adipose tissue function. Hence, ablation of retinol dehydrogenase 1 (rdh1) in mice, which results in alterations in vitamin A homeostasis, leads to enhanced size and adiposity. Further, fibroblasts with reduced expression of cellular retinol-binding protein I (CRBP-I) undergo adipocyte differentiation more readily than parental cells, and CRBP-I-null mice display increased adiposity. It was also reported that CRBP-III plays a role in lipid metabolism. Treatment of mice with RA at a pharmacological but non-toxic dose (~3 mg/kg/d) was reported to result in weight loss and improved glucose tolerance despite a larger food intake by treated animals. Additional reports showed that RA reduces adiposity in rodents, but RA reduces adiposity in rodents, and in muscle, where the hormone signals through both RAR and PPARβ/δ. It has thus been reported that, in cultured adipocytes, RA enhances energy expenditure by inducing the expression of PPARβ/δ target genes that trigger energy dissipation, e.g., UCP3, and lipolysis, e.g., hormone sensitive lipase. In vivo, administration of RA induces the expression of lipid- and sugar-processing PPARβ/δ target genes in adipose tissue and liver, and it recapitulates the reported activity of PPARβ/δ in increasing skeletal muscle mitochondrial content. Taken together with the −0.5 °C higher body temperature of mice treated with RA, these observations indicate that induction of weight loss by RA is associated with enhanced energy utilization.

In addition to its activities in mature adipocytes and muscle, RA is also closely involved in regulation of adipogenesis. Interestingly, it has been reported that RA induces commitment of embryonic stem cells to the adipocyte lineage and potently blocks differentiation of preadipocytes into mature adipose cells. It has been suggested that induction of adipocyte commitment of stem cells by RA involves glycogen synthase kinase 3 and that inhibition of adipocyte differentiation by the hormone involves Smad3. However, the identity of direct target genes that mediate these activities and the mechanisms by which the effects of RA on adipogenesis are propagated were unknown. Providing insight into some of these questions, our recent observations showed that inhibition of adipocyte differentiation by RA is mediated primarily by the RAR subtype RARα and that, in preadipocytes, RARγ directly controls the expression of several genes that encode known inhibitors of adipogenesis. One of these is the Kruppel-like factor KLF2, a transcription factor that inhibits adipogenesis by suppressing the expression of the adipogenic factors PPARγ, C/EBPα, and SREBP1c. Interestingly, the data showed that, while suppressing the expression of these genes, KLF2 upregulates the expression of both CRABP-II and RARγ in preadipocytes. Hence, KLF2 participates in a positive feedback loop that amplifies inhibition of adipocyte differentiation by RA. Other RA-regulated genes that block differentiation of preadipocytes into mature fat cells are the preadipocyte marker Pref-1, its activator ADAM17, and its downstream effector, the transcription factor SOX9. Pref-1, a plasma membrane protein exclusively expressed in preadipocytes, is cleaved by ADAM17 to produce an extracellular form that activates ERK signaling, leading to induction of SOX9. In turn, SOX9 impedes adipogenesis by repressing the expression of C/EBPβ and C/EBPδ.

Mice fed a high fat/high sucrose (HFHS) diet and treated with RA display a lower weight, lower adipose tissue mass, and lower adipocyte size as compared with animals fed a HFHS diet in the absence of administration of RA. RA treatment also blunts diet-induced elevation in levels of plasma cholesterol and plasma triglycerides. These effects emanate in part from increased expression of adipose and muscle proteins that enhance lipid oxidation and energy dissipation and that promote insulin signaling. However, the data also showed that adipose tissue of RA-treated mice contains fewer mature, lipid-containing, adipocytes, and displays a higher expression level of the preadipocyte marker Pref-1. The data thus establish that RA contributes to maintenance of preadipocytes and inhibits adipocyte differentiation in vivo. CRABP-II+/− mice, in which RA signaling through the CRABP-II/RAR pathway is reduced, were used to further examine whether inhibition of adipogenesis by RA contributes to its ability to protect animals from diet-induced obesity. Expression levels of adipocyte markers in WT and CRABP-II+/− mice were similar. Hence, in agreement with the report that many of the activities of RA in mature adipocytes are mediated by the FABP5/PPARβ/δ path, adipocytes of CRABP-II+/− mice retain normal phenotype. In contrast, the levels of expression of Pref-1, SOX9, and KLF2 were markedly lower in adipose tissue of CRABP-II+/− mice than in WT mice. These observations further support the identification of these genes as direct targets for the RA-activated CRABP-II/RAR path, and they indicate that the preadipocyte content of adipose tissue of CRABP-II+/− mice is lower than that of WT animals. These findings suggest that these mice are prone to excess adipogenesis and thus that they may display a propensity for enhanced adiposity. Indeed, CRABP-II+/− mice fed a HFHS diet gained more weight than WT animals although they displayed a lower food
consumption. Remarkably, the size of adipocytes in WT and CRABP-II+/− mice was similar, indicating that the increase in the weight of these animals did not result from enhanced adipocyte hypertrophy but directly reflected accelerated generation of mature adipocytes.

As summarized in the model presented in Figure 1, available information demonstrates that the ability of RA to protect animals from diet-induced obesity and from obesity-related pathologies is exerted by two distinct activities. In preadipocytes, RA activates the CRABP-II/RAR pathway and thereby inhibits adipocyte differentiation, moderating the formation of new fat cells in response to high fat feeding. In mature adipocytes and in muscle, the hormone activates both the CRABP-II/RAR and the FABP5/PPARβ/δ pathways to promote lipid oxidation and energy utilization. RA thus suppresses dietary-induced obesity by counteracting both adipogenesis and adipocyte hypertrophy.

Disclosure of Potential Conflicts of Interest
No potential conflicts of interest were disclosed.

Acknowledgments
This work was supported by NIH grant RO1-DK60684.

References
1. Ailhaud G, Grimaldi P, Négrel R. Cellular and molecular aspects of adipose tissue development. Annu Rev Nutr 1992; 12:207-33; PMID:1503804; http://dx.doi.org/10.1146/annurev.nutr.12.070192.001231
2. Spalding KL, Arner E, Westermark PO, Bernard S, Buchholz BA, Bergmann O, et al. Dynamics of fat cell turnover in humans. Nature 2008; 453:783-7; PMID:18494136; http://dx.doi.org/10.1038/nature06902
3. Lemmonnier D. Effect of age, sex, and sites on the cellularity of the adipose tissue in mice and rats rendered obese by a high-fat diet. J Clin Invest 1972; 51:2907-15; PMID:5080416; http://dx.doi.org/10.1172/JCI10715
4. Klyde BJ, Hirsch J. Increased cellular proliferation in adipose tissue of adult rats fed a high-fat diet. J Lipid Res 1979; 20:765-9; PMID:490049.
5. Tchoukalova YD, Votruba SB, Tkchonia T, Giorgadze N, Kirkland JL, Jensen MD. Regional differences in cellular mechanisms of adipose tissue gain with overfeeding. Proc Natl Acad Sci U S A 2010; 107:18226-31; PMID:20921416; http://dx.doi.org/10.1073/pnas.1009529107
6. Cristancho AG, Lazar MA. Forming functional fat: a growing understanding of adipocyte differentiation. Nat Rev Mol Cell Biol 2011; 12:722-34; PMID:21952500; http://dx.doi.org/10.1038/nrm3398
7. Green H, Kehinde O. An established preadipose cell line and its differentiation in culture. II. Factors affecting the adipose conversion. Cell 1975; 5:19-27; PMID:165899; http://dx.doi.org/10.1016/0092-8674(75)90087-2
8. Green H, Meurh M. An established pre-adipose cell line and its differentiation in culture. Cell 1974; 3:127-33; PMID:4426090; http://dx.doi.org/10.1016/0092-8674(74)90116-0
9. Tontonoz P, Hu E, Graves RA, Budavari AI, Spiegelman BM. mPPAR gamma 2: tissue-specific regulator of an adipocyte enhancer. Genes Dev 1994; 8:1224-34; PMID:7926726; http://dx.doi.org/10.1101/gad.8.10.1224
10. Shao D, Lazar MA. Peroxisome proliferator activated receptor gamma, CCAAT/enhancer-binding protein alpha, and cell cycle status regulate the commitment to adipocyte differentiation. J Biol Chem 1997; 272:21473-8; PMID:9261165; http://dx.doi.org/10.1074/jbc.272.34.21473
11. Farmer SR. Transcriptional control of adipocyte formation. Cell Metab 2006; 4:263-73; PMID:17011499; http://dx.doi.org/10.1016/j.cmet.2006.07.001
12. Germain P, Chambon P, Eichele G, Evans RM, Lazar MA, Leid M, et al. International Union of Pharmacology. LX. Retinoic acid receptors. Pharmacol Rev 2006; 58:712-25; PMID:17132850; http://dx.doi.org/10.1124/pr.58.4.4
13. Berry DC, Noy N. All-trans-retinoic acid represses obesity and insulin resistance by activating both peroxisome proliferation-activated receptor beta/delta and retinoic acid receptor. Mol Cell Biol 2009; 29:3286-96; PMID:19364826; http://dx.doi.org/10.1128/MCB.01742-08
14. Berry DC, Sohantian H, Noy N. Repression of cellular retinoic acid-binding protein II during adipocyte differentiation. J Biol Chem 2010; 285:15524-32; PMID:20228061; http://dx.doi.org/10.1074/jbc.M110.110635
15. Schug TT, Berry DC, Shaw NS, Travis SN, Noy N. Opposing effects of retinoic acid on cell growth result from alternate activation of two different nuclear receptors. Cell 2007; 129:723-33; PMID:17512406; http://dx.doi.org/10.1016/j.cell.2007.02.050

Figure 1. Mechanisms by which RA suppresses dietary-induced adiposity and insulin resistance. In preadipocytes, RA activates CRABP-II and RARy to induce expression of Pref-1, ADAM17, Sox9, and KLF2, all of which contribute to inhibition of adipogenesis. In turn, KLF2 upregulates RARy and CRABP-II, thereby propagating a positive feedback loop that further potentiates RA-induced inhibition of adipocyte differentiation. In mature adipocytes, RA functions through both RAR and PPARβ/δ to induce the expression of genes that enhance energy expenditure and that promote insulin responses.
16. Schug TT, Berry DC, Toshkov IA, Cheng L, Nikitin AY, Noy N. Overcoming retinoic acid-resistance of mammary carcinomas by diverting retinoid acid from PPARbeta/delta to RAR. Proc Natl Acad Sci U S A 2008; 105:7549-54; PMID:18499524; http://dx.doi.org/10.1073/pnas.0709981105
17. Shaw N, Elholm M, Noy N. Retinoic acid is a high affinity selective ligand for the peroxisome proliferator-activated receptor beta/delta. J Biol Chem 2003; 278:41589-92.; PMID:12963727; http://dx.doi.org/10.1074/jbc.C300368200
18. Dong D, Ruuska SE, Levinthal DJ, Noy N. Distinct roles for cellular retinoic acid-binding proteins I and II in regulating signaling by retinoid acid. J Biol Chem 1999; 274:25695-8; PMID:10464126; http://dx.doi.org/10.1074/jbc.274.34.25695
19. Budhu AS, Noy N. Direct channeling of retinoic acid between cellular retinoic acid-binding protein II and retinoic acid receptor sensitizes mammary carcinoma cells to retinoic acid-induced growth arrest. Mol Cell Biol 2002; 22:2632-41; PMID:11909957; http://dx.doi.org/10.1128/MCB.22.8.2632-2641.2002
20. Manor D, Shmidt EN, Budhu A, Flesken-Nikitin A, Zgola M, Page R, e al. Mammary carcinoma suppression by cellular retinoid acid binding protein-II. Cancer Res 2003; 63;4426-33; PMID:12997615.
21. Sessler RJ, Noy N. A ligand-activated nuclear localization signal in cellular retinoic acid binding protein-II. Mol Cell 2005; 18:343-53; PMID:15866176; http://dx.doi.org/10.1016/j.molcel.2005.03.026
22. Tan NS, Shaw NS, Vincenbosch N, Liu P, Yazmin R, Desvergne B, e al. Selective cooperation between fatty acid binding proteins and peroxisome proliferator-activated receptors in regulating transcription. Mol Cell Biol 2002; 22:5114-27; PMID:12077430; http://dx.doi.org/10.1128/MCB.22.14.5114-5127.2002
23. Zhang M, Hu P, Krois CR, Kane MA, Napol JL. Altered vitamin A homeostasis and increased size and adiposity in the edn1-null mouse. FASEB J 2007; 21:2886-96; PMID:17435714; http://dx.doi.org/10.1095/edn1.007-7964.com
24. Zisola CF, Frey SK, Jirgarmukku S, Kadereit B, Yan N, Vogel S. Cellular retinol-binding protein type I (CRBP-I) regulates adipogenesis. Mol Cell Biol 2010; 30:3412-20; PMID:20498279; http://dx.doi.org/10.1128/MCB.00110-10
25. Zisola CF, Schwartz GJ, Vogel S. Cellular retinol-binding protein type III is a PPARgamma target gene and plays a role in lipid metabolism. Am J Physiol Endocrinol Metab 2008; 295:E1358-68; PMID:18840764; http://dx.doi.org/10.1152/ajpendo.90464.2008
26. Bonet ML, Oliver J, Picó C, Felipe F, Ribot J, Cinti S, e al. Opposite effects of feeding a vitamin A-deficient diet and retinoic acid treatment on brown adipose tissue uncoupling protein 1 (UCP1), UCP2 and leptin expression. J Endocrinol 2000; 166:511-7; PMID:10976465; http://dx.doi.org/10.1605/01.JEc 0.1605051
27. Felipe F, Mercader J, Ribot J, Palou A, Boner ML. Effects of retinoic acid administration and dietary vitamin A supplementation on leptin expression in mice: lack of correlation with changes of adipose tissue mass and food intake. Biochim Biophys Acta 2005; 1740:258-65; PMID:15940693; http://dx.doi.org/10.1016/j.bbadis.2004.11.034
28. Mercader J, Ribot J, Murano I, Felipe C, Sinti S, Bonet ML, e al. Remodeling of white adipose tissue after retinoic acid administration in mice. Endocrinology 2006; 147:5325-32; PMID:16849543; http://dx.doi.org/10.1210/en.2006-0760
29. Amengual J, Ribot J, Bonet ML, Palou A. Retinoic acid treatment increases lipid oxidation capacity in skeletal muscle of mice. Obesity (Silver Spring) 2008; 16:585-91; PMID:18296960; http://dx.doi.org/10.1038/oby.2007.104
30. Wang YX, Zhang CL, Yu RT, Cho HK, Nelson MC, Bayuga-Ocampo CR, e al. Regulation of muscle fiber type and running endurance by PPARdelta. PLoS Biol 2004; 2:e294; PMID:15328533; http://dx.doi.org/10.1371/journal.pbio.0020294
31. Dana C, Smith AG, Dessolin S, Leroy P, Staccioli L, Villagçois P, e al. Differentiation of embryonic stem cells into adipocytes in vitro. J Cell Sci 1997; 110:1279-85; PMID:9202838.
32. Monteiro MC, Wdziekonski B, Villageois P, Vernoehler C, Iehle C, Billon N, e al. Commitment of mouse embryonic stem cells to the adipocyte lineage requires retinoic acid receptor beta and active GSK3. Stem Cells Dev 2009; 18:457-63; PMID:18607973; http://dx.doi.org/10.1089/scd.2008.0154
33. Murray T, Russell TR. Inhibition of adipose conversion in 3T3-L1 cells by retinoid. J Supramol Struct 1980; 14:255-66; PMID:6164877; http://dx.doi.org/10.1007/jss.40014024
34. Sato M, Hiragau A, Mizutani H. Preadipocytes possess cellular retinoid binding proteins and their differentiation in 3T3-L2 cells by retinoic acid. J Supramol Struct 1982; 18:402-6; PMID:6374245; http://dx.doi.org/10.1006/jbcs.2008.0154
35. Wu J, Srinivasan SV, Neumann JC, Lingrel JB. The KLF2 transcription factor does not affect the formation of preadipocytes but inhibits their differentiation into adipocytes. Biochemistry 2005; 44:11098-105; PMID:16101293; http://dx.doi.org/10.1021/bi050166e
36. Wang Y, Kim KA, Kim JH, Sul HS. Pref-1, a preadipocyte secreted factor that inhibits adipogenesis. J Nutr 2006; 136:2953-6; PMID:17167015.
37. Moon YS, Smas CM, Lee K, Villena JA, Kim KH, Yun EJ, e al. Mice lacking paternally expressed Pref-1/Dkk1 display growth retardation and accelerated adiposity. Mol Cell Biol 2002; 22:5585-92; PMID:12101250; http://dx.doi.org/10.1128/MCB.22.15.5585-5592.2002
38. Villena JA, Choi CS, Wang Y, Kim S, Hwang YJ, Kim YB, e al. Resistance to high-fat diet-induced obesity but exacerbated insulin resistance in mice overexpressing preadipocyte factor-1 (Pref-1): a new model of partial lipodystrophy. Diabetes 2008; 57:3258-66; PMID:18835937; http://dx.doi.org/10.2337/db07-1739
39. Smas CM, Chen L, Sul HS. Cleavage of membrane-associated pref-1 generates a soluble inhibitor of adipocyte differentiation. Mol Cell Biol 1997; 17:977-88; PMID:9001251.
40. Wang Y, Sul HS. Ectodomain shedding of preadipocyte factor 1 (Pref-1) by tumor necrosis factor alpha converting enzyme (TACE) and inhibition of adipocyte differentiation. Mol Cell Biol 2006; 26:5421-35; PMID:16809777; http://dx.doi.org/10.1128/ MCB.02437-05
41. Wang Y, Zhao L, Smas C, Sul HS. Pref-1 interacts with fibronectin to inhibit adipocyte differentiation. Mol Cell Biol 2010; 30:3480-92; PMID:20457810; http://dx.doi.org/10.1128/MCB.00575-10
42. Wang Y, Sul HS. Pref-1 regulates mesenchymal cell commitment and differentiation through Sox9. Cell Metab 2009; 9:287-302; PMID:19254573; http://dx.doi.org/10.1016/j.cmet.2009.01.013