Id3, E47, and SREBP-1c
Fat Factors Controlling Adiponectin Expression
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The epidemic of obesity has become among the most serious health problems worldwide because the weight gain carries a high risk of developing life-threatening diseases such as type 2 diabetes, hypertension, coronary artery disease, and heart failure. Recent work has highlighted the key role of adipokines in the adverse clinical consequences of excessive fat mass. The increase in circulating levels of leptin and other adipocyte-derived factors, resulting from the expansion of adipose tissue, has been shown to promote insulin resistance, inflammation, hypertension, and endothelial dysfunction.1,2 Unlike many other adipokines whose circulating levels increase in obesity, the circulating levels of adiponectin decrease in obese subjects, particularly among patients with excess visceral adiposity (Figure).3 Clinical studies suggest that the attendant hypo adiponectinemia that occurs in obesity correlates with the development of hyperglycemia and type 2 diabetes, hypertension, coronary artery disease, sympathetic nerve activation, and impaired vasoreactivity.1,3 Consistent with this, adiponectin null mice have impaired glucose homeostasis and severe insulin resistance.3 Adiponectin has beneficial effects on biological processes that are relevant to the pathogenesis of diabetes and cardiovascular diseases including an improvement in insulin sensitivity (Figure).3,4 In endothelial cells, adiponectin stimulates the activity of endothelial nitric oxide synthase and increases production of NO.5 Moreover, administration of adiponectin decreases arterial pressure and renal sympathetic nerve outflow in rats.6 Together, these findings implicate dysregulated production of adiponectin in obesity as a potential mediator of the metabolic and cardiovascular abnormalities associated with this condition. Therefore, understanding the mechanisms that regulate the expression and production of adiponectin is critical.

Transcriptional regulation of the adiponectin gene is complex involving a number of ubiquitously expressed and cell-specific transcription factors. Among these are peroxisome proliferator-activated receptor γ, C/EBP, and SREBP-1c, each of which plays an important role in adipogenesis, metabolism, and the regulation of a wide array of adipose-specific genes (reviewed elsewhere).7 Binding sites for each of these transcription factors are present in the adiponectin promoter. In addition, the proximal 1 kb of the adiponectin promoter contains 3 E-boxes. E-boxes carry the consensus sequence CANNTG (where N is any nucleotide), which is recognized as the binding site for class I basic helix–loop–helix (bHLH) proteins, also known as E-proteins, such as the founding member of the family E2A. The E2A gene encodes 2 separate bHLH transcription factors, 1 of which is E47. E-proteins are expressed in many cell types but can gain functional specificity by forming heterodimers with other classes of bHLH proteins that exhibit greater cell specificity. SREBP-1c is a member of another class of bHLH proteins which also contains an adjacent leucine zipper domain (bHLH/LZ). Leucine zippers are formed between 2 proteins that have evenly spaced leucine residues on parallel α-helices. SREBPs are synthesized as membrane bound precursors that undergo post translational protein production in response to cues induced by cholesterol depletion (reviewed elsewhere).8 The amino-terminal segment of the protein then enters the nucleus, where it can bind to response elements in the promoter region of target genes. SREBPs exhibit an interesting DNA specificity in that they can recognize both sterol response elements (SRE) containing the sequence TCACNCCAC and specific genes (reviewed elsewhere).9 Their function can be modulated by yet another class of bHLH proteins that exhibit greater cell specificity. SREBP-1c is a member of another class of bHLH proteins called “inhibitors of differentiation” (Ids). Ids such as Id3 have a HLH structure but lack the basic residues required for DNA binding. Consequently, as their name implies, Ids inhibit the activity of E-proteins by directly binding to them, thus preventing their association with DNA. Because the Id proteins do not themselves bind to DNA, they act dominant negatively by sequestering their targets (ie, E47). As a member of bHLH/LZ family, SREBP may also be subjected to regulation by Id proteins. Moldes et al10 have reported a direct interaction between Id2 and Id3 with SREBP-1c, and Id2 and Id3 inhibited SREBP-1c-mediated activation of the fatty acid synthase promoter.

In this issue of Circulation Research, McNamara and colleagues11 examined the importance of the E-box sequences present in the adiponectin promoter and their interaction with E-proteins, Id proteins, and SREBP-1c, focusing primarily on E47 and Id3. The authors first confirmed that Id3 acts as an
endogenous inhibitor of adiponectin expression by showing increased epididymal and mesenteric adipose adiponectin protein and mRNA and increased serum adiponectin in ApoE<sup>−/−</sup> mice also lacking Id3 (ApoE<sup>−/−</sup>, Id3<sup>/−</sup>). Expression of adiponectin and Id3 were also mutually exclusive, with Id3 but not adiponectin expressed in undifferentiated adipocytes and with adiponectin but not Id3 expressed in fully differentiated adipocytes. Moreover, overexpression of Id3 markedly reduced adiponectin protein and mRNA. Like expression of fatty acid synthase, Id3 also inhibited the stimulatory effect of SREBP-1c on adiponectin expression. The authors convincingly demonstrated that E47 could directly interact with Id3 and SREBP-1c, but, in contrast to a previous study, Id3 could not directly interact with SREBP-1c. These data suggest a paradigm by which E47 and SREBP-1c cooperate to induce adiponectin expression, whereas Id3 represses adiponectin transcriptional activity by decreasing the availability of E47 to interact with adiponectin promoter E-box sequences (Figure). Confirming this model were studies showing that: (1) the promoter proximal E-box was required to mediate induction of the adiponectin promoter by SREBP-1c and E47; (2) both proteins bound to sequences surrounding the proximal E-box in chromatin at the adiponectin locus; (3) the binding of SREBP-1c and E47 to chromatin was reduced in the presence of Id3; and (4) the binding of both proteins to the adiponectin promoter in chromatin was low in undifferentiated adipocytes when Id3 levels are high but increased in differentiating and fully differentiated adipocytes when Id3 levels are decreasing or low. Importantly, the authors returned in vivo to show an increase in the binding of E47 to the adiponectin promoter in epididymal adipose tissue from ApoE<sup>−/−</sup> Id3<sup>−/−</sup> compared with ApoE<sup>−/−</sup> Id3<sup>+/−</sup> mice.

The authors provide evidence that E47 binds to the promoter proximal E-box and that SREBP-1c and E47 can physically interact. Therefore, one unresolved question is the requirement for the SRE and the independent binding of SREBP-1c to that site. Recall SREBP-1c can bind both E-boxes and SRE sequences. It is interesting to note that the rat insulin promoter has a similar arrangement of overlapping SRE and E-box sequences. Amemiya-Kudo et al<sup>12</sup> reported that, like adiponectin, insulin gene expression was synergistically activated by SREBP-1c and E47 and that this interaction was mediated by 2 E-box sequences but not the closely linked SREs. These data are consistent with a model in which E47 binds to the E-box sequences and then SREBP-1c interacts with E47 without itself binding to DNA. They further suggest that in this context, and perhaps similarly with adiponectin, SREBP-1c may act as a coactivator by recruiting CBP/p300 to the transcription complex. Thus, the necessity of the SRE sequences in mediating the synergistic transactivation of the adiponectin promoter by E47 and SREBP-1c requires further investigation.

Perhaps the most fascinating finding from the present study was that Id3 can inhibit the binding of both SREBP-1c and E47 to adiponectin promoter in chromatin through interference with E47. As discussed earlier in this editorial, a unique aspect of adiponectin is that in obesity, its level in the circulation declines, whereas many other adipokines increase. Mechanisms underlying hypoadiponectinemia in obesity are unknown. Because Id3 levels decrease during adipogenesis, one would logically expect Id3 levels to be lower in fat in the obese state. Indeed, a role for Id3 in obesity remains to be directly investigated. Therefore, this apparent contradiction suggests that other factors such as E47 and SREBP-1c may influence adiponectin levels in obesity. Consistent with this hypothesis, SREBP-1c expression was shown to be decreased in adipose tissue of obese subjects.<sup>13</sup> Understanding the mechanisms leading to hypoadiponectinemia in obesity will expand our present knowledge concerning the factors that influence fat mass and the predisposition to obesity. This could also lead to new therapeutic strategies to normalize circulating levels of adiponectin in obese subjects and overcome the metabolic and cardiovascular disorders associated with this condition.
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References

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