Transcriptional Dysregulation of Adipose Tissue Autophagy in Obesity

There is growing interest in understanding how dysregulated autophagy may contribute to pathogenesis of disease. Most frequently, disease states are associated with diminished autophagy, mostly attributed to genetic variation in autophagy genes and/or to dysfunctional posttranscriptional mechanisms. In human adipose tissue (AT), in obesity, expression of autophagy genes is upregulated and autophagy is likely activated, associating with adipose dysfunctions. This review explores the emerging role of transcriptional mechanisms regulating AT autophagy in obesity.

Autophagy is an evolutionarily conserved cellular process that targets intracellular components for lysosomal degradation. Its main purposes are to recycle nutrients from existing cell components under nutrient deficiency and to eliminate damaged or unnecessary organelles and proteins. Thus autophagy serves as a housekeeping mechanism and is mostly implicated in disease states when its activity is low. However, an exaggerated consumption of cellular components by overactivated autophagy may also disrupt cellular functions and contribute to pathogenesis. Indeed, autophagy can ambivalently support either cell death or survival, depending on a delicate, context-dependent regulation (8). Thus cellular homeostasis depends on careful regulation of autophagy, and both its attenuation and overactivation may contribute to the pathogenesis of various diseases.

The molecular events for executing autophagy are complex, involving a core machinery consisting of several dozen autophagy-related genes (ATGs) (FIGURE 1A). The process takes place sequentially in the following main stages. Induction of autophagy classically involves de-phosphorylation of the serine/threonine kinase Ulk1 (ATG1) and assembly of a complex consisting of Atg13, Atg17 (FIP200), and Atg101. Vesicle nucleation is mediated by Ulk1-induced phosphorylation and activation of Beclin1, the formation of a multiprotein complex, and creation of the phagophore, which is the initial piece of the autophagosomal double membrane. Vesicle elongation is essentially the growth of the phagophore into the autophagosome, a process regulated by a complex of Atg5-Atg12-Atg16 and by LC3(Atg8). Cargo-recognition involves proteins like p62 (sequestosome1), which links relevant cargo into to the autophagic machinery. Finally, fusion and degradation is the process in which the complete autophagosome merges (by outer membrane fusion) with lysosome to generate the autolysosome. This is followed by acidification of the vesicle, which enables the degradation of the autophagosome’s inner membrane and cargo by lysosomal acid hydrolases (FIGURE 1A; more detailed description of the process is provided in Refs. 15, 21, 28).

Recently accumulated evidence suggests that autophagy regulates lipid metabolism, complementing its involvement in glycogen and protein degradation (31, 32, 38, 61, 63). Lipophagy, the autophagic uptake and hydrolysis of cytosolic lipid droplets, was shown to function as a metabolic pathway for the breakdown of lipid droplets and the mobilization of lipids, thereby constituting an alternative/complementary pathway to “classical lipolysis” by cytosolic lipases (70). However, this was mainly studied in non-adipocytes, leaving largely unexplored the role of autophagy in the major cell type responsible for whole body lipid storage and release, particularly in conditions of gross alterations in lipid storage, as in obesity. Adipose tissue (AT) development and differentiation now have been realized to depend on intact autophagy: genetic approaches to interfere with the process (such as knockout of the autophagy genes ATG5 in pre-adipocytes or adipocyte-specific deletion of ATG5 or ATG7) resulted in failure to complete adipocyte differentiation, and with AT that resembles brown fat (1, 11, 64).

Sparking interest in manipulating autophagy in the context of obesity was the finding that adipocyte-specific, autophagy-deficient mice exhibited high oxygen consumption, and enhanced AT beta-oxidation, and were protected from high-fat-diet-induced obesity and metabolic derangements (1, 64). But does abnormally activated AT autophagy contribute to obesity and/or to its metabolic consequences? A few groups including ours have indeed found supportive evidence for such notion: Although not supported by all (66, 74),
FIGURE 1. Transcriptional regulation of adipose tissue autophagy by putative and confirmed (E2F1) transcription factors in obesity

A: autophagy begins with an initiation of an isolation membrane near cellular components targeted for degradation, which is followed by an elongation and closure of the isolation membrane and generation of a double-membrane vesicle named autophagosome (or phagophore). Next, docking and fusion steps between the outer membrane of the autophagosome and lysosome result in the creation of autophagolysosome. Cellular components carried by the autophagosome are then degraded by the lysosomal compartment's acid hydrolases. A large number of proteins participate in the different stages of autophagy. Several key gene products participating in each step and transcriptionally regulated are depicted. *Autophagy gene products regulated by E2F1 as assessed by promoter activity assay /H11002 /H11001 /E2F1 overexpression.

B: transcription factors that regulate autophagy gene products depicted in A at the different stages of autophagy. E2F1 is a confirmed regulator in adipose tissue; other transcription factors are putative adipose tissue regulators of autophagy based on known autophagy gene regulation capacity in nonadipose tissue, and known (autophagy-unrelated) activities in adipose tissue.

C: E2F1’s expression is elevated in human visceral adipose tissue in obesity, correlating with several systemic/clinical cardio-metabolic risk parameters, and with increased autophagic flux in omental adipose tissue. BMI, body mass index; WC, waist circumference; TG, serum triglycerides; FFA, circulating free fatty acids; GIR, glucose infusion rate during hyperinsulinemic-euglycemic clamp studies (a measure of insulin sensitivity).
autophagy is thought to be upregulated in human AT in obesity, particularly when accompanied by insulin resistance and/or Type 2 diabetes (T2DM), more so in visceral than in subcutaneous fat depots, and in persons displaying mostly intra-abdominal fat distribution (23, 30, 33, 38, 45, 46). Possible explanations for this not being universally observed by all investigators may span beyond the specific model (mice vs. humans) and technical differences in tissue handling (that can greatly affect autophagic activity, which is highly activated by stress and inflammation): Visceral fat particularly demonstrates autophagic activation in obesity, and whereas Soussi et al. (66) mainly investigated autophagy in subcutaneous isolated adipocytes, others focused on omental (intra-abdominal) AT. Moreover, when assessing whole AT, one is oblivious to differences in autophagic activity among the various cell types that populate adipose tissue (further discussed below). Yet, even when human isolated adipocytes are considered, results differ between studies (Ref. 66 vs. Ref. 46).

Interestingly, there seems to be less disagreement on the finding that obesity is associated with elevated expression of ATGs in (visceral) AT, at both the protein and the mRNA levels (33, 38). These changes may disclose regulatory mechanisms such as transcriptional-level regulation, which could support chronic adaptation (upregulation?) of AT autophagy to obesity—a chronic condition.

Until recently, it was commonly thought that autophagy is primarily regulated by ATGs’ protein–protein interactions and by their posttranslational modifications, particularly phosphorylation (21, 28). However, it is now understood that transcriptional control of autophagy genes is also tightly regulated by a growing number of transcription factors, most of which are also known to regulate genes involved in other cellular pathways, including metabolism, stress response, and cell cycle (13, 15). As with other aspects of autophagy, such mechanisms may be highly cell-type and context dependent, specificity that may underlie some conflicting reports in the literature. In AT in obesity, several considerations/cautionary notes are particularly noteworthy.

1) Obesity is a chronic state of nutrient overabundance. Classically, as a degradative/catabolic pathway, autophagy is mostly known to be activated by pathways that mediate nutrient deficiency/low energy state. These include low activity of mammalian target of rapamycin complex 1 (mTORC1) and activated AMP-activated protein kinase (AMPK). Conversely, autophagy is inhibited by signals of nutrient abundance (like insulin).

Indeed, the first study to report activation of autophagy in adipocytes suggested that the underlying mechanism is low mTOR activity, a result of insulin resistance (46). The transcriptional level regulation may suggest an input for autophagy activation by other triggers, more associated with nutrient overload, such as various stress response/responsive pathways [oxidative and endoplasmatic reticulum (ER) stress, inflammation, etc.].

2) Although mechanisms for developmental origins of adult obesity are of great interest, obesity frequently arises in the post-developmental state. Thus it is mature adipocytes, rather than differentiating pre-adipocytes, in which autophagy is activated in obesity. Current genetic mouse models have not provided insight on how activated autophagy in mature adipocytes alters their function. Nevertheless, transcriptional regulation of autophagy in such cells likely engages transcription factors expressed in mature adipocytes.

3) AT is composed of a diverse cell population that includes adipocytes, vascular cells, and various immune cells, particularly in obesity. In mouse models, which may exaggerate the robustness of obesity-associated AT inflammation compared with human obesity, macrophages were estimated to account for up to 50% of the tissue’s cell population. The cell-type specificity of autophagy regulation requires careful consideration when it comes to AT in obesity: As mentioned earlier, whole-tissue changes may obscure unique regulation of autophagy in a specific AT cell population and/or may reflect obesity-associated changes in cellular composition of the tissue.

This review considers transcriptional regulatory mechanisms of autophagy in adipocytes and AT in obesity by summarizing recent information on the topic. First, we briefly report findings implicating E2F1, currently the only transcriptional factor experimentally implicated in activated AT autophagy gene expression. Then, we review several likely transcription-regulating mechanisms of AT autophagy by considering transcription factors known to regulate autophagy in other tissues but that are also known to be expressed and regulate gene expression in adipocytes and/or non-adipocyte cells present in AT. Finally, we consider potential mechanisms by which dysregulated AT autophagy may lead to adipose dysfunction and contribute to obesity-associated cardio-metabolic morbidity.

E2F1: A Cell-Cycle Regulator That Activates Adipose Autophagy in Obesity

The E2F family is a group of transcriptional regulators (activators or repressors) best known for their involvement in cell cycle regulation, and therefore in cancer biology. E2F transcription factor 1 (E2F1) is a transcriptional activator that, un-
der different cellular contexts, supports either cell cycle progression and cellular growth or apoptotic cell death (6, 52, 68). The best characterized regulator of E2F1 activity is the tumor suppressor retinoblastoma 1 (Rb1). Beyond cell-cycle regulation, in non-adipocytes, E2F1 was shown to upregulate several ATGs, both through direct promoter binding (BCL2/adenovirus E1B 19-kDa interacting protein 3 (BNIP3), autophagy related gene 1 (ATG1), microtubule-associated protein 1 light chain 3 beta (LC3B), DNA-damage regulated autophagy modulator 1 (DRAM1)) and indirectly (ATG5) (19, 49, 59, 76). In cancer cells and in cardiac myocytes, E2F1 was shown to induce autophagy by activating BNIP3. BNIP3 overexpression is sufficient for induction of autophagy, releasing the inhibition of autophagy by B-cell CLL/lymphoma 2 (BCL2), which in turn acts by binding (and inhibiting) coiled-coil myosin-like BCL2-interacting protein (BECN1) (59). In human embryonic kidney 293 T cells (HEK293T), E2F1 overexpression increased promoter activity of several autophagic genes but, in addition, also sensitized the cells to further promote activation in response to the prototypic inflammatory cytokine tumor necrosis factor-α (TNF-α) (19).

In human AT, we have recently shown that E2F1 protein and mRNA levels are upregulated in obesity, correlating with elevated expression of ATG5 and LC3B (19) (FIGURE 1). This was particularly evident in omental rather than subcutaneous AT of obese patients in whom obesity is associated with a high risk for cardio-metabolic morbidity. Further substantiating this connection at the molecular level, a chromatin-immunoprecipitation (ChIP) assay optimized for use with fresh whole human AT explants (20) revealed increased E2F1 binding to a putative E2F1 binding site in the LC3B promoter in obese persons (19, 20). Interestingly, AT E2F1 expression did not correlate with proliferation markers such as marker of proliferation Ki-67 (Mki67), E2F1 binding to its classical cell cycle E2F1 target (cyclin D1) was not increased in obesity, and the elevated E2F1 expression was contributed mainly by the adipocyte (supposedly non-proliferating) cell fraction.

Collectively, utilizing complementary models, the suggested capacity of E2F1 to transcriptionally regulate AT autophagy is exemplified by the consistency of the data from the mRNA to the protein expression level, promoter binding, promoter activity and, finally, autophagic flux measurements (FIGURE 2). Yet, to causally implicate E2F1 in autophagy regulation in adipocytes, a loss-of-function approach was applied: mouse embryonic fibroblasts (MEFs) from wild-type (WT) vs. E2F1 knockout (KO) mice were differentiated into adipocyte-like cells. The efficiency of the differentiation was not grossly affected in the E2F1-deficient MEFs, as revealed by morphological appearance of the cells, by Oil red O staining, and by expression of several adipogenic genes. Expression of ATG’s and autophagic flux were attenuated in E2F1-null adipocyte-like cells, and autophagy was less activated by exposure to inflammatory cytokines than in WT control cells (19). Overall, rather than cell cycle regulation, these results identify E2F1 as a transcriptional regulator of autophagy genes and of the ability to activate autophagy to induction to inflammatory conditions in AT and in adipocytes. The upregulated expression of E2F1 in high-risk obesity phenotypes suggests the following pathway: visceral adiposity → high E2F1 → high adipose ATG expression and autophagic flux, and sensitization of autophagy to induction by inflammatory signals. The potential functional consequence of this proposed path will be discussed later.

Putative Transcriptional Regulators of Adipose Autophagy

The following section describes selected possible regulators of AT ATG expression. We consider their plausible involvement in AT autophagy regulation based on two criteria: 1) published autophagy-regulatory roles of these transcription factors in nonadipose tissue cells; 2) their non-autophagy-related functions in AT biology. Additional potential transcriptional regulators of AT autophagy that are not detailed in the text are briefly presented in Table 1.

NF-κB-IkB

Nuclear factor-κ-light-chain-enhancer of activated B cells (NF-κB) is a transcription factor activated by extracellular stimuli such as cytokines, reactive oxygen species (ROS), and bacterial or viral products. Its main regulation is through interaction with the inhibitory protein inhibitor of κB (IkB) that blocks NF-κB’s ability to migrate to the nucleus, bind DNA, and induce transcription. Regarding autophagy, NF-κB may serve as a “molecular switch” governing BNIP3 activity, somewhat counteracting E2F1: when NF-κB is bound to the promoter of BNIP3, it prevents E2F1 binding and promoter activation of this gene (15, 59). Given that E2F1 also stabilizes IkB, it seems that NF-κB and E2F1 could competitively and reciprocally regulate BNIP3. However, NF-κB cannot be viewed simply as an autophagy transcriptional inhibitor: NF-κB was shown to facilitate autophagy through transcriptional activation of several autophagy genes, like BECN1 and sequestosome 1 (SQSTM1/ p-62) (15).
A. mRNA

- E2F1/RNA18S1 mRNA
  - Non obese: Sc, Om
  - Obese: Sc, Om
  - Significance: **, ***

- MAP1C3B/RNA18S1 mRNA
  - Non obese: Sc, Om
  - Obese: Sc, Om
  - Significance: ***, ***

B. Protein

- E2F1
- LC3B-I
- LC3B-II
- ACTB

C. ChIP

- Non obese: Sc, Om
- Obese: Sc, Om
- Significance: ****, ***

- Occupation of MAP1LC3B promoter by E2F1 (% of input)

D. Promoter activity assay

- Relative MAP1LC3B promoter activity (folds of mock)

E. Autophagic flux assessment

- ΔLC3B ([+B-A1]-[B-A1]) (A.U.)
- Significance: *, ***
In AT, NF-κB has not been demonstrated directly as a regulator of autophagy. However, being a major transcriptional regulator of inflammation, NF-κB is thought to play a role in the pathogenesis of obese AT. Indeed, it was shown that the IKKB/NF-κB pathway inhibitors (salicylates) are capable of reversing some adverse effects of obesity (e.g., hyperinsulinemia, hyperglycemia, triglycerides, and FFA levels) in vivo and that IKKB knockdown partly protects obese mice from insulin resistance (75). Although a substantial portion of these effects was attributed to IKKB activity in liver (75) and in skeletal muscle (27), it was also demonstrated in adipocytes, since salicylate treatment in 3T3-L1 cells was shown to prevent the attenuation of insulin signaling induced by TNF-α and calyculin A. Given the putative involvement of NF-κB in AT dysfunction in obesity and its known function as a transcriptional regulator of autophagy, it may play a vital role in the interphase between autophagy and inflammation in obese AT. In this regard, particular focus should be given to AT immune cells, in which NF-κB is undoubtedly a critical regulator of inflammation and in which autophagy may further interact with immune cell function (62).

**FoxO Family of Transcription Factors**

The forkhead box O (FoxO) proteins are a family of transcription factors that control the expression of genes involved in cell cycle, regulation of differentiation, oxidative-stress response, and metabolism (essentially fasting-state metabolism, including control of gluconeogenesis genes) (18, 71). A role for FoxOs in the regulation of autophagy, particularly FoxO1 and FoxO3, has been described in various cell types, mostly cardiomyocytes and skeletal muscle, and in vivo (36, 39, 54, 72, 78). Both proteins, when overexpressed, were shown to induce autophagosome formation and mRNA expression of core autophagic machinery genes (Table 1). Also, in fasting/starvation, both proteins displayed more nuclear localization, accompanied by increased LC3B lipidation and increased promoter binding to core autophagy genes (ATG12, ATG8) (54). Interestingly, a non-transcriptional function of FoxO1 in the regulation of autophagy was also proposed, mediated by its phosphorylated and acetylated form, which directly binds and activates ATG7 (77). This dual role of FoxO1 may seem contradicting, since its nuclear export diminishes transcriptional activation of autophagy, but then its cytosolic function activates the process. A plausible explanation is of this being a deliberately fine-tuned feedback mechanism or that it represents tissue- and/or context-dependent activities of FoxO1.

FoxO3 was demonstrated to be both sufficient and necessary to induce autophagy in skeletal muscle fibers: a constitutively active (ca)-FoxO3 increased, and inhibiting FoxO3 decreased, the biogenesis of autophagosomes in primary skeletal muscle fibers of fed mice (39, 40). This was attributed to FoxO3 binding to the promoters of LC3B and BNI3P. Another mechanism proposed to mediate the effect of FoxO3 is its capacity to transcriptionally activate FoxO1 (78). Yet another, somewhat counter-intuitive explanation is FoxO3’s capacity to also transcriptionally activate phosphatidylinositol-4,5-bisphosphate 3-kinase, catalytic subunit alpha (PIK3CA), the catalytic subunit of PI3K class I which phosphorylates FoxO1 and induces its nuclear exclusion, thereby decreasing its transcriptional activation of ATGs, but promotes its pro-autophagic cytosolic function (78). Regardless, FoxO1 may constitute only part of the autophagy-activating pathways induced by FoxO3, since (ca)-FoxO3 induced a greater increase in autophagosome biogenesis than (ca)-FoxO1 (54).

Although a role for FoxOs in regulating AT autophagy has not been established, FoxOs, particularly FoxO1, have confirmed non-autophagy-related functions in adipocytes/AT: FoxO1 is known to inhibit...
Table 1. **Key potential transcription factors in the regulation of adipose tissue autophagy**

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<tr>
<td><strong>Confirmed role in transcriptional regulation of adipose tissue autophagy genes</strong></td>
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<tr>
<td>E2F1</td>
<td>Cell-cycle regulation, apoptosis</td>
<td>ATG1, LC3, DRAM1, BNIP3</td>
<td>Human adipose tissue, HEK293T cells, rat cardiomyocytes, WI38 cells, human osteosarcoma cell line (USOS)</td>
<td>23, 65, 78, 100</td>
<td>Autophagy, adipogenesis, endocrine function, stress response</td>
<td>15, 23</td>
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<tr>
<td><strong>Proposed transcriptional regulators of adipose tissue autophagy</strong></td>
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<tr>
<td>FOXO1</td>
<td>Metabolic homeostasis, oxidative stress response</td>
<td>ATG5, ATG12, ATG14, BECN1, BNIP3, VPS34, LC3</td>
<td>Rat cardiomyocytes, HEK293T cells, MEFs, hepatocytes</td>
<td>44, 73, 95, 106</td>
<td>Adipogenesis, oxidative stress response, immune cell function, AT endothelial insulin resistance</td>
<td>21, 30, 94</td>
</tr>
<tr>
<td>FOXO3</td>
<td>Oxidative stress response, apoptosis</td>
<td>ATG4, ATG12, BECN1, BNIP3, VPS34, ULK1, ULK2</td>
<td>Rat cardiomyocytes, HEK293T cells, MEFs, skeletal muscle</td>
<td>49, 50, 73, 103, 106</td>
<td>Immune cell function, oxidative stress response.</td>
<td>21, 94</td>
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<tr>
<td>NF-κB</td>
<td>“Fast responding” nuclear receptor. Responds to inflammation, infection, oxidative stress, and other stimuli</td>
<td>BNIP3, BECN1, P62, BCL2</td>
<td>Rat cardiomyocytes, human pancreatic carcinoma cells (panc-1)</td>
<td>78</td>
<td>Cytokine secretion, inflammatory reaction, ER stress response, AT immune cell function</td>
<td>70</td>
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<td>FXR</td>
<td>Lipid and bile acid sensing, fed-state regulation</td>
<td>Multiple genes (possibly 178)</td>
<td>Murine liver tissue, mouse hepatoma cells</td>
<td>41, 74</td>
<td>Regulates adipocyte-secretion</td>
<td>82</td>
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<td>CREB</td>
<td>Neuroplasticity and neuropeptides transcription, circadian rhythm</td>
<td>Multiple genes (possibly 112)</td>
<td>Murine liver tissue, mouse hepatoma cells</td>
<td>74</td>
<td>Adipogenesis, adiponectin secretion</td>
<td>33, 47, 102</td>
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<td>PPARa</td>
<td>Fasting-state regulator in the liver</td>
<td>Multiple genes (possibly 124)</td>
<td>Murine liver tissue, murine hepatocytes (AML12)</td>
<td>41</td>
<td>Lipolysis, cell size regulation, adipore expression, insulin sensitizing</td>
<td>91</td>
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<td>TFEB</td>
<td>Lysosomal biogenesis</td>
<td>ATG4, ATG9, LC3, P62, UVRAG, WIPI, BCL2</td>
<td>HeLa cells, COS7 cells, MEFs, ARPE-19 cells, CF7 cells, murine liver tissue</td>
<td>51, 75–77</td>
<td>?</td>
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<td>P53</td>
<td>DNA repair, cell-cycle regulation, apoptosis</td>
<td>ATG2, ATG4, ATG7, ATG10, ULK1, UVRAG, BCL2 NIP3, LC3, ULK</td>
<td>MEFs, HeLa cells, HCT116 (colon cancer cells), HFF2 (human fibroblasts, and more</td>
<td>31, 32, 58</td>
<td>Adipogenesis, insulin resistance</td>
<td>25, 55</td>
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<td>C/EBPbeta</td>
<td>Inflammation and cytokine production</td>
<td>Multiple genes</td>
<td>Primary murine hepatocytes, murine liver tissue</td>
<td>46</td>
<td>Adipogenesis</td>
<td>22</td>
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<td>ATF4</td>
<td>Cellular stress adaptation</td>
<td>ATG5, BH3 only, LC3, ULK1</td>
<td>HeLa cells, MEFs, multiple cancer cell lines (MCF7, MDA231, HCT116, HT29, A431)</td>
<td>1, 54, 64, 72</td>
<td>ER stress, lipid metabolism and thermogenesis, adipogenesis</td>
<td>92, 99</td>
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peroxisome proliferator-activated receptor γ (PPARγ), oppose cell-cycle progression, and thereby inhibit “clonal expansion” of pre-adipocytes upon induction of adipogenesis (18). FoxO1 is also known to participate in the defense response to oxidative stress (6), which develops in AT in obesity. Furthermore, FoxO1 is positively regulated by TNF-α, a pro-inflammatory cytokine implicated in AT inflammation (22, 71). All of these suggest that FoxO1 is likely elevated in AT in obesity and contributes to its dysfunction. Indeed, FoxO1 deletion increases adipocyte expression of PPARγ and glucose transporter type 4 (GLUT4), genes promoting adipocytes’ insulin sensitivity (42). Furthermore, adipocyte-/AT-specific silencing of FoxO1 leads to a healthier phenotype under high-fat diet, reflected in smaller adipocyte size, improved insulin sensitivity, higher adiponectin, and lower TNF-α secretion (43). Interestingly, the cytosolic function of FoxO1 may also be active in obesity, since sirtuin 2 (SIRT2) is downregulated in obese AT due to oxidative stress and hypoxia-inducible factor-1α (HIF-1α) expression (34). Among non-adipocytes comprising AT, FoxO1 hyperactivity was connected to endothelial insulin resistance in obesity (24). Furthermore, cultured macrophages treated with adiponectin secreted lower levels of TNF-α, an effect that was mediated by increased FoxO3a-dependent autophagy (51). An attractive level of further complexity may engage signal transducer and activator of transcription 3 (STAT3) via regulation of the FoxO-autophagy “axis” (60) in AT immune and AT stem cell function (12, 50).

**FXR, PPARα, and CREB Axes**

Farnesoid X receptor (FXR) is a metabolic nuclear receptor that functions as an intracellular sensor that translates nutrient signals into transcriptional programming. It is a fed-state sensor activated by increased bile-acid levels in the postabsorptive state, which regulates postprandial metabolic response in hepatocytes (4, 55). cAMP responsive element binding protein (CREB) is a fasting-state effector protein, which, on stimulation, binds a cAMP-response-element DNA sequence to induce

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**Table 1.—Continued**

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<td>ATF5</td>
<td>Neuroprogenitor cells differentiation</td>
<td>mTOR</td>
<td>Chronic myeloid leukemia cells K562 cell line Human primary peripheral blood cells.</td>
<td>80</td>
<td>Adipogenesis</td>
<td>105</td>
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<td>HIF1</td>
<td>Adaptive response to hypoxia</td>
<td>Bnip3</td>
<td>MEFs</td>
<td>101</td>
<td>Adipogenesis, adipose dysfunction in obesity</td>
<td>90</td>
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<td>STAT1</td>
<td>Cellular response to inflammatory factors, mainly IFNs</td>
<td>ATG12, BECN1</td>
<td>Primary murine cardiomyocytes</td>
<td>52</td>
<td>Lipolysis</td>
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<tr>
<td>STAT3</td>
<td>Cellular response to growth factors and interleukines</td>
<td>ATG3, BNIP3, BCL2</td>
<td>Human neuroblastoma cells</td>
<td>43</td>
<td>Adipogenesis, body weight regulation</td>
<td>6, 93</td>
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<td>Beta-catenin</td>
<td>Effector of Wnt signaling pathway, adhesion junctions formation and cell adhesion regulation</td>
<td>SQSTM1</td>
<td>Human cancer cell-lines (HT29, RKO, HCT116) HEK293T</td>
<td>63</td>
<td>Adipogenesis</td>
<td>45, 71</td>
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<td>c-JUN</td>
<td>Cell cycle progression, apoptosis</td>
<td>BECN1, LC3</td>
<td>Human nasopharyngeal carcinoma cells (CNE2), human hepatocellular carcinoma cells (Hep3B), human vascular smooth muscle cells</td>
<td>29, 42, 87</td>
<td>Insulin resistance</td>
<td>97</td>
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or inhibit transcription (29). In primary mouse liver cells, chromatin immunoprecipitation and high throughput sequencing revealed FXR direct binding to 178 genes involved in key autophagy processes. This was further confirmed by decreased mRNA levels of most of these genes upon treatment with FXR-agonist, which also reduced autophagic vesicle biogenesis and mitochondrial double-membrane engulfment (mitophagy), and resulted in decreased levels of LC3-II and accumulation of SQSTM1/p-62 (55). These data suggest that FXR directly binds and inhibits multiple autophagic genes, thereby attenuating autophagy. Similar techniques used for CREB revealed its binding to 112 autophagy-related genes, 78 of which overlapped with FXR targets. Downregulation of CREB decreased expression of autophagy genes and GFP-LC3 puncta formation in mouse hepatocytes, and attenuated lipophagy induced by fasting (55). In contrast, downregulation of FXR increased lipophagy in nonfasted cells (55). Altogether, it seems that FXR and CREB antagonize each other’s transcriptional regulation of autophagy. Importantly, a mutated CREB site or downregulation of CREB blocked FXR-mediated inhibition of autophagy, suggesting that FXR-mediated inhibition of autophagy may be CREB mediated. Indeed, co-immunoprecipitation studies proved that although FXR promoter occupancy does not displace CREB’s binding to these promoters, the binding of CREB to its coactivator CRTC2 is decreased. These data (further detailed in Ref. 55) indicate that FXR trans-represses expression of autophagy genes by disrupting the CREB-CRTC2 complex.

FXR was further shown to be opposed by the activity of PPARα, another nutrient-sensing transcriptional regulator like FXR itself (35). Pharmacological activation of PPARα reverses the normal, FXR-induced suppression of autophagy in the fed state, inducing lipophagy in primary mouse hepatocytes (35). PPARα and FXR were suggested to compete over shared binding sites in autophagy gene promoters, with opposite transcriptional outcomes. These results indicate a complementary mechanism for the transcriptional regulation of autophagy induced by nutrient status.

Whether AT autophagy is regulated by FXR, CREB, and PPARα has not been explored. It is interesting that PPARα, a transcriptional activator of autophagy in the liver, was actually proven to exert beneficial effects on the metabolic state of obese and insulin-resistant AT (14, 69). It would be interesting to discover the roles of these factors in the seeming opposite regulation of autophagy gene expression in the liver vs. AT in obese states (38).

### TFEB and ZKSCAN3

Transcription factor EB (TFEB) is a basic helix-loop-helix leucine zipper transcription factor, which is regulated by mTOR or mitogen-activated protein kinase 1 (ERK2)-mediated phosphorylation that induces nuclear exclusion, thereby inhibiting its transcriptional activity (41, 58). It is a key regulator of a gene network for lysosomal biogenesis and function (coordinated lysosomal enhancement and regulation (CLEAR)) by binding to CLEAR-box sequences (5-GTCAGGTGAC-3) in respective gene promoters (48). As such, its involvement in other lysosomal-related processes has been explored, and, indeed, 11 autophagy-related genes, including LC3B, SQSTM1/p-62 ATG9B, and UV radiation resistance-associated protein (UVRAG), were significantly elevated following TFEB overexpression, with proven direct promoter binding of TFEB confirmed by ChIP (58). In vivo, nuclear localization of TFEB and induction of both lysosomal and autophagic TFEB targets preceded starvation-induced increase in GFP-LC3-positive vesicles in GFP-LC3 mice (56, 58). These results suggest that TFEB has a broad activating function on multiple autophagy steps. The potential metabolic outcome of such regulation has been explored in the liver: Liver-specific TFEB-KO resulted in impaired lipid breakdown under starvation, whereas liver-specific overexpression of TFEB upregulated expression of genes involved in lipid metabolism (57). While this could represent TFEB’s direct effects via the autophagosomal/lysosomal lipid degradation pathway (lipophagy), it may also affect lipid oxidative capacity by direct binding to PGC1α (57).

Zinc-finger protein with KRAB and SCAN domains 3 (ZKSCAN3), another DNA-binding protein, seems to function as TFEB’s counterpart, acting as a transcriptional repressor of multiple autophagic and lysosomal genes, including LC3B, ULK1, ATG18B, and others (5, 9). Its own expression is reciprocally regulated to TFEB, being inhibited by starvation. Downregulating ZKSCAN3 was sufficient to induce autophagosome and lysosome formation, and its overexpression attenuated rapamycin-induced autophagy. The role of TFEB and ZKSCAN3 in the activation of AT autophagy awaits future studies and is raised here mainly since AT macrophages have been shown to exhibit enhanced lysosomal biogenesis in response to caloric restriction (73). Furthermore, it raises a more general question: To what degree is autophagy activation a primary/unique event vs. more general regulatory mechanisms of the “lysosomal compartment”?
The Functional Consequence of Activated AT Autophagy in Obesity

Defining the functional consequence of altered AT autophagy is crucial for both basic mechanistic understanding of obesity-associated morbidity, as well as for considering the prospects of manipulating autophagy as a therapeutic strategy. Current understanding is limited and controversial. Pharmacological inhibition of autophagy induction using 3-methyladenine (3MA) in human adipose tissue explants increased secretion and expression of pro-inflammatory cytokines (23), suggesting that the activated AT autophagy in obesity restraits adipose tissue inflammation. In contrast, other lines of evidence suggest that activated AT autophagy in obesity contributes to pathogenesis, reminiscent of its role in chronic lung disease (7).

First, clinically, elevated AT autophagy associates with a high-risk obesity phenotype (visceral fat distribution, obesity that associates with insulin resistance) (33). A transcriptional basis for this regulation is offered by significant associations of E2F1 expression in visceral (omental) AT with clinical parameters indicating elevated cardio-metabolic risk, including higher circulating free fatty acids, interleukin 6 (IL-6), and insulin resistance, and lower circulating adiponectin levels (19) (FIGURE 1). These associations were attenuated when the statistical model was adjusted to the expression of ATG5 or LC3, indicating that elevated AT E2F1 and activated autophagy genes are on the same path linking obesity with its associated cardio-metabolic morbidities. More mechanistically, E2F1-null adipocytes were less lipolytic, tended to respond better to insulin, secreted less leptin and more adiponectin, and, importantly, were more resilient to the dysfunctioning effects of exposure to pro-inflammatory cytokines (19). Intriguingly, both siRNA-mediated ATG7 knockdown in mature adipocytes and pharmacological inhibition of autophagy in adipose tissue from obese mice recapitulated the effects of E2F1 absence, resulting in increased adiponectin secretion (65). Thus activated AT (and adipocyte) autophagy in obesity may causally contribute to dysfunctional endocrine function of this tissue, linking obesity to its comorbidities.

The functional significance of autophagy alterations in AT non-adipocyte (i.e., SVF) cells is even less clear. Autophagy may exert profound regulatory effects on immune cell function, but how obesity alters autophagy in these cells is not entirely clear. Bone marrow-derived macrophages from high-fat-fed mice exhibited attenuated autophagy (37), but this does not necessarily represent AT macrophages. Lysosomal biogenesis in AT macrophages under conditions of obesity reversal was shown to participate in intra-AT lipid mobilization (73). Yet, it was claimed that this effect is not mediated by enhanced autophagic flux. Indeed, how autophagy contributes to lipid handling by macrophages is incompletely understood. In atherosclerotic plaque, where macrophages accumulate lipid...
largely by endocytosing modified low-density lipoproteins (LDL), autophagy may be required for reverse cholesterol transport (47) and is therefore considered anti-atherogenic. This would be consistent with lipid droplet autophagy (lipophagy) as a mechanism for esterified lipids degradation. Yet, recent data suggest in MEFs that autophagy, supposedly of the endoplasmic reticulum, may actually “feed” lipid droplets, which in turn are degraded by cytosolic, rather than lysosomal, lipase [adipose triglyceride lipase (ATGL) and not lysosomal acid lipase (LAL)] during starvation (53). The functional significance of autophagy in AT macrophages, which supposedly accumulate lipids by either phagocytosis of adipocyte remnants and/or by de novo lipogenesis, remains to be elucidated. Beyond lipid metabolism, given the potential close, bidirectional links between autophagy and inflammation, once the dysregulation of autophagy in AT immune cells in obesity is clarified, it would be interesting to explore its contribution to their cell-autonomous immune-cell function and to AT dysfunction. Most commonly considered in immune cells is the link between attenuated autophagy and activated inflammation. It would be important to assess whether this link is also operational in AT immune cells or, conversely, that activated autophagy in AT cells acts to restrain “excessive” inflammatory response (as proposed by Ref. 23). Furthermore, it would be interesting to assess whether this involves transcriptional mechanisms and/or is linked to altered immune cell metabolism.

**Table 2. Key outstanding questions**

<table>
<thead>
<tr>
<th>Question</th>
<th>Discussion</th>
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<tr>
<td>How is autophagy and its transcriptional regulation affected by obesity in non-adipocyte cells of AT?</td>
<td>Existing data support autophagy activation in adipocytes in obesity/obesity + TZDM, but the data on cells composing AT SVF are scarce. A fundamental limitation in addressing this question stems from the fact that isolation procedures to obtain individual cell types from adipose tissue impose significant stress on the cells and may thereby affect autophagy and its transcriptional regulation, resulting in artifactual or even paradoxical results.</td>
</tr>
<tr>
<td>The “chicken and egg” question: Is increased adipose tissue autophagy the cause or the consequence of obesity-associated morbidity (like insulin resistance)?</td>
<td>The first report of elevated adipocyte autophagy (46) largely suggested it is a consequence of insulin resistance, but autophagy may also contribute to pathogenesis. These two possibilities are not mutually exclusive, since circular pathways and feedbacks, rather than linear pathways, are frequent in pathogenic processes.</td>
</tr>
<tr>
<td>Does increased ATG gene and protein expression actually drive autophagy?</td>
<td>Although basic reasoning, it is still worthwhile to emphasize that correlated increased gene expression and autophagic flux may still represent an association and does not, on its own, prove that the mechanism for enhanced autophagy is the elevated gene expression. Gain and loss of function approaches are required to prove causality, but challenge remains in “normalizing,” rather than completely knocking out, the suspected gene of interest. Moreover, recent evidence arguing that obesity attenuates, rather than activates, autophagy in isolated human adipocytes (66) further adds to remaining basic uncertainties in this field.</td>
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<tr>
<td>What is the relative contribution of transcriptional mechanisms vs. other levels of regulation in determining adipose tissue autophagy?</td>
<td>In addition to the most frequently considered protein-protein interactions and phosphorylation, other levels of regulation determine autophagic activity, including miRNA, epigenetic mechanisms like histone modifications, and different posttranslational pathways. The relative contribution of each of these regulatory levels in adipose tissue has yet to be defined in health and disease.</td>
</tr>
<tr>
<td>What is the functional consequence of activated adipose tissue autophagy in obesity? Is it a protective process or one that contributes to pathogenesis?</td>
<td>Evidence is beginning to accumulate on this question, which still remains unsettled. Lack of specific and efficient tools to manipulate autophagy, particularly in vivo, greatly limit elucidating the functional consequences of altered AT autophagy in obesity.</td>
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<tr>
<td>Can autophagy genes be utilized as therapeutic targets in treating obesity and its related disorders?</td>
<td>This is a key translational question that likely awaits more fundamental understanding of the complexity of autophagy regulation and function.</td>
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**Concluding Remarks**

Dysregulated AT autophagy emerges as a potential mechanistic link between obesity and its associated morbidities. Although autophagy was mainly thought to be regulated by posttranscriptional mechanisms, transcriptional (dys)regulation of AT autophagy is currently emerging as a potentially significant alteration in obesity. The exact transcriptional regulators and other key outstanding questions need to be addressed before the full impact of AT autophagy is elucidated (Table 2).
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10. Dunlop EA, Tee AR. The kinase triad, AMPK, mTORC1 and design of research; N.M., S.B., Z.V., N.G., Y.H., and A.R. approved final version of manuscript; Y.H. prepared figures.


