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Adipose tissue insulin sensitivity and macrophage recruitment

Does PI3K pick the pathway?

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In the United States, obesity is a burgeoning health crisis, with over 30% of adults and nearly 20% of children classified as obese. Insulin resistance, a common metabolic complication associated with obesity, significantly increases the risk of developing metabolic diseases such as hypertension, coronary heart disease, stroke, type 2 diabetes, and certain cancers. With the seminal finding that obese adipose tissue harbors cytokine secreting immune cells, obesity-related research over the past decade has focused on understanding adipocyte–macrophage crosstalk and its impact on systemic insulin sensitivity. Indeed, adipose tissue has emerged as a central mediator of obesity- and diet-induced insulin resistance. In this mini-review, we focus on a potential role of adipose tissue phosphoinositide 3-kinase (PI3K) as a point of convergence of cellular signaling pathways that integrates nutrient sensing and inflammatory signaling to regulate tissue insulin sensitivity.

Introduction

Obesity and its associated metabolic diseases are one of the greatest public health challenges in the United States.[1] Insulin resistance, a central and defining feature of obesity, has been identified as a primary contributor to the increase in many metabolic diseases including type 2 diabetes, cardiovascular disease, and hypertension.[2] Currently, obesity-induced insulin resistance is proposed to arise secondary to an inflammatory response that is caused by an infiltration of adipose tissue by monocytes and subsequent pro-inflammatory macrophage differentiation.[3,4] Adipose tissue macrophages release pro-inflammatory cytokines that act both locally on adipocytes and vascular cells and also circulate to distal tissues to stimulate intracellular pro-inflammatory pathways. In a feed-forward cycle, these cytokines can also stimulate adipose tissue macrophages to secrete chemokines that promote the recruitment and infiltration of additional monocytes/macrophages into adipose tissue. These combined actions result in cell-autonomous insulin resistance in adipocytes, exacerbation of the inflammatory state, and subsequent systemic insulin resistance.[3,4] While increased macrophage accumulation is the primary driver of the pro-inflammatory response, the cellular processes and signals within the adipose tissue that initiate and promote an unresolved pro-inflammatory state and systemic insulin resistance are not well understood. We propose that adipocyte insulin resistance per se initiates macrophage recruitment through dysregulation of adipocyte nutrient metabolism and adipose tissue vascular remodeling. Further, our recent findings suggest that these events are orchestrated by changes in phosphoinositide 3-kinase (PI3K) signaling.[5]

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PI3K: The great integrator. PI3K is essential for many cellular functions such as differentiation, apoptosis, cell growth, motility and is necessary for almost all of insulin's metabolic actions including glucose transport, lipid metabolism, and glycogen and protein synthesis.[6] In adipocytes, PI3K plays a key role in differentiation,[7] and is essential for insulin-stimulated glucose uptake and suppression of lipolysis. It also regulates insulin-stimulated remodeling of the adipocyte extracellular matrix,[8] collagen production,[9] vascular growth, and function.[10] Additionally, PI3K signaling is required for several key immune cell functions from cytokine production to proliferation and chemotaxis.[11,12] Various impairments in these PI3K-linked pathways are found in obesity, implicating it as a major contributor to the progression of insulin resistance and inflammation in adipose tissue.

The specificity of PI3K signaling for these diverse pathways within a cell is not fully understood but likely relies on the cellular distribution and specificity of the regulatory/adaptor subunit isoforms. The Class I PI3K is an obligate heterodimer consisting of a regulatory subunit and a p110 catalytic subunit, each of which have several isoforms. Class I is further subdivided into IA or IB, which have unique regulatory and catalytic subunits and signaling patterns. The class IA regulatory subunit isoforms are a group of related proteins encoded by the *Pik3r1* (p85α, p55α, p50α), *Pik3r2* (p85β), and *Pik3r3* (p55γ).[6,13] They control not only the subcellular localization of the enzyme but also the stability and activation of the catalytic subunits (p110α, p110β, and p110δ) through domain interactions.[14] The regulatory subunit isoforms share common N-terminal and C-terminal Src-homology-2 (SH2) domains with affinity for phosphorylated tyrosine resides linking the Class IA PI3Ks to tyrosine kinase receptor signaling

pathways.[15,16] Detailed information on the structure and function of PI3K subunit domains is reviewed elsewhere.[6]

For the purposes of this mini-review, we focus on the role of the class IA PI3K regulatory subunits in mediating obesity-induced insulin resistance and inflammation in adipose tissue. This mini-review highlights key PI3K-dependent pathways in adipose tissue adipocytes, vascular cells and macrophage that are dysregulated in obesity and implicated in the pro-inflammatory phenotype that contributes to systemic insulin resistance. We speculate that changes in PI3K signaling under conditions of nutrient excess (such as in obesity) coordinately lead to adipocyte insulin resistance, vascular remodeling, and macrophage recruitment.

Adaptor Molecules Define PI3K Signaling Specificity

Individual signaling roles for the different regulatory subunits has not been fully explored. However, differences in insulin activation and binding affinities between the *Pik3r1* subunits have been tested in differentiated adipocyte and muscle cell cultures.[17] Interestingly, PI3K activity associated with p50α was greater than that associated with p85α or p55α, while increasing the level of p85α or p55α, but not p50α, inhibited both phosphotyrosine-associated and p110-associated PI3K activities and downstream signaling at Akt when expressed either alone or in the presence of overexpression of p110α.[16] These data suggest that p85α and p55α act as both positive and negative regulators of insulin action whereas the p50α subunit lacks the inhibitory function. Consistent with in vitro studies, transgenic mouse models have demonstrated that deletion of the class IA regulatory isoforms (p85α only, p85β only, and p55α/p50α double knockout) or heterozygous *Pik3r1* deletion enhances PI3K activity and subsequent insulin sensitivity.[15,18-20]

Regulatory to catalytic subunit ratio: Are the regulatory subunits "free"? Despite nearly a decade of observation, the mechanism of action for improved insulin action with reduced subunits is still controversial. The original mechanism proposed that "excess" regulatory subunits not bound to a catalytic subunit act as negative inhibitors of insulin action by competing with functional heterodimers for IRS binding sites.[13] This idea has stemmed primarily from knockout mouse studies and immunodepletion assays in cells and tissues.[21,22] However, the idea of "free p85" has been disputed as unstable and non-existent in cells as measured by quantitative mass spectrophotometry.[23] While the report by Geering et al. is convincing, the p85 to p110 ratio was only tested in tissues from mice under normal conditions. Most studies that have found increased expression or abundance of the regulatory subunits and reduced insulin-stimulated PI3K activity were in tissues from mice or humans under conditions of physiological stress like obesity,[5,24] nutrient excess,[25] pregnancy,[26] or excess growth hormone.[27] To date, there have been no in vivo studies using a *Pik3r1* over-expression model to test directly whether increasing the regulatory subunits would have the opposite effects of subunit deletion on insulin sensitivity. Over-expression or knock-in mouse studies may also reveal novel signaling roles or binding partners for the different regulatory subunits.

PI3K-independent effects of the regulatory subunits. Several studies have identified PI3K-independent roles for p85α subunit that may better explain the inverse relationship between p85α abundance and insulin sensitivity. Such roles include binding and stabilization of the lipid phosphatase, PTEN (phosphatase and tensin homolog deleted on chromosome 10), which directly opposes PI3K action.[28,29] Additional kinase-independent roles for p85α include nuclear translocation of XBP-1, important in ER stress signaling[30,31] and insulin-activation of c-Jun N-terminal kinase (JNK) through association with Cdc42.[32] The p85β subunit has also been shown to bind and translocate XBP-1 into the nucleus.[30] Kinase-independent roles have not yet been elucidated for the shorter *Pik3r1* isoforms, p50α and p55α or for the *Pik3r3*, p55γ. However, novel, non-redundant PI3K signaling roles have been attributed to both p50α-PI3K and p55α-PI3K. For example, in activated T cells, p50α-PI3K was shown to accumulate at the immunological synapse to mediate ICOS signaling for cytokine production.[33] In addition, p55α expression is specifically induced in cells infected by Epstein-Barr virus and p55α-PI3K is necessary for cell proliferation as knockdown of p55α in these cells resulted in apoptosis.[34] In contrast, in mammary epithelial cells, signal transducer and activator of transcription 3 (STAT3) activation increases p55α and p50α expression and reduces PI3K activity and Akt signaling, leading to cell apoptosis, which is necessary for involution.[35] Taken together, these studies reveal that differential increases in p85α, p55α, and p50α abundance is another mechanism used by cells to confer specificity of PI3K signaling in response to different stimuli.

Metabolic functions of PI3K. The function of PI3K is to convert phosphatidylinositol-4,5-bisphosphate (PIP_2) into phosphatidylinositol-3,4,5-trisphosphate (PIP_3) creating true second messengers in cellular membranes. In an un-stimulated state, the heterodimer is held in the cytosol in a state of low kinase activity through its association with its regulatory subunit. With hormone or cytokine stimulation, PI3K regulatory subunits are recruited to adaptor proteins containing tyrosine phosphorylated (pYxxM) sites. In adipocytes, substrate metabolism is regulated by insulin activation of PI3K leading to increased glucose metabolism and decreased fat metabolism (**Fig. 1**). Insulin-stimulation leads to auto-phosphorylation of the insulin receptor and subsequent recruitment and tyrosine phosphorylation of insulin receptor substrate (IRS1–4) proteins. IRS proteins act as docking sites for the regulator subunits and binding leads to a conformational change that relieves kinase inhibition and brings the enzyme in close proximity to its lipid membrane substrate.[14] After differentiation, the p110β subunit is the predominant insulin-responsive catalytic isoform in adipocytes.[36] Increased PIP_3 in the membrane attracts two key signaling proteins with lipid binding pleckstrin homology (PH) domains, PDK1 (phosphoinositide-dependent kinase-1) and PKB/Akt (protein kinase B). Akt isoforms are major downstream effector molecules of PI3K and are activated through phosphorylation by PDK1 at threonine 308 and by mTORC2 at serine 473.[37] In adipocytes, Akt2 is the primary isoform that stimulates the translocation of the glucose transporter GLUT4 to the plasma membrane, thereby promoting the uptake of glucose into the cell.[38]

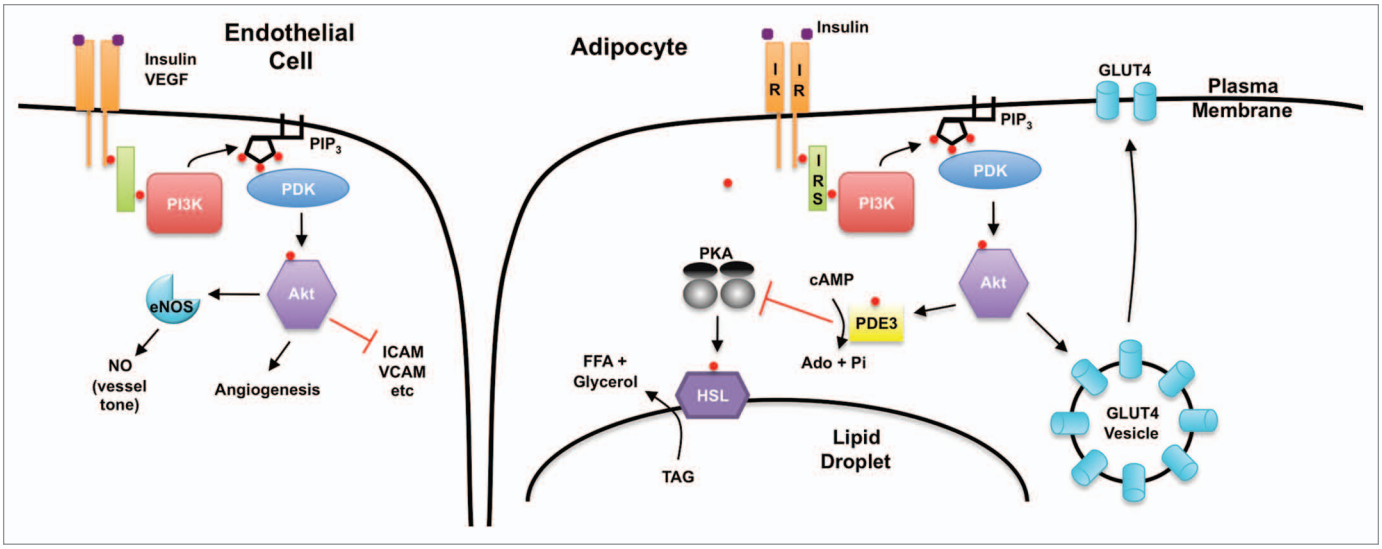

Figure 1. Insulin-stimulated PI3K signaling in adipocyte and endothelial cells. Insulin binding to the insulin receptor (IR) leads to auto-phosphorylation at tyrosine residues and subsequent recruitment and tyrosine phosphorylation of insulin receptor substrate (IRS) proteins. IRS acts as a docking protein for PI3K and binding by regulatory subunit relieves kinase inhibition and brings the enzyme in close proximity to the phospholipid membrane. PI3K phosphorylates the 3' site on the inositol ring forming phosphoinositol (3, 4, 5) phosphate (PIP_3), which attracts lipid binding proteins, PDK1 and Akt. In adipocytes, activation of Akt is essential for glucose clearance by enhancing signals necessary for GLUT4 translocation to the plasma membrane and for suppression of lipolysis through phosphorylation and activation of phosphodiesterase 3b (PDE3b). Activation of PDE3b leads to inhibition of protein kinase A (PKA) and hormone sensitive lipase (HSL) by depleting cyclic AMP (cAMP). In vascular endothelial cells, activation of Akt by PI3K promotes angiogenesis and nitric oxide (NO) production for vascular tone. Akt also represses the expression of adhesion molecules involved in leukocyte rolling and adhesion to the vascular luminal wall.

Insulin-activation of PI3K signaling is also important in suppression of lipolysis through activation of Akt and phosphorylation of phosphodiesterase 3b (PDE3b) at Ser273.[39] Activation of PDE3b catalyzes the hydrolysis of cAMP to 5'AMP, thereby attenuating PKA activity and decreasing the activity of hormone-sensitive lipase (HSL). Suppression of HSL inhibits the release of fatty acids from adipocytes triglycerides. A recent study by Choi et al. suggests that insulin may also regulate lipolysis through a PI3K-dependent but Akt-independent pathway in conditions of submaximal/physiological beta-adrenergic stimulation, potentially through subcellular localization of signaling to directly regulate perilipin phosphorylation.[40] This divergence in insulin signaling downstream of PI3K for glucose versus lipid metabolism has also been observed in liver, whereby insulin stimulates the PI3K–Akt axis to regulate gluconeogenesis and the PI3K-aPKC (atypical protein kinase C) λ/ζ to regulate lipogenesis.[41] Increased lipolysis in obese adipose tissue resulting in an increase in circulating free fatty acids (FFAs) is thought to be a major contributor to skeletal muscle and systemic insulin resistance. To this end, increasing FFAs flux in lean individuals to rates similar or greater than those found in obesity induces insulin resistance.[42] Pharmacological treatment with acipimox to reduce plasma FFAs improves insulin sensitivity in obese and diabetic subjects.[29] FFAs can directly induce insulin resistance in skeletal muscle, adipocytes and liver through increased production of fatty acid intermediates (diacylglyceride, ceramide, and long-chain acyl-CoA) that activate stress or inflammatory kinases and inhibit insulin signaling.[43] Activation of stress kinases, JNK, inhibitor of κB kinase (IKK), and atypical aPKCλ/ζ, inhibit insulin signaling through serine phosphorylation of IRS, which both prevents IRS tyrosine phosphorylation for PI3K association and signals for IRS degradation.[43]

PI3K Signaling: At the Heart of the Inflammatory Response in Obesity

Does NFκB link PI3K to the inflammatory response in obesity? Nuclear factor κB (NFκB) is a key transcriptional regulator of the inflammatory response associated with diet-induced obesity in various cell types, including adipocytes, and along with other transcription factors coordinates the increase in cytokine and chemokine production, inflammatory enzymes and adhesion molecules.[44] FFAs can increase pro-inflammatory cytokine and chemokine secretion via activation of Toll-like receptor (TLR) 2 or TLR4 and stimulation of signaling to NFκB in macrophage and other immune cells.[45] This suggests that FFAs may indirectly stimulate insulin resistance through recruitment and activation of macrophage. Knockout of IKKβ or TLR4, the major upstream regulators of NFκB, in myeloid cells ameliorates obesity induced insulin resistance through suppression of NFκB activation and reduced circulation of inflammatory cytokines.[46,47] In contrast, constitutive activation of IKKβ and subsequently NFκB activation in hepatocytes causes profound insulin resistance.[48]

Transduction of TLR4 signaling to NFκB requires PI3K activity for cytokine production in leukocytes.[49-51] Similarly, NFκB activation by IL-1β is also dependent on PI3K function, as pharmacological inhibition with wortmannin or LY294002 ameliorates NFκB activation.[52,53] Furthermore, deletion of the PI3K

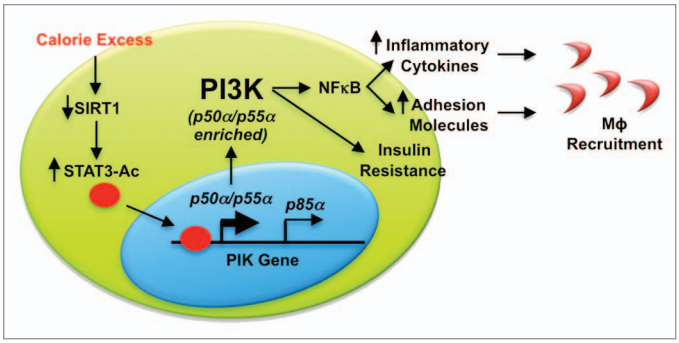

Figure 2. PI3K coordinates metabolic control and inflammatory signaling. Our proposed model for PI3K mediated signaling in obesity postulates that increased expression of the class IA regulatory subunits, p50α and p55α, inhibits insulin-stimulated PI3K activity for glucose uptake and suppression of lipolysis causing adipocyte insulin resistance. Concomitantly, reduced PI3K activity enhances activation of nuclear factor κ B (NFκB) to promote inflammatory cytokine secretion in adipocytes and increased secretion of adhesion molecules in the vascular endothelial cells. Combined, these signals promote adipose tissue recruitment and pro-inflammatory activation of macrophage causing a feed forward cycle that leads to infiltration of additional macrophages into adipose tissue. This results in the exacerbation of the inflammatory state and systemic insulin resistance.

catalytic subunits, p110δ or p110β, or the *Pik3r1* regulatory subunits, significantly impairs leukocyte (eosinophils, T cell, B cells, macrophage, and neutrophils) proliferation and chemotaxis in a cell type-dependent manner.[12] In contrast, deletion of the p85β subunit increased lymphocyte proliferation, accumulation at sites of infection and reduced cell death suggesting a unique role for p85β in limiting T cell expansion.[54] Relevant to obesity, these data suggest that inhibition of PI3K activity specifically in immune cells may potentially ameliorate the inflammatory response in adipose tissue as was recently described in obese mice with deletion of Class IB PI3Kγ.[55]

The role of PI3K in NFκB mediate cytokine secretion in adipocytes has not been thoroughly investigated. Several studies have found that wortmannin inhibited IL-1β-induced inflammatory response through reduced expression of NFκB regulated genes.[56,57] Similarly, Gustin et al.[58] found that cell lines, including 3T3-L1 adipocytes, that have a high proportion of IKKα to IKKβ were most sensitive to PI3K inhibitors to diminish the NFκB activation in response to TNFα exposure. Like leukocytes, palmitate exposure in 3T3-L1 adipocytes increased NFκB mediate cytokine secretion; however, in contrast to immune cells, inhibition of PI3K by wortmannin, alone or additively with palmitate, further activated the NFκB to induce cytokine (IL-6, TNFα) expression,[59] suggesting that PI3K may act constitutively to suppress inflammation. This is a very important point, and suggests that inhibition of PI3K may initiate and exacerbate the inflammation in adipose tissue that is associated with obesity and insulin resistance.

In agreement with this line of thinking, we have recently demonstrated that heterozygous knockdown of *Pik3r1* in obese mice, increased adipose tissue PI3K activity concomitant with reduced phosphorylation of IKKα/β and suppressed adipose tissue cytokine release.[5] These molecular changes were accompanied by significant improvements in systemic insulin sensitivity and reduced adipose tissue macrophage accumulation, again, despite marked obesity.[5] In obese adipose tissue from wild-type mice, we found a 4-fold increase in the PI3K regulatory subunits, p55α and p50α that was prevented by *Pik3r1* knockdown.[5] Thus, we propose that in obese adipose tissue increased p55α and p50α abundance inhibits insulin-stimulated PI3K activity, which increases both lipolysis and enhances FFA activation of NFκB to stimulate cytokine and chemokine production (**Fig. 2**). The NAD-dependent deacetylase sirtuin-1 (SIRT1) is an important cellular nutrient sensor that is down-regulated in obese adipose tissue.[60,61] We speculate that increased p55α and p50α abundance in obese adipose tissue is due to reduced SIRT1 activity and the subsequent increase in STAT3 acetylation, a known transcriptional regulator of *p55*/*p50*.[35] We have previously reported a similar mechanism for SIRT1 regulation of PI3K activity with decreased nutrient intake in skeletal muscle.[62] Others have found that SIRT1 interacts in an insulin-independent manner with the PI3K adapter subunit p85 and acts as a positive modulator of insulin signaling in muscle cells through PI3K.[63] Our proposed mechanism links nutrient sensing via SIRT1 with regulation of insulin action and inflammation through PI3K activity in obese adipose tissue (**Fig. 2**).

Control of Adipogenesis, Adipocyte Survival, and Adipose Tissue Development by PI3K

In addition to its central role in insulin signaling in adipocytes and other cells, PI3K also participates in adipogenesis and adipose tissue development. PI3K is activated in preadipocytes by insulin or insulin-like growth factor,[64] with subsequent stimulation of Akt. Some,[65] but not all,[64] pro-adipogenic effects of PI3K/Akt signaling are driven by the mammalian target of rapamycin (mTOR). Direct inhibition of PI3K with wortmannin or LY294002 blocked differentiation of immortalized preadipocytes in both 3T3-L1 and 3T3-F442A lines.[66] PI3K inhibits differentiation by suppressing transcription of pro-adipogenic factors including PPARγ[6] and Skp2,[67] and the phosphorylation of FoxO1.[68]

The importance of PI3K signaling in adipose tissue development is further exemplified by studies assessing the impact of Akt isoforms on adipocyte production. Mouse embryonic fibroblasts deficient in Akt1 exhibit a defect in adipocyte differentiation, and ectopic expression of Akt1, but not Akt2, restores adipogenic conversion in fibroblasts deficient in both forms of Akt.[68] As anticipated, mice deficient in Akt1 display reduced adiposity in response to high-fat feeding; however, this appears to be due to increased energy expenditure rather than defects in adipocyte production.[69] Interestingly, mice lacking Akt2 exhibit age-dependent loss of adipose tissue accompanied by insulin resistance, hyperglycemia, and elevated serum triglycerides.[70] Reusch and Klemm[71] have also demonstrated that inhibition of Akt in mature adipocytes elicits their apoptosis.

Finally, it is worth noting that PI3K may play a role in the commitment and differentiation of mesenchymal stem cells

to the adipocyte lineage. For example, activation of Exchange Protein Activated by cAMP (EPAC) promotes adipogenic rather than osteogenic gene expression and differentiation of human mesenchymal stem cells via PI3K/Akt signaling to CREB.[71] Dominant negative EPAC suppressed PI3K and Akt activity and CREB function, thereby stimulating transcription of osteogenic rather than adipogenic genes.[71] Results like these have led investigators to propose that components of the PI3K pathway may be targets for therapies to prevent osteoporosis by promoting the production of osteoblasts rather than adipocytes from marrow MSC.

PI3K in Vascular Endothelial Function

Normal vascular development requires appropriate activation of the PI3K signaling in endothelial cells (ECs).[10] During angiogenesis the PI3K pathway is stimulated in ECs in response to extracellular stimuli like VEGF and factors. Activation of PI3K/Akt signaling in ECs by these agents promotes the proliferation, differentiation and survival of resident cells in the vessel wall, and the recruitment of cells from other regions of the vasculature (**Fig. 1**). The importance of PI3K/Akt signaling in normal vascular development is exemplified by gene knockout studies such as the Akt1-null mouse which exhibits delayed vessel maturation and increased vascular permeability.[72] Alternately, endothelial-specific knockdown of PTEN, an inhibitor of PI3K/Akt, elicits increased angiogenesis.[73]

Since the growth and expansion of adipose tissue requires recruitment of new blood vessels,[74] defects in PI3K signaling may suppress normal adipose tissue development or adiposity with over-nutrition. Loss of PI3K signaling may also account for the decreased vascular density observed in "metabolically obese" insulin resistant individuals.[75] It has been proposed that the mismatch between adipocyte hypertrophy and O_2 delivery may contribute to adipose tissue inflammation and dysfunction.[76] EC PI3K is also crucial for maintenance of vascular tone and blood flow primarily through the production of nitric oxide.[77] Inhibition of PI3K in EC may in part explain the decreased arterial function observed in the adipose tissue of obese individuals,[78] which would be expected to further diminish adipose tissue oxygenation and promote adipose tissue dysfunction.

Finally, EC PI3K participates in adipose tissue inflammation observed with obesity and insulin resistance via the recruitment and transendothelial migration of macrophage. The normal transient activation of the PI3K pathway in EC represses the expression of adhesion molecules involved in leukocyte rolling and adhesion to the vascular luminal wall.[79] However, chronic insulin resistance results in upregulation of adhesion molecule expression.[80] Cell culture studies demonstrate that inhibition of PI3K signaling in ECs with agents like wortmannin or LY294002, alone or combination with TNFα, insulin, or VEGF, increase the expression of adhesion molecules like ICAM-1, VCAM, and E-selectin.[81] Alternately, ectopic expression of constitutively active Akt has been shown to induce ICAM-1 expression by ECs.[82] Interestingly, inhibiting PI3K with LY294002 decreased leukocyte transmigration through EC monolayers without altering the strength of adhesion or formation of the EC "docking structure" following ICAM ligation.[83] However, treatment of ECs with LY294002 prevented lymphocytes from extending processes below the level of VE-cadherin in the monolayers.

In summary, PI3K in ECs is crucial to the normal development and expansion of adipose tissue. Defects in EC PI3K signaling likely contribute to adipose tissue dysfunction by limiting angiogenesis and blood flow to the tissue, while promoting the recruitment of macrophage that further suppress insulin responsiveness.

PI3K in Macrophage Function

Macrophages are an important constituent of adipose tissue. These immune cells have been unambiguously linked to adipose tissue remodeling and changes in adipocyte insulin sensitivity in obesity.[3,4] The PI3K signaling system governs or participates in several signature macrophage functions including recruitment and invasion,[84,85] lipid uptake and accumulation,[86-88] inflammatory activation,[89,90] and glucose utilization.[91] Unfortunately, none of these studies examined macrophage residing in adipose tissue. However, experiments in other tissues and systems provide some potential insights into PI3K signaling in adipose tissue macrophage. For example, recruitment and invasion of macrophage into inflamed colon tissue,[84] or blood vessel walls in response to advanced glycosylation endproducts[85] requires PI3K activation. Similar recruitment to and invasion of adipose tissue occurs in obese humans and animals making it likely that PI3K signaling is involved in this process as well. The PI3K pathway also participates in the upregulation of CD36, LDL uptake and storage in macrophage expose to oxidized LDL[88] and lipid droplet formation and perilipin 2 expression in macrophage exposed to snake venom.[87] This suggests that the PI3K system may be engaged to facilitate the uptake of lipid from dying adipocytes by macrophage in adipose tissue of obese individuals. The absence of data to support these conjectures makes this an untapped field for future research.

An Old Idea, New Again: Does Insulin Action Direct the Inflammatory Response in Obesity?

Current dogma posits that obesity-induced insulin resistance arises secondary to the inflammatory response caused by infiltration of adipose tissue with pro-inflammatory immune cells.[92] This paradigm is strongly supported as interventions that reduce circulating inflammatory cytokines or prevent AT macrophage infiltration, not only reduce inflammatory cytokine production but also improve insulin action in rodent models of obesity.[3,4] Separating induction of insulin resistance from initiation of adipose tissue inflammation is difficult as both manifest rapidly, occurring within as little as 3 days of beginning high-fat diet (HFD) feeding.[25,93] However, a recent study by Lee et al. found that while 3 days of HFD caused both insulin resistance (tissue-specific and systemic) and macrophage infiltration in WT mice, deletion of macrophage/inflammatory component did not reverse IR associated with acute HFD. Rather, deletion of macrophage

component could only improve insulin sensitivity in already obese mice, which indicates that IR can occur independent of macrophage infiltration,[93] and suggests that inflammation plays a key role in the worsening of insulin resistance with chronic HFD and obesity.

While current research has focused on adipose tissue inflammation as a key regulator of insulin resistance in obesity, many studies have found that enhancing insulin signaling independent of known inflammatory pathways can restore or prevent insulin resistance in obesity. For example, adipocyte-specific GLUT4 over-expression normalizes glucose tolerance, expands fat mass, and improves whole body insulin resistance caused my muscle deletion of GLUT4.[94] Similarly, expansion of adipose tissue mass through modest over-expression of adiponectin, mimicking PPARγ agonist treatments, completely rescued the diabetic phenotype in ob/ob mice.[95] Enhancing PI3K activity through global genetic deletion of the *pik3r1* subunits improves systemic insulin sensitivity[5,96] and prevents macrophage accumulation.[5] Furthermore, these improvements in insulin action and reduced adipose tissue macrophage accumulation occur independent of *pik3r1* knockdown in bone marrow-derived cells.[5] Additional studies in adipocyte-specific knockout animals are essential in determining the relative contribution of adipocyte to the improved metabolic phenotype in these animals. Likewise, deletion of PTEN in adipocytes enhances insulin action despite hyperphagia.[97] Lastly, increasing adipose tissue lipid storage through adipocyte-specific over-expression of mitoNEET resulted in systemic improvements in insulin sensitivity and the metabolic profile despite massive obesity.[98] These studies and many others indicate that failure to adequately handle and store excess nutrients is a primary mechanism in the development of insulin resistance. These studies also indicate that there is coordination between adipocyte proliferation and vascular remodeling that may be directed by PI3K signaling. Further, improving lipid and glucose handling alone is sufficient to restore or prevent insulin resistance in obesity making a strong argument that defects in cellular metabolism and insulin signaling may initiate the signals necessary for macrophage chemotaxis. In obese adipose tissue, the inhibition of PI3K signaling in response to nutrient excess manifests as increased lipolysis, reduced vascular growth and proliferation, and increased chemokine signaling and expression of adhesion molecules like ICAM-1, VCAM, and E-selectin to upregulate the inflammatory response.

In this mini-review, we present a model in which PI3K is linked to cellular energy status through expression of the regulatory subunits, whose abundance can modulate enzyme activity to coordinate insulin resistance and inflammatory signaling in obese adipose tissue. Development of pharmaceuticals that could specifically target and suppress transcription of the PI3K regulatory subunits may be able to enhance PI3K activity, thus improving insulin sensitivity and the metabolic profile despite obesity.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

References

1. Flegal KM, Carroll MD, Kit BK, Ogden CL. Prevalence of obesity and trends in the distribution of body mass index among US adults, 1999-2010. JAMA 2012; 307:491-7; PMID:22253363; http://dx.doi.org/10.1001/jama.2012.39
2. Reaven GM. The insulin resistance syndrome: definition and dietary approaches to treatment. Annu Rev Nutr 2005; 25:391-406; PMID:16011472; http://dx.doi.org/10.1146/annurev.nutr.24.012003.132155
3. Osborn O, Olefsky JM. The cellular and signaling networks linking the immune system and metabolism in disease. Nat Med 2012; 18:363-74; PMID:22395709; http://dx.doi.org/10.1038/nm.2627
4. Romeo GR, Lee J, Shoelson SE. Metabolic syndrome, insulin resistance, and roles of inflammation--mechanisms and therapeutic targets. Arterioscler Thromb Vasc Biol 2012; 32:1771-6; PMID:22815343; http://dx.doi.org/10.1161/ATVBAHA.111.241869
5. McCurdy CE, Schenk S, Holliday MJ, Philp A, Houck JA, Patsouris D, et al. Attenuated Pik3r1 expression prevents insulin resistance and adipose tissue macrophage accumulation in diet-induced obese mice. Diabetes 2012; 61:2495-505; PMID:22698915; http://dx.doi.org/10.2337/db11-1433
6. Fruman DA. Regulatory subunits of class IA PI3K. Curr Top Microbiol Immunol 2010; 346:225-44; PMID:20563711; http://dx.doi.org/10.1007/82_2010_39
7. Kim SP, Ha JM, Yun SJ, Kim EK, Chung SW, Hong KW, et al. Transcriptional activation of peroxisome proliferator-activated receptor-gamma requires activation of both protein kinase A and Akt during adipocyte differentiation. Biochem Biophys Res Commun 2010; 399:55-9; PMID:20638365; http://dx.doi.org/10.1016/j.bbrc.2010.07.038
8. Mariman EC, Wang P. Adipocyte extracellular matrix composition, dynamics and role in obesity. Cell Mol Life Sci 2010; 67:1277-92; PMID:20107860; http://dx.doi.org/10.1007/s00018-010-0263-4
9. Urtasun R, Lopategi A, George J, Leung TM, Lu Y, Wang X, et al. Osteopontin, an oxidant stress sensitive cytokine, up-regulates collagen-I via integrin α(V)β(3) engagement and PI3K/pAkt/NFκB signaling. Hepatology 2012; 55:594-608; PMID:21953216; http://dx.doi.org/10.1002/hep.24701
10. Morello F, Perino A, Hirsch E. Phosphoinositide 3-kinase signalling in the vascular system. Cardiovasc Res 2009; 82:261-71; PMID:19038971; http://dx.doi.org/10.1093/cvr/cvn325
11. Fruman DA, Cantley LC. Phosphoinositide 3-kinase in immunological systems. Semin Immunol 2002; 14:7-18; PMID:11884226; http://dx.doi.org/10.1006/smim.2001.0337
12. Afonso PV, Parent CA. PI3K and chemotaxis: a priming issue? Sci Signal 2011; 4:pe22; PMID:21521877; http://dx.doi.org/10.1126/scisignal.2002019
13. Taniguchi CM, Emanuelli B, Kahn CR. Critical nodes in signalling pathways: insights into insulin action. Nat Rev Mol Cell Biol 2006; 7:85-96; PMID:16493415; http://dx.doi.org/10.1038/nrm1837
14. Yu J, Zhang Y, McIlroy J, Rordorf-Nikolic T, Orr GA, Backer JM. Regulation of the p85/p110 phosphatidylinositol 3'-kinase: stabilization and inhibition of the p110alpha catalytic subunit by the p85 regulatory subunit. Mol Cell Biol 1998; 18:1379-87; PMID:9488453.
15. Fruman DA, Cantley LC, Carpenter CL. Structural organization and alternative splicing of the murine phosphoinositide 3-kinase p85 alpha gene. Genomics 1996; 37:113-21; PMID:8921377; http://dx.doi.org/10.1006/geno.1996.0527
16. Inukai K, Anai M, Van Breda E, Hosaka T, Katagiri H, Funaki M, et al. A novel 55-kDa regulatory subunit for phosphatidylinositol 3-kinase structurally similar to p55PIK Is generated by alternative splicing of the p85alpha gene. J Biol Chem 1996; 271:5317-20; PMID:8621382; http://dx.doi.org/10.1074/jbc.271.10.5317
17. Ueki K, Algenstaedt P, Mauvais-Jarvis F, Kahn CR. Positive and negative regulation of phosphoinositide 3-kinase-dependent signaling pathways by three different gene products of the p85alpha regulatory subunit. Mol Cell Biol 2000; 20:8035-46; PMID:11027274; http://dx.doi.org/10.1128/MCB.20.21.8035-8046.2000
18. Chen D, Mauvais-Jarvis F, Bluher M, Fisher SJ, Jozsi A, Goodyear LJ, et al. p50alpha/p55alpha phosphoinositide 3-kinase knockout mice exhibit enhanced insulin sensitivity. Mol Cell Biol 2004; 24:320-9; PMID:14673165; http://dx.doi.org/10.1128/MCB.24.1.320-329.2004
19. Ueki K, Yballe CM, Brachmann SM, Vicent D, Watt JM, Kahn CR, et al. Increased insulin sensitivity in mice lacking p85beta subunit of phosphoinositide 3-kinase. Proc Natl Acad Sci U S A 2002; 99:419-24; PMID:11752399; http://dx.doi.org/10.1073/pnas.012581799
20. Terauchi Y, Tsuji Y, Satoh S, Minoura H, Murakami K, Okuno A, et al. Increased insulin sensitivity and hypoglycaemia in mice lacking the p85 alpha subunit of phosphoinositide 3-kinase. Nat Genet 1999; 21:230-5; PMID:9988280; http://dx.doi.org/10.1038/6023
21. Ueki K, Fruman DA, Yballe CM, Fasshauer M, Klein J, Asano T, et al. Positive and negative roles of p85 alpha and p85 beta regulatory subunits of phosphoinositide 3-kinase in insulin signaling. J Biol Chem 2003; 278:48453-66; PMID:14504291; http://dx.doi.org/10.1074/jbc.M305602200

22. Ueki K, Fruman DA, Brachmann SM, Tseng YH, Cantley LC, Kahn CR. Molecular balance between the regulatory and catalytic subunits of phosphoinositide 3-kinase regulates cell signaling and survival. Mol Cell Biol 2002; 22:965-77; PMID:11784871; http://dx.doi.org/10.1128/MCB.22.3.965-977.2002
23. Geering B, Cutillas PR, Nock G, Gharbi SI, Vanhaesebroeck B. Class IA phosphoinositide 3-kinases are obligate p85-p110 heterodimers. Proc Natl Acad Sci U S A 2007; 104:7809-14; PMID:17470792; http://dx.doi.org/10.1073/pnas.0700373104
24. Bandyopadhyay GK, Yu JG, Ofrecio J, Olefsky JM. Increased p85/55/50 expression and decreased phosphotidylinositol 3-kinase activity in insulin-resistant human skeletal muscle. Diabetes 2005; 54:2351-9; PMID:16046301; http://dx.doi.org/10.2337/diabetes.54.8.2351
25. Cornier MA, Bessesen DH, Gurevich I, Leitner JW, Draznin B. Nutritional upregulation of p85alpha expression is an early molecular manifestation of insulin resistance. Diabetologia 2006; 49:748-54; PMID:16491394; http://dx.doi.org/10.1007/s00125-006-0148-0
26. Kirwan JP, Varastehpour A, Jing M, Presley L, Shao J, Friedman JE, et al. Reversal of insulin resistance postpartum is linked to enhanced skeletal muscle insulin signaling. J Clin Endocrinol Metab 2004; 89:4678-84; PMID:15356080; http://dx.doi.org/10.1210/jc.2004-0749
27. del Rincon JP, Iida K, Gaylinn BD, McCurdy CE, Leitner JW, Barbour LA, et al. Growth hormone regulation of p85alpha expression and phosphoinositide 3-kinase activity in adipose tissue: mechanism for growth hormone-mediated insulin resistance. Diabetes 2007; 56:1638-46; PMID:17363744; http://dx.doi.org/10.2337/db06-0299
28. Taniguchi CM, Tran TT, Kondo T, Luo J, Ueki K, Cantley LC, et al. Phosphoinositide 3-kinase regulatory subunit p85alpha suppresses insulin action via positive regulation of PTEN. Proc Natl Acad Sci U S A 2006; 103:12093-7; PMID:16880400; http://dx.doi.org/10.1073/pnas.0604628103
29. Santomauro AT, Boden G, Silva ME, Rocha DM, Santos RF, Ursich MJ, et al. Overnight lowering of free fatty acids with Acipimox improves insulin resistance and glucose tolerance in obese diabetic and nondiabetic subjects. Diabetes 1999; 48:1836-41; PMID:10480616; http://dx.doi.org/10.2337/diabetes.48.9.1836
30. Park SW, Zhou Y, Lee J, Lu A, Sun C, Chung J, et al. The regulatory subunits of PI3K, p85alpha and p85beta, interact with XBP-1 and increase its nuclear translocation. Nat Med 2010; 16:429-37; PMID:20348926; http://dx.doi.org/10.1038/nm.2099
31. Winnay JN, Boucher J, Mori MA, Ueki K, Kahn CR. A regulatory subunit of phosphoinositide 3-kinase increases the nuclear accumulation of X-box-binding protein-1 to modulate the unfolded protein response. Nat Med 2010; 16:438-45; PMID:20348923; http://dx.doi.org/10.1038/nm.2121
32. Taniguchi CM, Aleman JO, Ueki K, Luo J, Asano T, Kaneto H, et al. The p85alpha regulatory subunit of phosphoinositide 3-kinase potentiates c-Jun N-terminal kinase-mediated insulin resistance. Mol Cell Biol 2007; 27:2830-40; PMID:17283057; http://dx.doi.org/10.1128/MCB.00079-07
33. Fos C, Salles A, Lang V, Carrette F, Audebert S, Pastor S, et al. ICOS ligation recruits the p50alpha PI3K regulatory subunit to the immunological synapse. J Immunol 2008; 181:1969-77; PMID:18641334.
34. Spender LC, Lucchesi W, Bodelon G, Bilancio A, Karstegl CE, Asano T, et al. Cell target genes of Epstein-Barr virus transcription factor EBNA-2: induction of the p55alpha regulatory subunit of PI3-kinase and its role in survival of EREB2.5 cells. J Gen Virol 2006; 87:2859-67; PMID:16963743; http://dx.doi.org/10.1099/vir.0.82128-0
35. Abell K, Bilancio A, Clarkson RWE, Tiffen PG, Altaparmakov AI, Burdon TG, et al. Stat3-induced apoptosis requires a molecular switch in PI(3)K subunit composition. Nat Cell Biol 2005; 7:392-8; PMID:15793565; http://dx.doi.org/10.1038/ncb1242
36. Asano T, Kanda A, Katagiri H, Nawano M, Ogihara T, Inukai K, et al. p110beta is up-regulated during differentiation of 3T3-L1 cells and contributes to the highly insulin-responsive glucose transport activity. J Biol Chem 2000; 275:17671-6; PMID:10748220; http://dx.doi.org/10.1074/jbc.M910391199
37. Franke TF. Intracellular signaling by Akt: bound to be specific. Sci Signal 2008; 1:pe29; PMID:18560018; http://dx.doi.org/10.1126/scisignal.124pe29
38. Bae SS, Cho H, Mu J, Birnbaum MJ. Isoform-specific regulation of insulin-dependent glucose uptake by Akt/protein kinase B. J Biol Chem 2003; 278:49530-6; PMID:14522993; http://dx.doi.org/10.1074/jbc.M306782200
39. Kitamura T, Kitamura Y, Kuroda S, Hino Y, Ando M, Kotani K, et al. Insulin-induced phosphorylation and activation of cyclic nucleotide phosphodiesterase 3B by the serine-threonine kinase Akt. Mol Cell Biol 1999; 19:6286-96; PMID:10454575.
40. Choi SM, Tucker DF, Gross DN, Easton RM, DiPilato LM, Dean AS, et al. Insulin regulates adipocyte lipolysis via an Akt-independent signaling pathway. Mol Cell Biol 2010; 30:5009-20; PMID:20733001; http://dx.doi.org/10.1128/MCB.00797-10
41. Taniguchi CM, Kondo T, Sajan M, Luo J, Bronson R, Asano T, et al. Divergent regulation of hepatic glucose and lipid metabolism by phosphoinositide 3-kinase via Akt and PKClambda/zeta. [zeta]. Cell Metab 2006; 3:343-53; PMID:16679292; http://dx.doi.org/10.1016/j.cmet.2006.04.005
42. Bachmann OP, Dahl DB, Brechtel K, Machann J, Haap M, Maier T, et al. Effects of intravenous and dietary lipid challenge on intramyocellular lipid content and the relation with insulin sensitivity in humans. Diabetes 2001; 50:2579-84; PMID:11679437; http://dx.doi.org/10.2337/diabetes.50.11.2579
43. Zick Y. Ser/Thr phosphorylation of IRS proteins: a molecular basis for insulin resistance. Sci STKE 2005; 2005:pe4; PMID:15671481.
44. Barnes PJ, Karin M. Nuclear factor-kappaB: a pivotal transcription factor in chronic inflammatory diseases. N Engl J Med 1997; 336:1066-71; PMID:9091804; http://dx.doi.org/10.1056/NEJM199704103361506
45. Shi H, Kokoeva MV, Inouye K, Tzameli I, Yin H, Flier JS. TLR4 links innate immunity and fatty acid-induced insulin resistance. J Clin Invest 2006; 116:3015-25; PMID:17053832; http://dx.doi.org/10.1172/JCI28898
46. Arkan MC, Hevener AL, Greten FR, Maeda S, Li ZW, Long JM, et al. IKK-beta links inflammation to obesity-induced insulin resistance. Nat Med 2005; 11:191-8; PMID:15685170; http://dx.doi.org/10.1038/nm1185
47. Saberi M, Woods NB, de Luca C, Schenk S, Lu JC, Bandyopadhyay G, et al. Hematopoietic cell-specific deletion of toll-like receptor 4 ameliorates hepatic and adipose tissue insulin resistance in high-fat-fed mice. Cell Metab 2009; 10:419-29; PMID:19883619; http://dx.doi.org/10.1016/j.cmet.2009.09.006
48. Cai D, Yuan M, Frantz DF, Melendez PA, Hansen L, Lee J, et al. Local and systemic insulin resistance resulting from hepatic activation of IKK-beta and NF-kappaB. Nat Med 2005; 11:183-90; PMID:15685173; http://dx.doi.org/10.1038/nm1166
49. Santos-Sierra S, Deshmukh SD, Kalnitski J, Küenzi P, Wymann MP, Golenbock DT, et al. Mal connects TLR2 to PI3Kinase activation and phagocyte polarization. EMBO J 2009; 28:2018-27; PMID:19574958; http://dx.doi.org/10.1038/emboj.2009.158
50. Lee JY, Ye J, Gao Z, Youn HS, Lee WH, Zhao L, et al. Reciprocal modulation of Toll-like receptor-4 signaling pathways involving MyD88 and phosphatidylinositol 3-kinase/AKT by saturated and polyunsaturated fatty acids. J Biol Chem 2003; 278:37041-51; PMID:12865424; http://dx.doi.org/10.1074/jbc.M305213200
51. Hochdörfer T, Kuhny M, Zorn CN, Hendriks RW, Vanhaesebroeck B, Bohnacker T, et al. Activation of the PI3K pathway increases TLR-induced TNF-α and IL-6 but reduces IL-1β production in mast cells. Cell Signal 2011; 23:866-75; PMID:21262348; http://dx.doi.org/10.1016/j.cellsig.2011.01.012
52. Reddy SAG, Huang JH, Liao WSL. Phosphatidylinositol 3-kinase in interleukin 1 signaling. Physical interaction with the interleukin 1 receptor and requirement in NFkappaB and AP-1 activation. J Biol Chem 1997; 272:29167-73; PMID:9360994; http://dx.doi.org/10.1074/jbc.272.46.29167
53. Haller D, Russo MP, Sartor RB, Jobin C. IKK beta and phosphatidylinositol 3-kinase/Akt participate in non-pathogenic Gram-negative enteric bacteria-induced RelA phosphorylation and NF-kappa B activation in both primary and intestinal epithelial cell lines. J Biol Chem 2002; 277:38168-78; PMID:12140289; http://dx.doi.org/10.1074/jbc.M205737200
54. Deane JA, Trifilo MJ, Yballe CM, Choi S, Lane TE, Fruman DA. Enhanced T cell proliferation in mice lacking the p85beta subunit of phosphoinositide 3-kinase. J Immunol 2004; 172:6615-25; PMID:15153476.
55. Kobayashi N, Ueki K, Okazaki Y, Iwane A, Kubota N, Ohsugi M, et al. Blockade of class IB phosphoinositide-3 kinase ameliorates obesity-induced inflammation and insulin resistance. Proc Natl Acad Sci U S A 2011; 108:5753-8; PMID:21436039; http://dx.doi.org/10.1073/pnas.1016430108
56. Busch F, Mobasheri A, Shayan P, Lueders C, Stahlmann R, Shakibaei M. Resveratrol modulates interleukin-1β-induced phosphatidylinositol 3-kinase and nuclear factor κB signaling pathways in human tenocytes. J Biol Chem 2012; 287:38050-63; PMID:22936809; http://dx.doi.org/10.1074/jbc.M112.377028
57. Buhrmann C, Mobasheri A, Busch F, Aldinger C, Stahlmann R, Montaseri A, et al. Curcumin modulates nuclear factor kappaB (NF-kappaB)-mediated inflammation in human tenocytes in vitro: role of the phosphatidylinositol 3-kinase/Akt pathway. J Biol Chem 2011; 286:28556-66; PMID:21669872; http://dx.doi.org/10.1074/jbc.M111.256180
58. Gustin JA, Ozes ON, Akca H, Pincheira R, Mayo LD, Li Q, et al. Cell type-specific expression of the IkappaB kinases determines the significance of phosphatidylinositol 3-kinase/Akt signaling to NF-kappa B activation. J Biol Chem 2004; 279:1615-20; PMID:14585846; http://dx.doi.org/10.1074/jbc.M306976200
59. Ajuwon KM, Spurlock ME. Palmitate activates the NF-kappaB transcription factor and induces IL-6 and TNFalpha expression in 3T3-L1 adipocytes. J Nutr 2005; 135:1841-6; PMID:16046706.
60. Chalkiadaki A, Guarente L. High-fat diet triggers inflammation-induced cleavage of SIRT1 in adipose tissue to promote metabolic dysfunction. Cell Metab 2012; 16:180-8; PMID:22883230; http://dx.doi.org/10.1016/j.cmet.2012.07.003
61. Liang F, Kume S, Koya D. SIRT1 and insulin resistance. Nat Rev Endocrinol 2009; 5:367-73; PMID:19455179; http://dx.doi.org/10.1038/nrendo.2009.101
62. Schenk S, McCurdy CE, Philp A, Chen MZ, Holliday MJ, Bandyopadhyay GK, et al. Sirt1 enhances skeletal muscle insulin sensitivity in mice during caloric restriction. J Clin Invest 2011; 121:4281-8; PMID:21985785; http://dx.doi.org/10.1172/JCI58554

63. Fröjdö S, Durand C, Molin L, Carey AL, El-Osta A, Kingwell BA, et al. Phosphoinositide 3-kinase as a novel functional target for the regulation of the insulin signaling pathway by SIRT1. Mol Cell Endocrinol 2011; 335:166-76; PMID:21241768; http://dx.doi.org/10.1016/j.mce.2011.01.008
64. Gagnon A, Chen CS, Sorisky A. Activation of protein kinase B and induction of adipogenesis by insulin in 3T3-L1 preadipocytes: contribution of phosphoinositide-3,4,5-trisphosphate versus phosphoinositide-3,4-bisphosphate. Diabetes 1999; 48:691-8; PMID:10102683; http://dx.doi.org/10.2337/diabetes.48.4.691
65. Yu W, Chen Z, Zhang J, Zhang L, Ke H, Huang L, et al. Critical role of phosphoinositide 3-kinase cascade in adipogenesis of human mesenchymal stem cells. Mol Cell Biochem 2008; 310:11-8; PMID:18060476; http://dx.doi.org/10.1007/s11010-007-9661-9
66. Tomiyama K, Nakata H, Sasa H, Arimura S, Nishio E, Watanabe Y. Wortmannin, a specific phosphatidylinositol 3-kinase inhibitor, inhibits adipocytic differentiation of 3T3-L1 cells. Biochem Biophys Res Commun 1995; 212:263-9; PMID:7541986; http://dx.doi.org/10.1006/bbrc.1995.1965
67. Auld CA, Caccia CD, Morrison RF. Hormonal induction of adipogenesis induces Skp2 expression through PI3K and MAPK pathways. J Cell Biochem 2007; 100:204-16; PMID:16924675; http://dx.doi.org/10.1002/jcb.21063
68. Yun SJ, Kim EK, Tucker DF, Kim CD, Birnbaum MJ, Bae SS. Isoform-specific regulation of adipocyte differentiation by Akt/protein kinase Balpha. Biochem Biophys Res Commun 2008; 371:138-43; PMID:18423396; http://dx.doi.org/10.1016/j.bbrc.2008.04.029
69. Wan M, Easton RM, Gleason CE, Monks BR, Ueki K, Kahn CR, et al. Loss of Akt1 in mice increases energy expenditure and protects against diet-induced obesity. Mol Cell Biol 2012; 32:96-106; PMID:22037765; http://dx.doi.org/10.1128/MCB.05806-11
70. Garofalo RS, Orena SJ, Rafidi K, Torchia AJ, Stock JL, Hildebrandt AL, et al. Severe diabetes, age-dependent loss of adipose tissue, and mild growth deficiency in mice lacking Akt2/PKB beta. J Clin Invest 2003; 112:197-208; PMID:12843127.
71. Reusch JE, Klemm DJ. Inhibition of cAMP-response element-binding protein activity decreases protein kinase B/Akt expression in 3T3-L1 adipocytes and induces apoptosis. J Biol Chem 2002; 277:1426-32; PMID:11694510; http://dx.doi.org/10.1074/jbc.M107923200
72. Chen J, Somanath PR, Razorenova O, Chen WS, Hay N, Bornstein P, et al. Akt1 regulates pathological angiogenesis, vascular maturation and permeability in vivo. Nat Med 2005; 11:1188-96; PMID:16227992; http://dx.doi.org/10.1038/nm1307
73. Hamada K, Sasaki T, Koni PA, Natsui M, Kishimoto H, Sasaki J, et al. The PTEN/PI3K pathway governs normal vascular development and tumor angiogenesis. Genes Dev 2005; 19:2054-65; PMID:16107612; http://dx.doi.org/10.1101/gad.1308805
74. Rupnick MA, Panigrahy D, Zhang CY, Dallabrida SM, Lowell BB, Langer R, et al. Adipose tissue mass can be regulated through the vasculature. Proc Natl Acad Sci U S A 2002; 99:10730-5; PMID:12149466; http://dx.doi.org/10.1073/pnas.162349799
75. Srdić B, Stokić E, Korać A, Ukropina M, Veličković K, Breberina M. Morphological characteristics of abdominal adipose tissue in normal-weight and obese women of different metabolic profiles. Exp Clin Endocrinol Diabetes 2010; 118:713-8; PMID:20533176; http://dx.doi.org/10.1055/s-0030-1254165
76. Wood IS, de Heredia FP, Wang B, Trayhurn P. Cellular hypoxia and adipose tissue dysfunction in obesity. Proc Nutr Soc 2009; 68:370-7; PMID:19698203; http://dx.doi.org/10.1017/S0029665109990206
77. Oudit GY, Sun H, Kerfant BG, Crackower MA, Penninger JM, Backx PH. The role of phosphoinositide-3 kinase and PTEN in cardiovascular physiology and disease. J Mol Cell Cardiol 2004; 37:449-71; PMID:15276015; http://dx.doi.org/10.1016/j.yjmcc.2004.05.015
78. Farb MG, Ganley-Leal L, Mott M, Liang Y, Ercan B, Widlansky ME, et al. Arteriolar function in visceral adipose tissue is impaired in human obesity. Arterioscler Thromb Vasc Biol 2012; 32:467-73; PMID:22095978; http://dx.doi.org/10.1161/ATVBAHA.111.235846
79. Aljada A, Saadeh R, Assian E, Ghanim H, Dandona P. Insulin inhibits the expression of intercellular adhesion molecule-1 by human aortic endothelial cells through stimulation of nitric oxide. J Clin Endocrinol Metab 2000; 85:2572-5; PMID:10902810; http://dx.doi.org/10.1210/jc.85.7.2572
80. Madonna R, De Caterina R. Prolonged exposure to high insulin impairs the endothelial PI3-kinase/Akt/nitric oxide signalling. Thromb Haemost 2009; 101:345-50; PMID:19190820.
81. Kim I, Moon SO, Kim SH, Kim HJ, Koh YS, Koh GY. Vascular endothelial growth factor expression of intercellular adhesion molecule 1 (ICAM-1), vascular cell adhesion molecule 1 (VCAM-1), and E-selectin through nuclear factor-kappa B activation in endothelial cells. J Biol Chem 2001; 276:7614-20; PMID:11108718; http://dx.doi.org/10.1074/jbc.M009705200
82. Minhajuddin M, Bijli KM, Fazal F, Sassano A, Nakayama KI, Hay N, et al. Protein kinase C-delta and phosphatidylinositol 3-kinase/Akt activate mammalian target of rapamycin to modulate NF-kappaB activation and intercellular adhesion molecule-1 (ICAM-1) expression in endothelial cells. J Biol Chem 2009; 284:4052-61; PMID:19074768; http://dx.doi.org/10.1074/jbc.M805032200
83. Nakhaei-Nejad M, Hussain AM, Zhang QX, Murray AG. Endothelial PI 3-kinase activity regulates lymphocyte diapedesis. Am J Physiol Heart Circ Physiol 2007; 293:H3608-16; PMID:17890432; http://dx.doi.org/10.1152/ajpheart.00321.2007
84. Khan MW, Keshavarzian A, Gounaris E, Melson JE, Cheon E, Blatner NR, et al. PI3K/AKT signaling is essential for communication between tissue infiltrating mast cells, macrophages, and epithelial cells in colitis-induced cancer. Clin Cancer Res 2013; 19:2342-54; PMID:23487439; http://dx.doi.org/10.1158/1078-0432.CCR-12-2623
85. Qin Q, Niu J, Wang Z, Xu W, Qiao Z, Gu Y. Heparanase induced by advanced glycation end products (AGEs) promotes macrophage migration involving RAGE and PI3K/AKT pathway. Cardiovasc Diabetol 2013; 12:37; PMID:23442498; http://dx.doi.org/10.1186/1475-2840-12-37
86. Cristina Giannotti K, Leiguez E, Moreira V, Nascimento NG, Lomonte B, Gutiérrez JM, et al. A Lys49 Phospholipase A2, Isolated from Bothrops asper Snake Venom, Induces Lipid Droplet Formation in Macrophages Which Depends on Distinct Signaling Pathways and the C-Terminal Region. Biomed Res Int 2013; 2013:807982; PMID:23509782; http://dx.doi.org/10.1155/2013/807982
87. Kruth HS. Fluid-Phase Pinocytosis of LDL by Macrophages: A Novel Target to Reduce Macrophage Cholesterol Accumulation in Atherosclerotic Lesions. Curr Pharm Des 2013; In press; PMID:23438954.
88. Rios FJ, Koga MM, Ferracini M, Jancar S. Co-stimulation of PAFR and CD36 is required for oxLDL-induced human macrophages activation. PLoS One 2012; 7:e36632; PMID:22570732; http://dx.doi.org/10.1371/journal.pone.0036632
89. Deng H, Maitra U, Morris M, Li L. Molecular mechanism responsible for the priming of macrophage activation. J Biol Chem 2013; 288:3897-906; PMID:23264622; http://dx.doi.org/10.1074/jbc.M112.424390
90. Hyam SR, Lee IA, Gu W, Kim KA, Jeong JJ, Jang SE, et al. Arctigenin ameliorates inflammation in vitro and in vivo by inhibiting the PI3K/AKT pathway and polarizing M1 macrophages to M2-like macrophages. Eur J Pharmacol 2013; 708:21-9; PMID:23375938; http://dx.doi.org/10.1016/j.ejphar.2013.01.014
91. Ouro A, Arana L, Gangoiti P, Rivera IG, Ordoñez M, Trueba M, et al. Ceramide 1-phosphate stimulates glucose uptake in macrophages. Cell Signal 2013; 25:786-95; PMID:23333242; http://dx.doi.org/10.1016/j.cellsig.2013.01.009
92. Olefsky JM, Glass CK. Macrophages, inflammation, and insulin resistance. Annu Rev Physiol 2010; 72:219-46; PMID:20148674; http://dx.doi.org/10.1146/annurev-physiol-021909-135846
93. Lee YS, Li P, Huh JY, Hwang IJ, Lu M, Kim JI, et al. Inflammation is necessary for long-term but not short-term high-fat diet-induced insulin resistance. Diabetes 2011; 60:2474-83; PMID:21911747; http://dx.doi.org/10.2337/db11-0194
94. Carvalho E, Kotani K, Peroni OD, Kahn BB. Adipose-specific overexpression of GLUT4 reverses insulin resistance and diabetes in mice lacking GLUT4 selectively in muscle. Am J Physiol Endocrinol Metab 2005; 289:E551-61; PMID:15928024; http://dx.doi.org/10.1152/ajpendo.00116.2005
95. Kim JY, van de Wall E, Laplante M, Azzara A, Trujillo ME, Hofmann SM, et al. Obesity-associated improvements in metabolic profile through expansion of adipose tissue. J Clin Invest 2007; 117:2621-37; PMID:17717599; http://dx.doi.org/10.1172/JCI31021
96. Terauchi Y, Matsui J, Kamon J, Yamauchi T, Kubota N, Komeda K, et al. Increased serum leptin protects from adiposity despite the increased glucose uptake in white adipose tissue in mice lacking p85alpha phosphoinositide 3-kinase. Diabetes 2004; 53:2261-70; PMID:15331535; http://dx.doi.org/10.2337/diabetes.53.9.2261
97. Komazawa N, Matsuda M, Kondoh G, Mizunoya W, Iwaki M, Takagi T, et al. Enhanced insulin sensitivity, energy expenditure and thermogenesis in adipose-specific Pten suppression in mice. Nat Med 2004; 10:1208-15; PMID:15489860; http://dx.doi.org/10.1038/nm1117
98. Kusminski CM, Holland WL, Sun K, Park J, Spurgin SB, Lin Y, et al. MitoNEET-driven alterations in adipocyte mitochondrial activity reveal a crucial adaptive process that preserves insulin sensitivity in obesity. Nat Med 2012; 18:1539-49; PMID:22961109; http://dx.doi.org/10.1038/nm.2899