**ABSTRACT**
Molecular scaffolds are often viewed as passive signaling molecules that facilitate protein-protein interactions. However, new evidence gained from the use of loss-of-function or gain-of-function models is dispelling this notion. Our own recent discovery of 14-3-3\(z\) as an essential regulator of adipogenesis highlights the complex roles of this member of the 14-3-3 protein family. Depletion of the 14-3-3\(z\) isoform affected parallel pathways that drive adipocyte development, including pathways controlling the stability of key adipogenic transcription factors and cell cycle progression. Going beyond adipocyte differentiation, this study opens new avenues of research in the context of metabolism, as 14-3-3\(z\) binds to a variety of well-established metabolic proteins that harbor its canonical phosphorylation binding motifs. This suggests that 14-3-3\(z\) may contribute to key metabolic signaling pathways, such as those that facilitate glucose uptake and fatty acid metabolism. Herein, we discuss these novel areas of research, which will undoubtedly shed light onto novel roles of 14-3-3\(z\), and perhaps its related family members, on glucose homeostasis.

**Physiological roles of molecular scaffolds**
Molecular scaffolds are well suited to coordinate complex signaling networks due to their ability to promote interactions between signaling effectors and to control the subcellular localization of transcription factors and kinases.\(^6\) Despite these critical functions, the requirement of scaffolds in physiology, and more specifically in the regulation of glucose homeostasis and lipid metabolism, has not been investigated in great detail. This is partly due to the paucity of adequate genetic knockout or transgenic models or the assumption that such proteins have only minor roles in the physiological pathways governing glucose homeostasis and metabolism. Scaffolds that have been examined for roles in glucose homeostasis include Akap150, \(\beta\)-arrestin-1, and NLRP,\(^7\)\(^-\)\(^9\) but there are still numerous unexplored families of scaffolds, such as what are the endogenous molecules present within the cell to ensure the accurate transduction of downstream signals.
as 14-3-3 proteins, that may contribute to key events that control metabolism.

**14-3-3ζ is a critical regulator of adipocyte differentiation**

Since their discovery from brain extracts, all seven 14-3-3 proteins, which are encoded by distinct genes, have been examined as potential biomarkers for various neurological diseases and some forms of cancer.\textsuperscript{10-12} The scaffolding function of 14-3-3 proteins stems from their unique ability to bind to phosphorylated proteins harboring canonical phosphorylated serine or threonine motifs.\textsuperscript{13} This permits their interaction with receptors, kinases, transcription factors, and ion channels to regulate their activity, stability, and localization within a cell.\textsuperscript{10,14}

When taken together, these pleiotropic functions emphasize that 14-3-3 proteins go beyond being ‘simple’ scaffolds within cells. Loss-of-function and gain-of-function studies have also identified critical roles of 14-3-3 proteins in cell survival and the regulation of autophagy in numerous cell types.\textsuperscript{15-17} Prior to our study, it was not known if 14-3-3 proteins were involved in metabolic signaling pathways \textit{in vivo}.

Our efforts to understand the role of 14-3-3 proteins in metabolism started with our report that 14-3-3ζ was essential for pancreatic β-cell survival \textit{in vitro}\textsuperscript{16} and we hypothesized that deletion of this isoform \textit{in vivo} would lead to profound β-cell death and frank diabetes. Instead, the primary defect \textit{in vivo} was decreased visceral adiposity and the presence of poorly differentiated adipocytes.\textsuperscript{18} This was due in part to the aberrant expression of the hedgehog signaling effector, Gli3, and the cyclin-dependent kinase inhibitor, p27\textsuperscript{kip1}, in mesenchymal adipose precursor cells and also from the increase in autophagy-mediated degradation of C/ebpα, a key transcription factor that promotes Pparγ expression.\textsuperscript{18,19} The ability of 14-3-3ζ to control differentiation was not surprising given the ability of 14-3-3 proteins to control differentiation in other cell types,\textsuperscript{20-22} but prior to our study, the role of 14-3-3ζ in the adipocyte had not been examined.

Given its ability to interact with multiple phosphorylated proteins harboring its canonical target motifs, a 14-3-3ζ may also coordinate other parallel pathways that drive the development of a mature adipocyte. For example, TAZ (transcriptional coactivator with PDZ-binding motif) is a 14-3-3 binding protein that is known to modulate mesenchymal stem cell differentiation by competing with Pparγ, the canonical adipogenic transcription factor (Fig. 1).\textsuperscript{19,23} Binding of TAZ to 14-3-3 proteins induces its sequestration and permits Pparγ to initiate the adipogenic program. Deletion of 14-3-3ζ may promote the anti-adipogenic actions of TAZ in adipose precursor cells, but how the activity of TAZ relates to those of Gli3 and p27\textsuperscript{kip1} is not known. Thus, further work is required to identify the interactome of 14-3-3ζ during adipogenesis, and this will define which pathways are regulated by 14-3-3ζ and potentially lead to the identification of new targets to affect adipocyte differentiation.

The idea that distinct pools of adipose precursor cells exist in various adipose depots has been confirmed by elegant studies using \textit{in vivo} lineage tracing or fluorescence-activated cell sorting approaches.\textsuperscript{24,25} Remarkably, we found that the requirement for 14-3-3ζ was specific to visceral adipose tissue depots, while having no apparent effect on the sub-cutaneous fat we examined. Our study demonstrated that 14-3-3ζ regulates one distinct group of precursor cells (or all precursor cells in a partial manner) because systemic deletion of 14-3-3ζ did not fully ablate all visceral adipose tissue \textit{in vivo}.\textsuperscript{18} Progenitor cell heterogeneity in visceral adipose tissue depots has been recently reported by Chau and colleagues, whereby they identified a subset of progenitors that express Wt1.\textsuperscript{26} In our study, knockout of 14-3-3ζ promoted the aberrant expression of Gli3 and p27\textsuperscript{kip1} in adipose precursor cells, but the fact that adipogenesis still occurred suggests that not all adipocytes rely on 143–3ζ for

![Figure 1. Potential involvement of 14-3-3ζ in alternative pathways controlling adipogenesis.](image-url)
differentiation. This apparent heterogeneity is further reinforced by a recent finding from the Scherer lab, where they identified distinct transcriptional programs that control embryonic versus adult adipocyte maturation. Although both Pparγ and C/ebpα have long been postulated to be critical for adipogenesis during embryogenesis or adulthood, mice deficient in C/ebpα displayed essential functions for this transcription factor only in compensatory adipocyte hypertrophy or hyperplasia in adulthood, and not in terminal adipocyte differentiation during embryogenesis. The observation that 14-3-3 proteins found to complex with C/ebpβ prior to binding to the Pparγ promoter suggests a bias of 14-3-3 proteins in the embryonic development of adipocytes. Our study reinforces this notion because the expansion of fat mass following high-fat diet feeding was normal in 14-3-3z knockout mice, which indicates normal function of residual adult adipocytes. Nonetheless, further work is required to determine the identity of precursors that are dependent on 14-3-3z activity.

**Indirect role in browning of white adipose tissue?**

Factors and mechanisms controlling the conversion of subcutaneous white adipocytes to brown-like, or beige, adipocytes have been an area of great research in adipocyte biology, but it is still not fully understood what are the endogenous processes that regulate this event. Reduced circulating insulin, intermittent cold exposure and circulating FGF21 are known to promote browning of white adipocytes, and it has recently been shown that FTO may also have critical roles in mediating this process in human adipocytes. Elegant work from Qiu and colleagues described the contributions of the innate immune system on the development of beige fat. They identified the importance to catecholamine production from macrophages on browning of subcutaneous fat through the use of mice with macrophages that lack tyrosine-hydroxylase, a key enzyme in catecholamine production. Interestingly, one of the earliest established functions of 14-3-3 proteins is their ability to modulate the activity of tyrosine hydroxylase. 14-3-3z knockout mice did not display differences in energy expenditure; however, this observation was obtained at 23°C. Further studies performed with intermittent or prolonged cold exposure are required to examine the requirement of 14-3-3z on tyrosine-hydroxylase-dependent browning of white adipocytes.

**Regulation of insulin-stimulated glucose uptake by 14-3-3z**

Given the ability of 14-3-3 proteins to bind to various components of the insulin signaling network
14-3-3 proteins could have important roles in various insulin-mediated metabolic effects in adipocytes. This was apparent in 14-3-3ζKO mice, as they displayed decreased insulin sensitivity following administration of peripheral exogenous insulin, and likely indicates defects in GLUT4-mediated glucose uptake in skeletal muscle or fat. It is uncertain as to whether deletion of 14-3-3ζ in vivo affects proximal or distal events in the insulin signaling pathway that control glucose uptake, but 14-3-3 proteins are known to bind to and promote the stability of insulin receptor substrate (IRS) molecules, which are key proximal effectors in this pathway. Deletion of 14-3-3ζ in adipocytes may also impact distal events controlling in the translocation of GLUT4 to the plasma membrane for glucose uptake. The Rab GAP AS160/TBC1D4 controls the activity of Rab molecules involved in transport of GLUT4 containing vesicles to the plasma membrane, following its phosphorylation by Akt, its inhibitory activity is attenuated by interacting with 14-3-3 proteins. Mutations of key residues on AS160/TBC1D4 that mediate 14-3-3 binding promotes reduced insulin sensitivity and glucose uptake. Thus, deletion of 14-3-3ζ in adipocytes and muscle may promote the inhibitory actions of AS160/TBC1D4 on insulin-stimulated glucose uptake.

**Does 14-3-3ζ have a role in lipolysis?**

One of the key functions of adipose tissue is to store lipids. Gonadal adipocytes in 14-3-3ζKO mice were still able to expand in size following high-fat diet feeding, which demonstrates that processes underlying lipid storage were not affected by deletion of 14-3-3ζ. In contrast, 14-3-3β has been implicated in lipid droplet formation in 3T3-L1 adipocytes by regulating actin remodeling. Mobilization of fatty acids from adipocytes following exposure to adrenergic stimuli requires the activities of lipases such as lipoprotein lipase (Lpl), adipose triglyceride lipase (Atgl), and hormone sensitive lipase (Hsl), and although we did not test this directly in our study, the presence of 14-3-3 binding sites on both Atgl and Hsl suggest the ability of 14-3-3ζ, and other isoforms, to regulate lipolysis (Fig. 2B). The phosphorylation of ATGL by AMPK generates phosphorylation sites specific to 14-3-3 proteins, and in C. Elegans, this interaction has been shown to control the localization of Atgl from lipid droplets. Following its phosphorylation by Akt, Foxo1 has been shown to interact with various 14-3-3 protein isoforms where it gets retained in the cytoplasm. While it has been shown that Foxo1 is required for adipocyte differentiation, our study demonstrates that the pro-adipogenic roles of 14-3-3ζ lie upstream of Foxo1 and 14-3-3ζ controls Foxo1 expression. This does not exclude the possibility that 14-3-3ζ may regulate the function of Foxo1 in mature adipocytes. Gonadal adipose tissue from mice lacking 14-3-3ζ had significantly decreased Atgl mRNA levels. Although we speculated that this could be due to residual gonadal white adipocytes being in a poorly differentiated state, it is possible that altered Foxo1 activity stemming from deficiency of 14-3-3ζ could be responsible for this observed decrease in Atgl mRNA. Foxo1 has been found to directly regulate Atgl transcription.

**Conclusion**

The discovery of novel physiological roles of 14-3-3ζ has opened exciting, new avenues of metabolic research. Our studies have dispelled the notion that 14-3-3ζ is a casual signaling bystander and instead revealed its critical function in adipogenesis. More work is clearly warranted to better understand which processes are regulated by 14-3-3ζ in the adipocyte and whether other related isoforms may have over-lapping or unique roles. In the end, a better understanding of how these integral signaling proteins coordinate signal transduction networks may aid in the development of highly specific therapeutics to treat various aspects of the metabolic syndrome.

**Disclosure of potential conflicts of interest**

No potential conflicts of interest were disclosed.

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