will be interesting to investigate whether oxytocin is also required for the subsequent vascular specialization in the pituitary, where the endothelium needs to become fenestrated instead of forming a blood brain barrier (Figure 1D) (reviewed by Burbach et al., 2001). Such studies will likely be facilitated by the beautiful double-transgenic zebrafish line that Levkowitz and colleagues created to covisualize oxytocin-secreting axons and blood vessels from development into adulthood (Gutnick et al., 2011).

From a physiological point of view, it appears sensible that a neurohormone such as oxytocin should function as a vascular guidance cue to promote the growth of blood vessels that will ultimately help its release into the circulation. A different type of neurovascular relationship promotes the function of the anterior pituitary of mammals, where the gonodotropin-releasing hormone GnRH induces the release of the gonadotropins LH and FSH into the circulation. In this system, the vascular growth factor VEGF promotes the survival of GnRH-secreting neurons during their migration from the nasal placode to the hypothalamus, independently of blood vessels (Cariboni et al., 2011). Taken together with the new study by Levkowitz and colleagues, it appears that the development of the hypothalamic hormonal response system relies on neurovascular congruence at different levels. Because the general function of the neurohypophysis and the GnRH neuron system are conserved in vertebrates, findings made in one model organism are likely to be relevant for other vertebrates. Hence, using optically transparent transgenic zebrafish embryos may help to elucidate how vascular growth factors promote the development of the GnRH neuronal system, while the oxytocin-deficient mouse (Nishimori et al., 1996) should enable further research into neuropeptide-mediated vascular patterning in the mammalian neurohypophysis.

REFERENCES

Burbach, J.P., Luckman, S.M., Murphy, D., and Gainer, H. (2001). Physiol. Rev. *81*, 1197–1267.

Cariboni, A., Davidson, K., Dozio, E., Memi, F., Schwarz, Q., Stossi, F., Parnavelas, J.G., and Ruhrberg, C. (2011). Development *138*, 3723–3733.

Cassoni, P., Marrocco, T., Bussolati, B., Allia, E., Munaron, L., Sapino, A., and Bussolati, G. (2006). Mol. Cancer Res. *4*, 351–359.

Gutnick, A., Blechman, J., Kaslin, J., Herwig, L., Belting, H., Affolter, M., Bonkowski, J.L., and Levkowitz, G. (2011). Dev. Cell *21*, this issue, 642–654.

Makita, T., Sucov, H.M., Gariepy, C.E., Yanagisawa, M., and Ginty, D.D. (2008). Nature *452*, 759–763.

Mukouyama, Y.S., Gerber, H.P., Ferrara, N., Gu, C., and Anderson, D.J. (2005). Development *132*, 941–952.

Neumann, I.D. (2008). J. Neuroendocrinol. *20*, 858–865.

Nishimori, K., Young, L.J., Guo, Q., Wang, Z., Insel, T.R., and Matzuk, M.M. (1996). Proc. Natl. Acad. Sci. USA *93*, 11699–11704.

Segura, I., De Smet, F., Hohensinner, P.J., Ruiz de Almodovar, C., and Carmeliet, P. (2009). Trends Mol. Med. *15*, 439–451.

Thibonnier, M., Conarty, D.M., Preston, J.A., Plesnicher, C.L., Dweik, R.A., and Erzurum, S.C. (1999). Endocrinology *140*, 1301–1309.

Long, Saturated Chains: Tasty Domains for Kinases of Insulin Resistance

Vladimir Lizunov,[1] Petr Chlanda,[1] Mary Kraft,[2] and Joshua Zimmerberg[1,*]
[1]Program in Physical Biology, Eunice Kennedy Shriver National Institute of Child Health and Human Development, National Institutes of Health, Bethesda, MD 20892, USA
[2]School of Chemical Sciences, University of Illinois, Urbana, IL 61801, USA
*Correspondence: zimmerbj@mail.nih.gov
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The mechanistic basis of how cells respond to increased fatty acids (FAs) is murky but potentially involves receptor-mediated activation or inhibition by different FA classes. Holzer et al. (2011) recently propose in *Cell* that expansion of intracellular membrane microdomains induced by saturated FA recruit and activate c-Src for JNK activation.

In this era of unprecedented caloric excess, we face increased incidences of obesity, metabolic syndrome, and diabetes mellitus; natural selection has left us ill equipped for unrestricted food. The first adverse sign is insulin resistance—decreased glucose transport into cells that is matched by an increase in serum insulin at the cost of elevated blood insulin, free fatty acids (FAs), and inflammatory mediators to maintain blood glucose homeostasis. Although the insulin receptor signaling cascade is redundant, with one insulin receptor substrate compensating for the loss of the other's function, c-Jun n-terminal kinase family members 1 and 2 (JNK, aka stress-activated protein kinases, a subset of mitogen-activated protein kinases), when activated, act as intracellular mediators of insulin resistance by disrupting both arms of this cascade. The Randle hypothesis links increased free FA to insulin resistance and proposes that FA compete

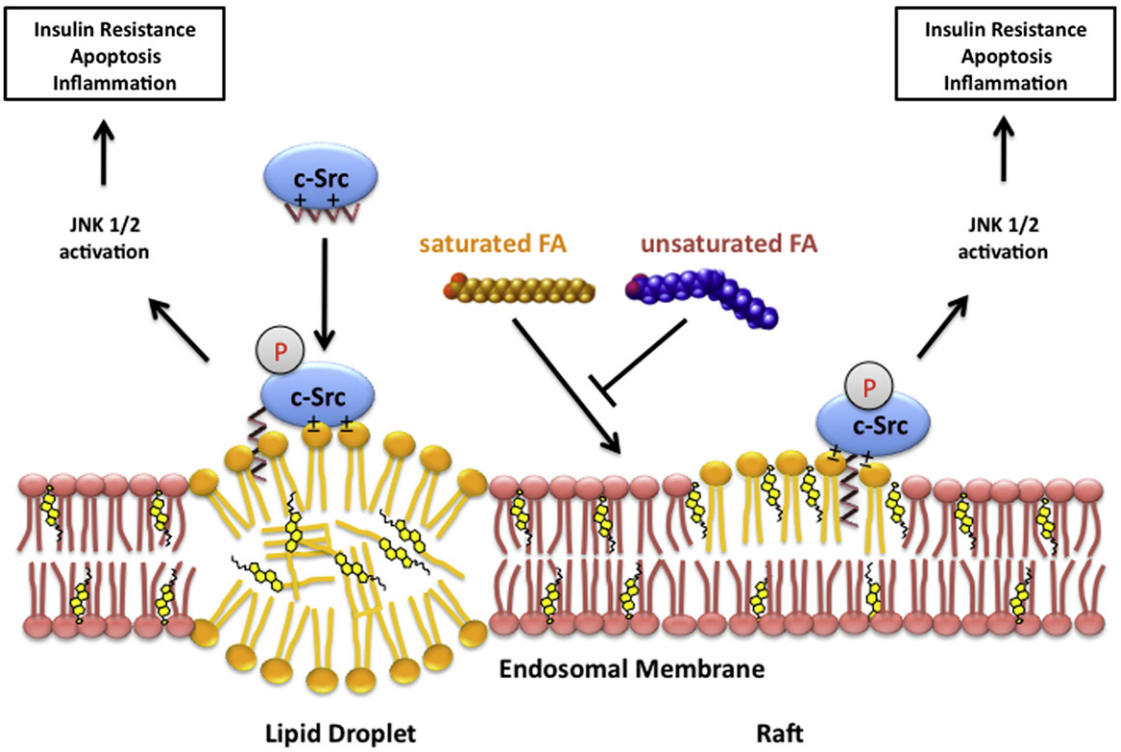

Figure 1. Hypothetical Mechanisms Linking Fatty-Acid-Induced Activation of c-Src and JNK to Insulin Resistance and Inflammatory Response

Holzer et al. (2011) propose that saturated long chain FAs can induce new intracellular membranes to form that bind c-Src, leading to its autophosporylation and activation and starting a JNK signaling cascade giving rise to insulin resistance. These new membrane domains result from an imbalance in the incorporation of saturated (orange) and unsaturated (blue) fatty acids from the diet. Dietary fat can also induce new lipid droplets. Rich in saturated chains, these new surfaces, bilayer or monolayer, could induce the accumulation of dually myristoylated and palmitoylated proteins via interaction of their saturated alkyl chains. c-Src is myristoylated, not palmitoylated, and binds to surfaces with anionic lipids. Long chain saturated fatty acids may also overload sphingomyelin synthesis pathway and lead to the accumulation of ceramides, lipid signaling molecules also capable of induction of insulin resistance.

with glucose as the major energy substrate for mitochondrial oxidation. But not all FA are alike. They vary by chain length, number of double bonds per chain (degree of unsaturation), and configuration (*cis* or *trans*). In the diet, they have very different effects on health; the mechanism underlying their adverse or beneficial effects, as well as how they are distinguished, is unknown. Holzer et al. (2011) in a recent issue of *Cell* propose that long chain fatty acids ($\geq$C16) lead to formation of intracellular lipid domains that recruit and activate c-Src kinase, linking the elevated saturated FA of obesity to insulin resistance via downstream activation of c-Jun N-terminal kinase 1 (Figure 1).

FA activation of JNK is not stringent—a class, rather than one molecular species, of FA causes JNK activation, i.e., two long chain saturated FA (palmitic and stearic acids) but not unsaturated FA can induce robust JNK activation. Even the addition of a single double bond to the saturated palmitic acid is sufficient to prevent JNK activation. Following up on the finding that the activation of the upstream JNK activator mixed-lineage kinase-3 (MLK3) is dependent upon saturated fatty acids (Jaeschke and Davis, 2007), Holzer et al. (2011) tested other tyrosine kinases known to interact with microtubule-associated protein kinases. Clearly, FA activation of both MLK3 and JNK is dependent upon FA saturation, due to the activation of one of these tyrosine kinase, the membrane-associated c-Src. Moreover, they explain the mystery of soluble MLK3 activating membrane-bound JNK: the activation of c-Src promotes the translocation of MLK3 to the same FA-induced internal membrane fractions that c-Src and JNK reside in.

How is this selectivity between saturated and unsaturated FA for colocalization and activation of these kinases achieved? In general, we can envision both a purely proteinaceous mechanism and, now, a lipidic mechanism. In a proteinaceous mechanism, a FA binding protein (FABPs) selective for saturated FA can signal the translocation of these molecules through protein-protein interactions. For example, FABPs selective for unsaturated lipids can interact with downstream targets that include nuclear regulators and transcription factors like PPARs (Hostetler et al., 2010). Additionally, recently identified G protein coupled receptors like GPR40-43 and GPR120 can bind FA, and GPR120 is able to selectively interact with omega-fatty acids (Oh et al., 2010). A lipidic mechanism is unprecedented but attractive. Increased substrate for lipidic membrane components could lead to increased synthesis of membranes whose cytoplasmic surface facilitates binding and activation of c-Src. Selectivity can arise on the enzymatic level since serine palmitoyltransferase uses palmitate but not palmitoleate as a substrate (Chavez et al., 2003).

Palmitate is a precursor of ceramides that in turn gives rise to sphingomyelins, presumably the building blocks of raft domains (Yeung et al., 2008). Rafts by definition accumulate high densities of saturated chains; palmitoylating c-Src by mutation activates it constitutively. However, the entire notion of rafts is questionable. While the plasma membrane is undoubtedly highly organized and heterogeneous with respect to proteins, the nature of lipid domains in cell membranes is unclear. The ease that cell membranes are fractionated into domains by simple density gradients with or without detergent is provocative, but there are many possible interpretations of such results (Heerklotz, 2002). Membrane fluidity, the reciprocal of viscosity, is a property of the lipid bilayer. But the large differences in measured mobilities of cell membrane components are due to interactions with proteins. Lipid mobilities are similarly fluid everywhere in living cells at physiological temperature. It is possible that as yet poorly characterized protein fences, which impede large-scale motion of lipids and proteins, could generate lipid domains, but this remains to be tested. Clearly more specific language concerning fluidity and lipid domains, and future studies that directly assess lipid composition near proteins of interest without cell disruption, would be valuable.

What is the identity of the membrane domain for the signaling pathway proposed by Holzer et al. (2011)? c-Src targets to endosomes, plasma membranes, and focal adhesions, requiring RhoB endosome-associated actin assembly (Sandilands and Frame, 2008). Most intracellular membranes, of endoplasmic reticulum and Golgi origin, are excluded, as c-Src has a polybasic domain that binds to anionic lipids, which these membranes are relatively devoid of. The cellular structures whose membranes harboring the putative c-Src signaling domains accumulate intracellularly, like endosomes. Could they include lipid droplets (LDs), organelles composed of neutral lipids and covered with a phospholipid monolayer (Fujimoto and Parton, 2011)? LD too are induced upon FA uptake by cells with similar timescales. Notably, the LD monolayer also contains the typical raft markers flotilin-1 and caveolin-1, and the LD monolayer may function as a "monolayer domain" and a signaling hub. It is conceivable that LDs enriched in saturated FA may possess biophysical properties positive for c-Src selectivity in a similar way as suggested for rafts. However, LD monolayers derived from ER may have insufficient anionic lipid, despite sufficient phosphatidyl inositol (PI) for signaling. Nevertheless, saturated FA may differentially induce lipid droplets from membranes of the endosomal system, rich in anionic phospholipids (C. Jackson and K. Soni, personal communication). Thus the special domains for activation may be on endosomal-derived LD.

Beyond the kinase activation step, toxicity of FA may trigger endoplasmic reticulum (ER) stress and apoptotic pathways. Indeed, palmitate but not palmitoleate is reported to induce ER stress, with mono- and polyunsaturated FA protecting (Diakogiannaki et al., 2008). Whether ER stress precedes or follows c-Src and JNK activation is not clear, as both processes have timescales of a few hours after palmitate treatment. Clearly, more work is needed to understand the effect of dietary lipid on the lipids of our own membranes and on signaling processes that modulate our metabolism. If you are what you don't metabolize or excrete, we can start to fathom some of the aspects of human nutrition that depend upon FA chain chemistry.

REFERENCES

Chavez, J.A., Knotts, T.A., Wang, L.P., Li, G., Dobrowsky, R.T., Florant, G.L., and Summers, S.A. (2003). J. Biol. Chem. *278*, 10297–10303.

Diakogiannaki, E., Welters, H.J., and Morgan, N.G. (2008). J. Endocrinol. *197*, 553–563.

Fujimoto, T., and Parton, R.G. (2011). Cold Spring Harb. Perspect. Biol. *3*, a004838.

Heerklotz, H. (2002). Biophys. J. *83*, 2693–2701.

Holzer, R.G., Park, E.-J., Li, N., Tran, H., Chen, M., Choi, C., Solinas, G., and Karin, M. (2011). Cell *147*, 173–184.

Hostetler, H.A., Balanarasimha, M., Huang, H., Kelzer, M.S., Kaliappan, A., Kier, A.B., and Schroeder, F. (2010). J. Lipid Res. *51*, 3103–3116.

Jaeschke, A., and Davis, R.J. (2007). Mol. Cell *27*, 498–508.

Oh, D.Y., Talukdar, S., Bae, E.J., Imamura, T., Morinaga, H., Fan, W., Li, P., Lu, W.J., Watkins, S.M., and Olefsky, J.M. (2010). Cell *142*, 687–698.

Sandilands, E., and Frame, M.C. (2008). Trends Cell Biol. *18*, 322–329.

Yeung, T., Gilbert, G.E., Shi, J., Silvius, J., Kapus, A., and Grinstein, S. (2008). Science *319*, 210–213.