

Not the average view of adipogenesis

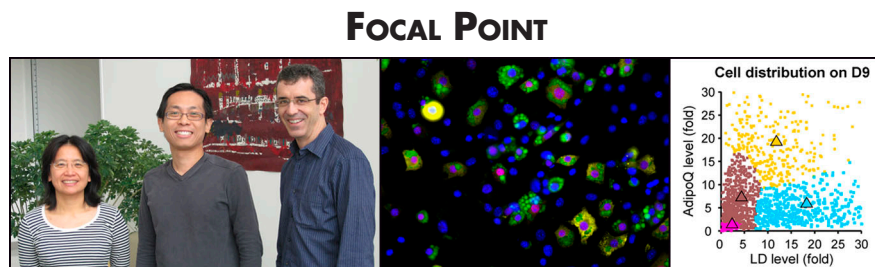
Study on cell heterogeneity offers a surprising twist to adipocyte differentiation.

American men are 176.3 cm tall and weigh 86.6 kg. This statement isn't actually true, of course—it's just an average that masks a huge diversity in body shape and size. Loo et al. reveal that only looking at the population average of differentiating adipocytes hides a similar heterogeneity, and limits our ability to understand adipogenesis (1).

Adipocytes are the body's fat-storing cells. As they develop from precursors, they express specific proteins such as adiponectin, and form large lipid droplets in their cytosol (2). Judged by methods—such as Western blotting—that measure the average values of a cell population, these markers all increase in conjunction with one another. Lit-Hsin Loo, Steve Altschuler, Lani Wu, and their colleagues at UT Southwestern Medical Center, Dallas, Texas, were trying to model the signaling pathways that control fat metabolism in 3T3-L1 adipocytes when they realized that the cells were too heterogeneous to treat as a uniform population. Immunofluorescence revealed widely divergent levels of adiponectin and lipid droplets in each cell as they progressed through differentiation.

"We realized that we had to go to a different resolution," says Altschuler. But while considering the cells as a single homogenous group was too simple, analyzing each cell individually would make things too complicated. Therefore, based on the adiponectin and lipid droplet levels measured by automated microscopy, Loo et al. clustered the cells into four different subpopulations using an unbiased computer algorithm. "We looked at a very large number of cells," explains Loo. "So we could see patterns that wouldn't have been obvious if we'd only analyzed a small number."

Surprisingly, none of the four subpopulations consisted of cells with high amounts of both adiponectin and lipid



Lani Wu, Lit-Hsin Loo, Steve Altschuler, and colleagues identified four phenotypically distinct subpopulations within a heterogeneous mix of differentiating adipocytes (middle). Clustering individual cells together according to their levels of particular markers (right) offered a markedly different view of adipogenesis than seen with measurements averaged across the whole population—markers thought to be closely connected are actually inversely correlated at the single cell level.

droplets, even though the two markers increase simultaneously when measured in the population as a whole. Instead, the researchers identified one subpopulation (labeled S3) with higher adiponectin and lower lipid droplet levels, and another (S4) with low adiponectin but more lipid droplets. Several other markers thought to be linked were also shown to have distinct expression patterns in S3 and S4 cells. "It's a lesson to us that there can be this illusion of correlation," Altschuler admits.

The four subpopulations probably represent particular stages of adipogenesis, consistent with the idea that differentiating cells transit through distinct phenotypic states (3, 4). The proportion of adipocytes in each subpopulation changed over time, and time-lapse microscopy suggested that the cells shift from one state to another. S3 cells seem to develop into S4 cells but, Wu explains, "people haven't seen this before because the cells differentiate asynchronously."

Thus, at the population level, adiponectin and lipid droplets increase together, even though they peak at different points during adipogenesis.

If the subpopulations correspond to distinct stages of differentiation, the researchers reasoned that they would react to drug treatments in different ways.

"Adipocytes respond to a variety of compounds, hormones, and fatty acids," says Loo and, indeed, many of these factors had specific effects on the subpopulations in ways that are obscured by population-averaged measurements. The next question is to determine how these results on cultured adipocytes relate to the situation in vivo. One step will be to better define the different cellular states, perhaps by identifying unique biomarkers for each subpopulation.

Meanwhile, the group is interested in many other systems—including cancer—in which heterogeneity among cells is a notable feature. "Our ultimate goal is to build better models of signal transduction," says Altschuler. "To do this, we have to understand whether our data are coming from a heterogeneous system or not. When can you justify using a mean? We're finding that in many cases, you actually can't. You have to decompose your population into more homogenous groups, and only then can you build your model."

1. Loo, L.-H., et al. 2009. *J. Cell Biol.* doi:10.1083/jcb.200904140.
2. Lefterova, M.I., and M.A. Lazar. 2009. *Trends Endocrinol. Metab.* 20:107–114.
3. Huang, S., and D.E. Ingber. 2006. *Breast Dis.* 26:27–54.
4. Waddington, C.H. 1957. *The Strategy of the Genes.* Allen & Unwin, London.

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