ADIPOSE TISSUE

Insulin sensitive human adipocytes for in vitro studies

Leon G. Straub and Philipp E. Scherer

Adipocyte insulin resistance is a driving force for systemic insulin resistance. Advances in human stem cell culture have established conditions for human adipocytes that give rise to a reproducible population of adipocytes that retain a high level of insulin sensitivity, paving the way for high throughput screening approaches.

Refers to Friesen, M. et al. Development of a physiological insulin resistance model in human stem cell–derived adipocytes. *Science Advances* **8**, eabn7298 (2022).

With two landmark clinical trials that were successfully concluded in 2021 and 2022[1,2], anti-obesity treatment regimens have taken centre stage to fight a plethora of obesity-associated co-morbidities. Central control of energy homeostasis occurs through critical circuits in the hypothalamus, which exert essential functions towards restoring energy homeostasis in obesity; however, intricate crosstalk with insulin-sensitive adipose tissue is essential for this process to be successful[3]. To pharmacologically intervene in obesity at the level of adipose tissue, high-throughput drug screening methods for adipocytes are key to diversify drug targets. A major limitation for this process is the lack of reliable human adipocyte tissue culture systems. Furthermore, establishing in vitro culture conditions that properly reflect the complex architecture and cellular microenvironment that these adipocytes encounter in vivo is challenging.

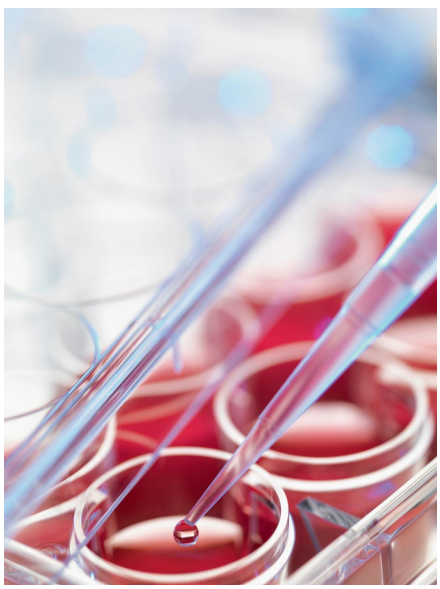
In a recent article in *Science Advances*, Friesen and colleagues established an optimized protocol for in vitro cultures of adipocytes[4]. Their starting materials were human pluripotent stem cells (hPSCs). These cells are readily available and can be reproducibly pushed through an adipogenic programme using transient exposure to ectopic expression of peroxisome proliferator activated receptor gamma. In the present study, the authors undertake a systematic approach towards optimizing insulin sensitivity in these cells[4], providing an improved culture protocol compared with previous hPSC models[5].

Ahfeld and colleagues[5], as well as other laboratories, established the initial protocol conditions to effectively turn hPSCs into adipocytes. However, the cells obtained had similar disadvantages to tissue culture systems derived from mouse models, with a limited response to insulin. Earlier work from Lin and colleagues in 2005 determined that standard tissue culture conditions involving 25 mM glucose in the culture medium were a major contributing factor towards the generation of insulin resistant cells[6]. They demonstrated that mouse 3T3-L1 fibroblast cells can effectively be differentiated into adipocytes with 5 mM glucose, with a dramatic improvement in insulin sensitivity[4]. Another common approach to sensitize adipocytes towards insulin is an overnight insulin and serum starvation. These established conditions were systematically tested in the present study[4]. The extension of the sensitization period to five days optimized the insulin response assays best, but had a minimal effect on cell numbers and differentiation states[4].

In Friesen et al., a large set of experimental readouts, including phospho-AKT2 over total AKT2 protein quantification, glucose uptake, total internal reflection fluorescence imaging of GLUT4 membrane translocation and lipolysis assays, as well as RNA sequencing, were used to create a multi-dimensional picture of the insulin response in human hPSC-derived adipocytes[4]. The findings were validated in a number of different hPSC lines, including Hues9, WA09/H9, DiPS, 1016 SevA and WIBR599. The optimized protocol was also used in the context of two other adipocyte models, mouse 3T3-L1 derived adipocytes and primary stromal vascular fractions-derived human adipocytes.

> “…high-throughput drug screening methods for adipocytes are key to diversify drug targets”

Cell culture systems help reduce the number of variables that have an impact in a cell. Friesen and colleagues elegantly explain that high glucose and high insulin concentrations can on their own cause insulin insensitivity in isolated human adipocytes. However, despite the fact that 100 years have passed since the discovery of insulin, many questions remain regarding the term ‘insulin resistance’. The field in general talks about selective insulin resistance in the liver with differential effects on lipid and glucose metabolism[7]. Ongoing debates rage about in vivo progression to an insulin resistant state, in which progressive insulin insensitivity leads to a compensatory increase in insulin production and release from pancreatic β-cells that eventually causes insulin resistance. Is this progressive increase in endogenous insulin production the driver for progressive insulin resistance? Is the addition of exogenous pharmacological levels of insulin, while effectively lowering glucose levels in the short term, leading to progressive



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> Friesen and colleagues established an optimized protocol for in vitro cultures of adipocytes

worsening of insulin sensitivity? Similar scenarios have been proposed for leptin, and leptin lowering has been shown to enhance leptin sensitivity[8].

The difference between leptin and insulin resistance is that leptin resistance rapidly progresses to a point where supra-physiological levels of leptin result in a complete absence of a response. By contrast, a progressive increase in insulin levels continues to elicit a minimal response, with downstream insulin action. We might find an explanation with the old rule of thumb that under normal physiological conditions, only 5% of all cell surface insulin receptors need to be engaged for a full response[9]. The only way to get a complete lack of insulin signalling is an elimination of the receptor. The insulin receptor can be inducibly knocked down in mature adipocytes of heavily obese mice. Interestingly, upon elimination of the insulin receptor in adipocytes, the diabetic state further deteriorates[10]. This experiment suggests that under conditions of obesity, adipocytes could be insulin insensitive and not fully resistant to insulin.

Friesen and colleagues are now well positioned to characterize the transition from insulin sensitive to insulin resistant states in adipocytes in vitro. How does cell signalling and metabolism change in an adipocyte undergoing this transition? The challenge remains, of course, that it will be difficult to translate the findings into an in vivo setting in the context of a complex interplay between many different cell types in adipose tissue. This crosstalk includes not only the adipocytes, but also a host of immune cells, including macrophages, endothelial cells, adipocyte precursors, fibroblasts and in many cases myofibroblasts. Nevertheless, along a reductionist line of experimental approaches, the system established by Friesen and colleagues will be very helpful.

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Competing interests
The authors declare no competing interests.