transition. The authors then showed that, in mice lacking expression of the core autophagy regulators *Atg5* and *Atg12* in UCP1-positive beige adipocyte, there is no loss of beige phenotype after withdrawal of β3-AR agonist treatment. The authors thus conclude that autophagy-mediated mitochondrial clearance controls beige adipocyte maintenance.

These two papers provide interesting information on the dynamics of beige adipocytes, and they also focus on the uniqueness of the subcutaneous adipose organ. Is the plasticity of this organ unique to the adipocytes found here, or is this a feature common to all adipocytes—albeit at different threshold levels? Is the Cx43-mediated cell-to-cell communication specifically a way to facilitate the spread of cAMP signaling, or is this communication also open for other types of messengers? To a large extent, adipocytes have previously been characterized based on cellular morphology; these studies show that this might be an oversimplification. We have learned that what looks like a white adipocyte in the microscope can be either a bona fide white adipocyte or an unstimulated dormant beige adipocyte. Exactly what makes a white adipocyte a white adipocyte at the cellular level and how this differs from a beige adipocyte is an interesting area for future experiments. Is this difference a quantitative trait, or is this a cell-autonomous property? A detailed mapping of the mechanisms regulating the developmental fate of mesenchymal progenitors giving rise to "pure" white adipocytes versus beige adipocytes would be an interesting step to take in finding out more about this. These two papers have described new and interesting properties of the subcutaneous adipose organ—it is very likely that there are more to come.

REFERENCES

Altshuler-Keylin, S., Shinoda, K., Hasegawa, Y., Ikeda, K., Hong, H., Kang, Q., Yang, Y., Perera, R.M., Debnath, J., and Kajimura, S. (2016). Cell Metab. *24*, this issue, 402–419.

Kazak, L., Chouchani, E.T., Jedrychowski, M.P., Erickson, B.K., Shinoda, K., Cohen, P., Vetrivelan, R., Lu, G.Z., Laznik-Bogoslavski, D., Hasenfuss, S.C., et al. (2015). Cell *163*, 643–655.

Koza, R.A., Hohmann, S.M., Guerra, C., Rossmeisl, M., and Kozak, L.P. (2000). J. Biol. Chem. *275*, 34486–34492.

Lee, Y.H., Petkova, A.P., Mottillo, E.P., and Granneman, J.G. (2012). Cell Metab. *15*, 480–491.

Rosenwald, M., Perdikari, A., Rülicke, T., and Wolfrum, C. (2013). Nat. Cell Biol. *15*, 659–667.

Wu, J., Boström, P., Sparks, L.M., Ye, L., Choi, J.H., Giang, A.H., Khandekar, M., Virtanen, K.A., Nuutila, P., Schaart, G., et al. (2012). Cell *150*, 366–376.

Zeng, W., Pirzgalska, R.M., Pereira, M.M., Kubasova, N., Barateiro, A., Seixas, E., Lu, Y.H., Kozlova, A., Voss, H., Martins, G.G., et al. (2015). Cell *163*, 84–94.

Zhu, Y., Gao, Y., Tao, C., Shao, M., Zhao, S., Huang, W., Yao, T., Johnson, J.A., Liu, T., Cypess, A.M., et al. (2016). Cell Metab. *24*, this issue, 420–433.

Specialized Hub Beta Cells Trade Maximal Insulin Production for Perfect Timing

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The pulsatility of insulin release is disturbed early in type 2 diabetes, but it is not clear whether specialized pacemaker cells drive islet oscillations. In this issue of *Cell Metabolism*, Johnston et al. (2016) show that specialized hubs, identified as 1%–10% of beta cells with more active mitochondria and less insulin, synchronize beta cell oscillations.

Beta cells within individual healthy islets display coordinated rhythmic firing leading to pulsatile insulin release that may prevent insulin resistance (Pørksen et al., 2002). Cell-cell interactions in situ are thought to be important because dispersed beta cells release less insulin (Zhang et al., 2003). In the heart, specialized pacemaker cells drive collective rhythmicity. In the pancreas, the existence of specialized beta cells that coordinate islet oscillatory behavior has been postulated but not directly tested due to imaging limitations and the inability to manipulate single beta cells within islets. In this issue of *Cell Metabolism*, Johnston et al. (2016) employ large-scale functional cell mapping and optogenetics to demonstrate for the first time that synchronized rhythmicity in intact pancreatic islets is controlled by 1%–10% of an islet's beta cell population.

These specialized pacemaker beta cells, termed "hubs" (Figure 1), are defined by their high degree of connectivity to other beta cells and their rapid reaction to elevated glucose, which precedes the populous of "follower" beta cells. Hubs are present in both mouse and human islets, despite their reported

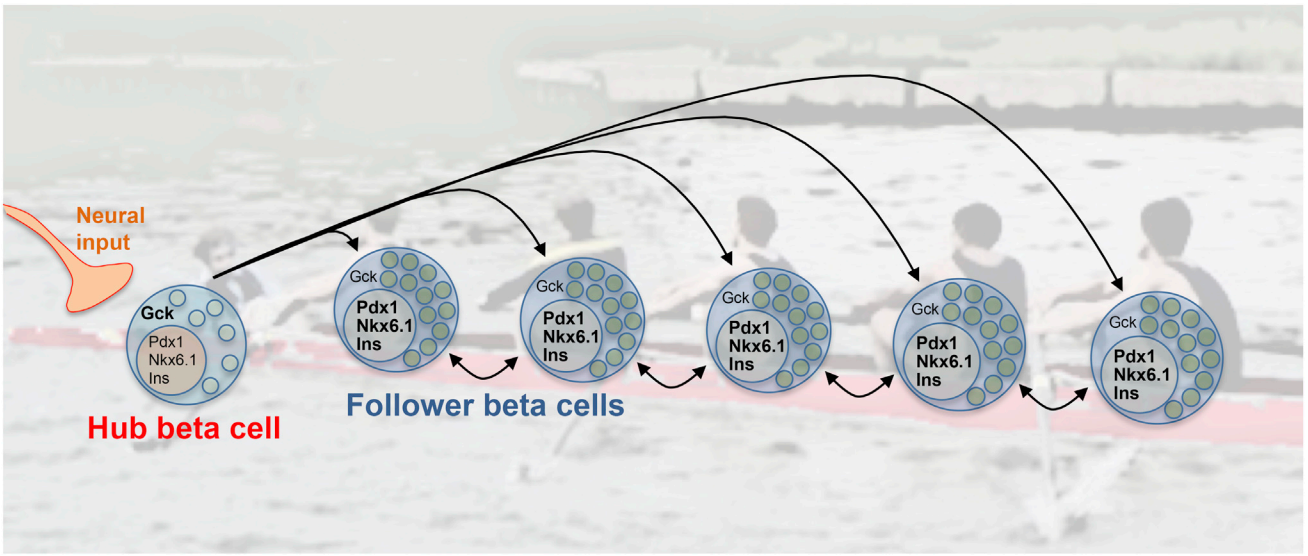

Figure 1. Hub Beta Cells Set the Pace for Synchronized Beta Cell Work
Hub cells have more glucokinase (Gck) but less insulin, possibly due to the lower expression of the key transcription factors Pdx1 and Nkx6.1. Hubs serve to set the islet's pace, likely in response to neuronal stimulation. Follower cells produce more insulin and are connected to each other via gap junctions. Rowing image courtesy of Wikimedia Commons.

differences in structure. Silencing individual hubs via the light-gated Cl^- pump, halorhodopsin, abolished synchronization and reduced insulin granule exocytosis from within isolated islets. This work is a technical tour de force, which ushers in a new era of optogenetic manipulation of intact islets.

With the existence and importance of hub cells established, Johnston et al. (2016) sought to determine what makes these cells unique by immunostaining islets with hubs marked by a photoactivatable fluorescent protein. Johnston et al. (2016) examined Gck, which sets the threshold for glucose responsiveness, and found that it was increased significantly in hub cells. This provides one mechanism for the more rapid responses of hubs relative to follower beta cells. It has been proposed that a critical fraction of beta cells needs to be excited before global islet activity occurs (Hraha et al., 2014). While there was no obvious difference in mitochondrial number, TMRE (tetramethylrhodamine, ethyl ester) imaging suggested increased mitochondrial activity in hubs, also consistent with their ability to robustly sense glucose. The reduced SERCA2 ATP-dependent Ca^{2+} pumps may increase reaction time of the hubs by sparing ATP and reducing the initial depression phase of Ca^{2+} response (Zhang et al., 2003). Thus, finely tuned hub cells have characteristics allowing them to respond robustly to glucose ahead of follower beta cells. An evolutionary biologist might ask what the trade-off is for this specialization. Remarkably, hub beta cells had lower Pdx1 and Nkx6.1, beta cell transcription factors that are required to maintain insulin production (Szabat et al., 2012). Accordingly, hubs had less insulin and fewer secretory granules. Reducing insulin production by ~50% increases ATP in beta cells, without adversely affecting glucose sensing or cell survival (Szabat et al., 2016), thus suggesting that hubs can shunt energy away from the beta cell's major ATP-consuming process to remain nimble.

This paper adds to the multi-decade literature on islet cell heterogeneity. Hubs may be a sub-group of a previously characterized beta cell population. Recently, it was found that ~20% of mouse beta cells negative for *Fltp* promoter activity have less *Pdx1*, *Nkx6.1*, and *Gck* (Bader et al., 2016). In a series of studies, it was shown that ~20%–30% of human or mouse beta cells with low *Ins1* promoter activity had lower insulin production/secretion, lower Pdx1 and Nkx6.1 expression, and qualitatively faster Ca^{2+} responses to glucose (Szabat et al., 2012). Statistical clustering of a human islet cell Ca^{2+} response dataset distinguished two types of Ca^{2+} responses to glucose: a majority group with oscillations and a smaller group of non-oscillatory cells with more rapid response initiation (Wills et al., 2016). While previous studies mostly investigated functional heterogeneity of dispersed cells, this work is one of the first to examine beta cell functional specialization in the intact islet. It is unclear whether properties of hubs persist after islet dispersion.

It is important to understand how a beta cell becomes a hub and maintains its designated hub status. The experiments in the current paper showed that hubs are stable for at least 3 hr on a microscope stage (Johnston et al., 2016). It is possible that a specific microenvironment niche influences whether a cell is a hub, but Johnston et al. (2016) did not uncover a positional bias within the islets. It is not clear whether hubs are a stable sub-population of beta cells or whether long-lived beta cells transition between hub and follower states over time. Perhaps all beta cells have the potential to be hubs early in their lives, when insulin production has not yet ramped up. It would be interesting to determine whether more hubs could be induced in beta cells with a forced reduction in insulin production (Szabat et al., 2016). Understanding how hubs and followers are formed, and whether there

is plasticity between these states, has important implications for efforts to preserve islet functionality in diabetes.

Hubs are important; but are they also a weak link in islet function? Beta cell diversity was previously shown to be clinically relevant since insults may preferentially target specific cell populations to induce beta cell failure (Rutter and Hodson, 2015). In the current work, Johnston et al. (2016) show that acutely challenging mouse or human islets with pro-inflammatory cytokines or a glucolipotoxic milieu reduces the number of hubs, which is consistent with the hypothesis that hub cells may be especially vulnerable. However, Johnston et al. (2016) did not examine whether loss of connectivity consequently reduced insulin secretion, or alternatively whether dysfunctional diabetic human islets also show loss of hubs. Furthermore, it is unclear whether non-hub beta cells are also sensitive to these insults, or whether hubs represent the islet's Achilles' heel. One would expect hub cells with <20% of the normal levels of Pdx1 to be highly susceptible to apoptosis (Johnson et al., 2003). In vivo studies will require new markers to understand how hubs and their followers change over longer periods of time and how the hub-follower ratio affects glucose homeostasis. Unbiased discovery of hub markers in the future may be possible with light-dependent CRISPR-mediated genomic barcoding and single-cell transcriptomics.

In the meantime, Johnston et al. (2016) have made a major breakthrough in islet biology and established an exciting new research field. It is remarkable that such a small number of beta cells exerts such dominant control over connectivity and islet function.

REFERENCES

Bader, E., Migliorini, A., Gegg, M., Moruzzi, N., Gerdes, J., Roscioni, S.S., Bakhti, M., Brandl, E., Irmler, M., Beckers, J., et al. (2016). Nature *535*, 430–434.

Hraha, T.H., Westacott, M.J., Pozzoli, M., Notary, A.M., McClatchey, P.M., and Benninger, R.K.P. (2014). PLoS Comput. Biol. *10*, e1003819.

Johnson, J.D., Ahmed, N.T., Luciani, D.S., Han, Z., Tran, H., Fujita, J., Misler, S., Edlund, H., and Polonsky, K.S. (2003). J. Clin. Invest. *111*, 1147–1160.

Johnston, N.R., Mitchell, R.K., Haythorne, E., Pessoa, M.P., Semplici, F., Ferrer, J., Piemonti, L., Marchetti, P., Bugliani, M., Bosco, D., et al. (2016). Cell Metab. *24*, this issue, 389–401.

Pørksen, N., Hollingdal, M., Juhl, C., Butler, P., Veldhuis, J.D., and Schmitz, O. (2002). Diabetes *51* (*Suppl 1*), S245–S254.

Rutter, G.A., and Hodson, D.J. (2015). Cell. Mol. Life Sci. *72*, 453–467.

Szabat, M., Lynn, F.C., Hoffman, B.G., Kieffer, T.J., Allan, D.W., and Johnson, J.D. (2012). Diabetes *61*, 1365–1371.

Szabat, M., Page, M.M., Panzhinskiy, E., Skovsø, S., Mojibian, M., Fernandez-Tajes, J., Bruin, J.E., Bround, M.J., Lee, J.T.C., Xu, E.E., et al. (2016). Cell Metab. *23*, 179–193.

Wills, Q.F., Boothe, T., Asadi, A., Ao, Z., Warnock, G.L., Kieffer, T.J., and Johnson, J.D. (2016). Islets *8*, 48–56.

Zhang, M., Goforth, P., Bertram, R., Sherman, A., and Satin, L. (2003). Biophys. J. *84*, 2852–2870.

Fueling Performance: Ketones Enter the Mix

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Ketone body metabolites serve as alternative energy substrates during prolonged fasting, calorie restriction, or reduced carbohydrate (CHO) availability. Using a ketone ester supplement, Cox et al. (2016) demonstrate that acute nutritional ketosis alters substrate utilization patterns during exercise, reduces lactate production, and improves time-trial performance in elite cyclists.

Exercise intensity is the primary determinant of substrate utilization during exercise. As intensity increases, the contribution of substrates to energy provision shifts from blood-borne free fatty acids (FFAs) and glucose toward increased reliance on intramuscular triglyceride (IMTG) and glycogen (Egan and Zierath, 2013). At moderate-to-high exercise intensities (>75% of maximal oxygen uptake, VO_2max), muscle glycogen is the main source of energy provision. This hierarchy of fuel selection in exercising muscle is long established, and, consequently, nutrition strategies for fueling for most competitive athletic performance are based around optimizing CHO provision before and during performance. Nevertheless, training with reduced CHO intake may be a means to improve body composition and enhance metabolic and mitochondrial adaptations to exercise (Burke, 2015). Moreover, there is increasing interest in the purported benefits of ketogenic diets (KDs) and the use of exogenous ketone supplements within the athletic community, particularly in ultra-endurance sports. Using a ketone ester food supplement initially developed for enhancing warfighter performance, the work by Clarke and colleagues (Cox et al., 2016) comprises a series of experiments employing acute ingestion of ketone ester by highly trained cyclists prior to and during intense cycling exercise, while examining outcomes related to fuel selection, mitochondrial bioenergetics, intramuscular substrate utilization, and time-trial performance. The provocative findings