Glucose: not always the bad guy.

"Glucose: not always the bad guy"

Commentary on "Glucose Uptake and Runx2 Synergize to Orchestrate Osteoblast Differentiation and Bone Formation" by Wei et al, Cell. 2015 Jun 18;161(7):1576-91.

Tara C Brennan-Speranza[1] and Itamar Levinger[2]

[1] Department of Physiology, Bosch Institute for Medical Research, University of Sydney, Australia.
[2] Clinical Exercise Science Program, Institute of Sport, Exercise and Active Living (ISEAL), Victoria University, Melbourne, Australia.

Corresponding Author:
Tara Brennan-Speranza
Department of Physiology,
University of Sydney, NSW, Australia, 2006
Fax: +61 9351 2510
Email: tara.speranza@sydney.edu.au

The type 2 diabetes epidemic currently faced by increasing numbers of countries has led to many investigations that analyse the negative effects of glucose on the health, function and fate of cells, tissues, organs and body systems. This is certainly true for skeletal health, as patients suffering from type 2 diabetes mellitus have increased fracture risk, despite normal to high bone mineral density [1-4]. There is no doubt that long term hyperglycemia adversely affects bone, yet a recent paper by Wei and colleagues published in Cell highlights the importance of glucose for functional health and perhaps even commitment of mesenchymal cells to the osteoblast lineage and bone formation [5].

Glucose is a major source of energy for active bone forming osteoblasts. Interestingly, Wei *et al* have demonstrated that in the mouse, the uptake of glucose into osteoblasts accounts for a large proportion of total body glucose uptake, being one-fifth of that taken up by skeletal muscle and half of what is taken up by white adipose tissue. Secondly, they show osteoblastic glucose uptake is insulin-independent, and finally, that glucose uptake is likely to be responsible for the commitment of ostoeprogenitors and bone development [5]. The mRNA level of Glut1 was almost 100-times higher than that of other glucose transporters in osteoblasts [5]. The authors characterized the role of GLUT1 in skeletal development and bone formation through a series of genetic knockouts in mice.

Commitment of osteoprogenitor cells is the first stage in osteoblast formation and development. The condensation of mesenchymal cells during embryonic development is followed by rapid differentiation of these cells into their lineages, including chondroblasts and osteoblasts, for skeletal development[6]. This process is tightly controlled so that mesenchymal cells differentiate to chondroblasts or pre-osteoblasts, then mature osteoblasts that in turn increase bone matrix deposition and mineralization. The particular progression of mesenchymal cells into osteoblasts is known as osteoblastogenesis. Osteoblastogenesis is tightly regulated by a choreographed expression pattern of particular transcription factors.

Runt-related transcription factor 2 (RUNX2), also known as core-binding factor subunit alpha-1 (CBF-alpha-1) is encoded by the *Runx2* gene. RUNX2 has been identified as the major transcription factor in the control of osteoblastogenesis and osteoblast function during both endochondral and intramembranous ossification [7,8]. Like other members of the RUNX family of transcription factors, RUNX2 contains a Runt DNA-binding domain that can bind DNA either alone or as a complex with other transcription factors. The early commitment of mesenchymal stem cells into osteoblasts requires the expression of *Runx2* that regulates the expression of several important bone proteins, including type I collagen, bone sialoprotein (BSP), osteopontin (OP), transforming growth factor β (TGFβ), alkaline phosphatase (ALP) and osteocalcin (OCN)[9] amongst others. *Runx2* displays haploinsufficiency in humans where patients with a mutation in one allele are affected with a skeletal condition known as cleidocranial dysplasia characterized

by suppressed bone formation [10]. It has become clear that certain transcription factors lead to *Runx2* expression at different time points during the commitment process of mesenchymal cells to the osteoblast lineage, including Hoxa2, a member of the Hox homeodomain family of transcription factors, SABT2 [11] and even the suppression of chondroblastogenic factors including Sox9 [9] and certain microRNAs that act as inhibitors of bone formation[12]. The exact chronology and identification of all the required factors for expression of *Runx2* are still unclear.

Type I collagen is synthesized by osteoblasts and is the most abundant organic component of the extracellular bone matrix (ECM). It consists of two α1 and one α2 chains, encoded by separate genes. The promotor region of the most highly expressed α1 chain has a specific RUNX2 binding domain [13], leading to the supposition that the initial expression of type I collagen was driven by RUNX2. However, Wei *et al* use in situ hybridization to show that, *in vivo*, type I collagen synthesis occurs in developing hind limbs prior to the expression of *Runx2*. This indicates there is another trigger for the movement of mesenchymal cells into the osteoblast lineage. Examination of the Glut1 transporters in these same developing limbs identified that these transporters were also expressed before *Runx2*. GLUT1 is an insulin-independent glucose (and Vitamin C) transporter, encoded by the gene: *SLC2A1* (or *Glut1*)[14]. GLUT 1 is known to facilitate basal glucose uptake in the brain and eurythrocytes, for example [14]. Other studies have also shown an important role for GLUT1 in insulin-independent glucose metabolism in osteoblasts [15].

Glut1 transporter knockouts in both early and later embryonic development verified the importance of these glucose transporters in osteoblastogenesis and bone formation [5]. Both models resulted in reduced ECM mineralization, decreased osteoblast differentiation, reduced trabecular thickness and delayed osteocalcin expression. Interestingly, *Runx2* and *α1 collagen* expression were normal in the osteoblast-specific Glut1 knockout mice, yet, accumulation of RUNX2 and collagen Iα1 protein was decreased. Induction of Glut1 transporter knockout either at the post-natal and 6 week stage resulted in mice with low bone mass, reduced osteoblast proliferation, reduced osteocalcin expression and reduced glucose and insulin tolerance at 3 months of age. As these effects are a consequence of knocking out the most abundant glucose transporter in osteoblasts, the authors conclude they are likely due to an overall reduction in energy supply leading to a reduction in total protein synthesis.

Mammalian target of rapamycin complex 1, or mTORc1, is a nutrient-sensitive kinase complex that regulates, in particular, nucleotide and protein synthesis and thus orchestrates cell growth and proliferation. mTORc1 and AMPK are reciprocally regulated via nutrient availability [16]. Considering the effect of reduced glucose uptake in the Glut1 knockout mice, Wei *et al* analyzed the effect of the knockout on mTORc1 and confirmed its involvement via increased AMPK activity [5]. Further, in an elegant mouse model in which AMPK activity was reduced by

deletion of one AMPK subunit allele in the Glut1 osteoblast knockout model, they observed restored ATP levels, as well as restored RUNX2 and type I collagen protein accumulation.

The observed normal *Runx2* expression alongside low levels of RUNX2 protein accumulation in Glut1-null osteoblasts led the authors to consider whether the lack of Glut1 and the reduced glucose uptake in these cells led to increased RUNX2 ubiquitination and thus increased proteasomal degradation. Indeed, the ubiquitin ligase, SMURF1, was shown to trigger this degradation via AMPK activity [17]. To assess the role of glucose uptake in RUNX2-induced osteoblast differentiation, they crossed their embryonic models of osteoblastic Glut1-null with mice lacking a single *Smurf1* allele. While this model restored RUNX2 accumulation, mTORc1 activity and collagen synthesis remained low. In essence, restoring RUNX2 accumulation was not sufficient to restore embryonic skeletal development or bone formation when glucose uptake remained impaired. Evidence that extracellular glucose alone may trigger the synthesis of collagen came from *in vitro* studies in induced *Runx2*-null osteoblasts with high, but not physiological glucose levels increased energy consumption by the osteoblasts. This also normalized AMPK and mTORc1 activity, improved the stability of the collagen triple helix and resulted in normal type 1 collagen protein accumulation. These results were confirmed *in vivo* in heterozygote *Runx2* knockout mice. These mice had reduced Glut1 expression and lower glucose consumption. Inducing hyperglycemia in these embryos led to improved intramembranous bone development and type 1 collagen expression, but did not improve mineralization, likely due to the lack of RUNX2-induced alkaline phosphatase expression. Careful note should be taken with the interpretation of these data as it is known that high glucose adversely affects non-genetically modified osteoblasts [18] and reduces markers of bone formation in both normal and obese patients [19]. Furthermore, inducing short term hyperglycemia in mice also increases insulin, and other factors, which may be contributing to the observed effects.

Lastly, using *in vitro* chromatin immunoprecipitation and co-transfection methods, the authors demonstrated that RUNX2 binds to a canonical Runx binding site on the Glut1 promoter region and increases the activity of the promoter. Runx2 expression is correlated with Glut1 expression, likely explaining the low glucose uptake and undetectable mTORc1 signaling in osteoblasts lacking *Runx2.* These data imply that there is a feedforward loop that regulates the uptake of glucose by Glut1 transporters and the functions of the master osteoblast transcription factor, RUNX2. Indeed skeletal preparations of heterozygote *Runx2* knockout mice crossed with mice lacking one allele of osteoblastic-specific *Glut1* mimic $Runx2^{-/-}$ mice at E16.5, and continue with problematic skeletal formation and mineralization beyond that time point. These malformations were improved in the embryos of hyperglycemic mothers.

Altogether, this comprehensive study relates the energy requirements of early pre-osteoblastic stem cells to their fate. These cells require glucose uptake, via insulin-independent GLUT1 facilitation, to trigger the stable expression and accumulation of the major transcription factors involved in osteoblastogenesis and proteins involved in bone formation. GLUT3 transporters have also been identified in bone and may play a role in insulin-dependent glucose transport in the developing skeleton [15]. Importantly, however, the data presented in the study by Wei *et al* indicate that it is glucose uptake in these cells that drives the expression of type I collagen prior to RUNX2 expression and accumulation. While the study showed that RUNX2 is clearly necessary for osteoblast differentiation and function, the most notable result of this investigation was that increased glucose exposure in osteoblasts lacking *Runx2*, but not increased RUNX2 accumulation in osteoblasts lacking *Glut1*, improved collagen expression. Future studies, including in humans, are necessary to conclude whether nutritional-based treatments in patients with skeletal dysplasia might be useful and careful consideration of the negative effects of high glucose and high fat diets on the skeleton is required.

Acknowledgements: Dr Brennan-Speranza is supported by an NHMRC Early Career Research Fellowship (ID: 1013295) and A/Prof Levinger is supported by a Future Leader Fellowship (ID: 100040) from the National Heart Foundation of Australia.

References:

1 Vestergaard, P., Rejnmark, L. & Mosekilde, L. Diabetes and its complications and their relationship with risk of fractures in type 1 and 2 diabetes. *Calcified tissue international* **84**, 45-55, (2009).

2 Leslie, W. D., Rubin, M. R., Schwartz, A. V. & Kanis, J. A. Type 2 diabetes and bone. *Journal of bone and mineral research : the official journal of the American Society for Bone and Mineral Research* **27**, 2231-2237, (2012).

3 Schwartz, A. V., Vittinghoff, E., Bauer, D. C., Hillier, T. A., Strotmeyer, E. S., Ensrud, K. E. *et al.* Association of BMD and FRAX score with risk of fracture in older adults with type 2 diabetes. *Jama* **305**, 2184-2192, (2011).

4 Bonds, D. E., Larson, J. C., Schwartz, A. V., Strotmeyer, E. S., Robbins, J., Rodriguez, B. L. *et al.* Risk of fracture in women with type 2 diabetes: the Women's Health Initiative Observational Study. *J Clin Endocrinol Metab* **91**, 3404-3410, (2006).

5 Wei, J., Shimazu, J., Makinistoglu, M. P., Maurizi, A., Kajimura, D., Zong, H. *et al.* Glucose Uptake and Runx2 Synergize to Orchestrate Osteoblast Differentiation and Bone Formation. *Cell* **161**, 1576-1591, (2015).

6 Mariani, F. V. & Martin, G. R. Deciphering skeletal patterning: clues from the limb. *Nature* **423**, 319-325, (2003).

7 Komori, T., Yagi, H., Nomura, S., Yamaguchi, A., Sasaki, K., Deguchi, K. *et al.* Targeted disruption of Cbfa1 results in a complete lack of bone formation owing to maturational arrest of osteoblasts. *Cell* **89**, 755-764, (1997).

8 Ducy, P., Zhang, R., Geoffroy, V., Ridall, A. L. & Karsenty, G. Osf2/Cbfa1: A Transcriptional Activator of Osteoblast Differentiation. *Cell* **89**, 747-754, (1997).

9 Marie, P. J. Transcription factors controlling osteoblastogenesis. *Archives of Biochemistry and Biophysics* **473**, 98-105, (2008).
10 Mundlos, S., Otto, F., Mundlos, C., Mulliken, J. B., Aylsworth, A. S., Albright, S. *et al.* Mutations Involving the Transcription Factor CBFA1 Cause Cleidocranial Dysplasia. *Cell* **89**, 773-779, (1997).
11 Dobreva, G., Chahrour, M., Dautzenberg, M., Chirivella, L., Kanzler, B., Fariñas, I. *et al.* SATB2 Is a Multifunctional Determinant of Craniofacial Patterning and Osteoblast Differentiation. *Cell* **125**, 971-986, (2006).
12 Hassan, M. Q., Gordon, J. A., Beloti, M. M., Croce, C. M., van Wijnen, A. J., Stein, J. L. *et al.* A network connecting Runx2, SATB2, and the miR-23a~27a~24-2 cluster regulates the osteoblast differentiation program. *Proceedings of the National Academy of Sciences of the United States of America* **107**, 19879-19884, (2010).
13 Kern, B., Shen, J., Starbuck, M. & Karsenty, G. Cbfa1 Contributes to the Osteoblast-specific Expression of type I collagen Genes. *Journal of Biological Chemistry* **276**, 7101-7107, (2001).
14 Mueckler, M., Caruso, C., Baldwin, S. A., Panico, M., Blench, I., Morris, H. R. *et al.* Sequence and structure of a human glucose transporter. *Science (New York, N.Y.)* **229**, 941-945, (1985).
15 Zoidis, E., Ghirlanda-Keller, C. & Schmid, C. Stimulation of glucose transport in osteoblastic cells by parathyroid hormone and insulin-like growth factor I. *Mol Cell Biochem* **348**, 33-42, (2011).
16 Hardie, D. G. AMPK—Sensing Energy while Talking to Other Signaling Pathways. *Cell Metabolism* **20**, 939-952, (2014).
17 Zhao, M., Qiao, M., Oyajobi, B. O., Mundy, G. R. & Chen, D. E3 ubiquitin ligase Smurf1 mediates core-binding factor alpha1/Runx2 degradation and plays a specific role in osteoblast differentiation. *The Journal of biological chemistry* **278**, 27939-27944, (2003).
18 Cunha, J. S., Ferreira, V. M., Maquigussa, E., Naves, M. A. & Boim, M. A. Effects of high glucose and high insulin concentrations on osteoblast function in vitro. *Cell and tissue research*, (2014).
19 Levinger, I., Brennan-Speranza, T. C., Jerums, G., Stepto, N. K., Serpiello, F. R., McConell, G. K. *et al.* The effect of hyperinsulinaemic-euglycaemic clamp and exercise on bone remodeling markers in obese men. *BoneKEy reports* **4**, 731, (2015).

Figure Legends

Figure 1: Mechanisms leading to glucose-induced osteoblast differentiation and bone formation. Glucose uptake via insulin-independent GLUT1 transporters in the preosteoblast inhibits the phosphoryltion of AMPK. Phosphorylation of AMPK activates the ubiquitin ligase, Smurf1, which in turn targets RUNX2 for proteasomal degradation. Thus glucose uptake via GLUT1 promotes RUNX2-mediated transcription of Glut1 itself and osteoblast differentiation markers including osteocalcin. AMPK activity also decreases mTORc1 activation. Glucose uptake via GLUT1 therefore also promotes mTORc1 activity, which leads to collagen synthesis bone and formation. (Ub: ubiquitin, ℗: phosphate).