TBC1D1 and TBC1D4 (also known as Akt Substrate of 160 kDa, AS160) are paralogue Rab GTPase activating proteins (Rab-GAPs) that have attracted a great deal of interest because they are implicated in the regulation of the trafficking of specialized GLUT4 glucose transporter storage vesicles that are found in adipose and muscle tissue. TBC1D4 was originally linked to the control of insulin-stimulated glucose transport on the basis of experiments using cultured adipocytes that expressed TBC1D4 with mutations to prevent phosphorylation on specific serine and threonine residues (Sano et al. 2003). These seminal experiments revealed that the ability to phosphorylate key Akt-phosphomotifs on TBC1D4 was important for controlling insulin-regulated GLUT4 translocation. These observations were extended by subsequent research that focused on the phosphorylation of TBC1D4 in cultured myocytes or rodent skeletal muscle. Expressing TBC1D4 that was mutated to prevent phosphorylation on specific serine or threonine residues resulted in attenuated insulin-stimulated glucose uptake by both cultured myocytes and rodent skeletal muscles.

Both TBC1D1 and TBC1D4 are expressed in human skeletal muscle, and each of these Rab-GAPs in human muscle can become phosphorylated on selected sites in response to elevated plasma insulin. The standard approach for assessing insulin’s regulation of TBC1D1 and TBC1D4 in human skeletal muscle has been to infuse insulin into the vasculature (e.g. during a euglycaemic–hyperinsulinaemic clamp). Accordingly, Treebak et al. (2014) also included an in vivo exercise component to their study to assess the influence of exercise with and without a meal-induced increase in plasma insulin levels. Participants performed a one-legged exercise protocol (with one leg resting while the other leg performed knee extensor exercise) in the fasted and meal-fed conditions. With this experimental design, the separate and combined effects of a physiological insulin level and in vivo exercise on TBC1D1 and TBC1D4 site-specific phosphorylation levels were determined for each participant.

Treebak et al. (2014) also included experiments using mice. Each of TBC1D1’s four phospho-sites that were studied exhibited greater phosphorylation after one-legged exercise in human vastus lateralis muscle. For mice that performed in vivo exercise (treadmill running), phosphorylation was increased for three of the four TBC1D1 phospho-sites in the extensor digitorum longus (EDL) muscle, but for none of the four sites in the soleus (phosphorylation decreased on two sites). TBC1D4 phosphorylation did not increase for either the EDL or soleus of mice with treadmill running on any of the five phospho-sites tested (phosphorylation decreased on several sites), whereas exercise resulted in greater phosphorylation on each of these sites in human vastus lateralis. Treebak et al. (2014) concluded that ‘transferring findings between species can be difficult’, but they also acknowledge that neither the intensity nor mode of the exercise was matched for humans versus mice. Earlier studies using humans have demonstrated that exercise effects on TBC1D4 phosphorylation depend on the intensity and mode of exercise, and the current results for mice revealed that effects of a given exercise protocol on TBC1D1 can differ between different muscles. What are the ‘real’ reasons for the distinct effects on TBC1D1 and TBC1D4 elicited by exercise in mice versus humans? Based on available data, it is unclear the extent to which differences in experimental designs (exercise protocols, muscles studied, etc.) contributed to the different results in mice versus humans. Regardless, Treebak et al. (2014) have provided a substantial amount of interesting and novel data with relevance to the regulation of TBC1D1 and TBC1D4 phosphorylation, and their study illustrates that the thoughtful use of multiple approaches along with careful interpretation of the results will be required to fully understand the regulation of these two ‘really’ important signalling proteins.

References

Let’s get real about the regulation of TBC1D1 and TBC1D4 phosphorylation in skeletal muscle
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This approach provides useful information with a great deal of experimental control, but a sustained and high level of plasma insulin does not replicate the ‘real’ condition during which dynamic changes in the rate of insulin secretion occur after the ingestion of a meal.

In this issue of The Journal of Physiology, Treebak et al. (2014) aimed to use a more physiologically relevant approach for increasing plasma insulin by probing the regulation of TBC1D1 and TBC1D4 site-specific phosphorylation in skeletal muscle (vastus lateralis) sampled from a group of men on two occasions: (1) after an overnight fast (characterized by low plasma insulin), and (2) after an overnight fast that was followed by eating a small carbohydrate-rich meal corresponding to about 5% of their daily caloric intake (leading to a modest and brief increase in plasma insulin).

A number of earlier studies demonstrated that ex vivo contractile activity of rodent skeletal muscles in the absence of insulin can lead to greater phosphorylation of each protein (Cartee & Wojtaszewski, 2007; Sakamoto & Holman, 2008; Cartee & Funai, 2009). Genetic and pharmacological approaches have implicated contraction effects on TBC1D1 and TBC1D4 as participating in the increased glucose transport in muscle during and shortly after contractile activity. Electrically stimulated contractions by isolated muscle preparations can provide valuable information about the direct effects of contractile activity, but this model does not perfectly mimic the more complex condition of ‘real’ in vivo exercise.

Earlier research has also demonstrated that in vivo exercise can result in greater phosphorylation of TBC1D1 and TBC1D4 in human muscle, but the previous studies were performed either with basal insulin levels or using a euglycaemic–hyperinsulinaemic clamp. Accordingly, Treebak et al. (2014) also included an in vivo exercise component to their study to assess the influence of exercise with and without a meal-induced increase in plasma insulin levels. Participants performed a one-legged exercise protocol (with one leg resting while the other leg performed knee extensor exercise) in the fasted and meal-fed conditions. With this experimental design, the separate and combined effects of a physiological insulin level and in vivo exercise on TBC1D1 and TBC1D4 site-specific phosphorylation levels were determined for each participant.
Additional information

Competing interests

None declared.