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JOURNAL CLUB

New insight into the mechanism by which acute physical exercise ameliorates insulin resistance

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Insulin resistance is a major metabolic defect leading to type 2 diabetes. The precise mechanisms involved in the onset of insulin resistance are yet to be fully elucidated. However, many studies have demonstrated that insulin signalling, which is responsible for activating glucose transporter translocation to the plasma membrane, becomes defective in its activation/transduction in the insulin-resistant state (Petersen & Shulman, 2006). Activation of the insulin signalling pathway involves post-translational phosphorylation of tyrosine (e.g. insulin receptor (IR) and insulin receptor substrate (IRS)) and serine/threonine (e.g. protein kinase B (Akt) and Akt substrate of 160 kDa (AS160)) residues, which induces alterations in kinase activity and translocation of intermediates in this pathway. S-Nitrosation, the addition of nitrosonium ion (NO^+) to cysteine residues resulting in the formation of a SNO group, can also regulate protein function, including kinase/phosphorylase activity in an analogous fashion to post-translational phosphorylation. Intriguingly in rodent models of insulin resistance, S-nitrosation of key insulin signalling intermediates including IR, IRS-1 and Akt has recently been shown to have an antagonistic effect on insulin signalling and may be a novel molecular mechanism of insulin resistance (Carvalho-Filho *et al.* 2005).

Physical exercise is a well-known treatment for type 2 diabetes as it improves insulin sensitivity in major insulin target tissues, predominantly skeletal muscle. The mechanisms underpinning the beneficial effects of exercise on insulin action are at present not fully resolved. In a recent report in *The Journal of Physiology*, Pauli *et al.* (2008) demonstrate that exercise may acutely improve insulin sensitivity in high-fat-fed rats, in part, by reversing

S-nitrosation of key insulin signalling intermediates in skeletal muscle.

Male Wistar rats fed a high-fat diet for 3 months exhibited decreased whole-body insulin sensitivity, as indicated by the glucose disappearance rate during an insulin tolerance test. A significant reduction in activating phosphorylation of IR, IRS-1 and Akt in gastrocnemius muscle was also observed in these animals. Rats were then subjected to an exercise protocol which involved two 3 h swimming sessions, separated by 45 min. Measurements of insulin action were conducted either 2 h or 16 h after the cessation of exercise. At both time points, animals displayed similar improvements in whole-body insulin sensitivity and this was associated with increased activating phosphorylation of insulin signalling intermediates. Notably the improvements in insulin signalling were beginning to diminish at 16 h post-exercise, perhaps due to the fact that the animals were returned to the high-fat diet. Interestingly, S-nitrosation of IR, IRS-1 and Akt displayed a tight inverse relationship with activating phosphorylation, being elevated in the high-fat control animals and being reduced at both time points post-exercise, although more substantially at 2 h when restoration of insulin signalling was greater. The alterations in S-nitrosation correlated with changes in the protein levels of inducible nitric oxide synthase (iNOS), which is considered to be the major NOS isoform mediating S-nitrosation of insulin signalling proteins (Carvalho-Filho *et al.* 2005). Whether additional mechanisms are also involved in modulating the levels of S-nitrosation in this acute time frame is currently unknown.

These initial experiments provided correlative evidence for a role of reduced S-nitrosation in the beneficial effects of exercise. To extend their findings, Pauli *et al.* conducted an elegant series of experiments in which they pretreated high-fat-fed rodents with either the NO donor S-nitrosoglutathione (GSNO) or the iNOS inhibitor L-N⁶-(l-iminoethyl)lysine (L-NIL) prior to the swimming exercise. Two hours post-exercise insulin sensitivity in GSNO-treated high-fat-fed animals was reduced to a similar level to high-fat-fed animals which did not undergo the exercise regime. This reduced insulin sensitivity was associated with reduced phosphorylation

and increased S-nitrosation of IR, IRS-1 and Akt. In the non-exercised group, treatment with GSNO did not significantly exacerbate the reduced insulin sensitivity and defective insulin signalling compared to non-GSNO-treated animals. Inhibition of iNOS activity (but not protein expression) with L-NIL, restored insulin sensitivity to a similar extent to swimming exercise in rodents fed a high-fat diet and this was associated with increased phosphorylation and decreased S-nitrosation of insulin signalling intermediates. Furthermore, there was no additive effect of iNOS inhibition with swimming exercise. Collectively these experiments suggest that alterations in S-nitrosation are important factors involved in diet and exercise-induced changes in insulin signalling and sensitivity.

Previous studies have shown that AMP-activated protein kinase (AMPK) is activated following exercise and may contribute to exercise-associated improvements in insulin sensitivity and glucose metabolism (Hawley & Lessard, 2008). Pauli *et al.* observed reduced activation of the AMPK pathway in high-fat-fed rats, which was restored to control levels following the exercise. Since AMPK can down-regulate iNOS expression, these findings suggest that in addition to its role to increase fatty acid oxidation and glucose uptake, increased AMPK activity may potentially contribute to exercise-induced improvements in insulin sensitivity by indirectly decreasing S-nitrosation of signalling intermediates in the insulin signalling pathway.

Insulin resistance is a multifactorial disorder and accordingly Pauli *et al.* investigated if alterations in other pathways, which have been linked to reduced insulin action, were also altered under their experimental conditions. The protein level of PTP-1B, which is the tyrosine phosphatase which dephosphorylates IRS-1, thereby attenuating signalling at this node of the insulin signalling cascade, was examined. PTP-1B protein expression was increased by the high-fat diet, remained elevated 2 h post-exercise in fat-fed rats and was not affected by GSNO or L-NIL treatment. Another prominent pathway which has recently been implicated as a major mediator of insulin resistance involves the c-Jun N-terminal kinase (JNK) (Hirosumi *et al.* 2002). Activation of this

inflammatory pathway has been linked with phosphorylation of IRS-1 at Ser-307 and a subsequent inhibition of its activating tyrosine phosphorylation, thereby limiting insulin signal transduction. In these experiments Pauli *et al.* observed increased p-JNK and Ser-307 phosphorylation of IRS-1 with high-fat feeding, but again these parameters were not restored 2 h post-exercise and did not change with the GSNO or L-NIL treatments. It should be noted that the authors of the present study do report elsewhere that 16 h after an acute exercise bout, some reductions are observed in the levels of PTP-1B, p-JNK and p-Ser-307 IRS-1 in skeletal muscle of high-fat-fed rats (Ropelle *et al.* 2006). However, collectively, the current findings support the notion that reductions in S-nitrosation of insulin signalling proteins contribute to the improved insulin sensitivity observed after an acute bout of exercise.

The study of Pauli *et al.* reveals a number of interesting findings concerning the role of exercise in counteracting high-fat diet-induced insulin resistance:

(1) It demonstrates that alterations in insulin signalling in muscle may be important in mediating the improved insulin sensitivity observed following acute exercise. Other studies have failed to see substantial differences in activation of the insulin signalling cascade following exercise (Hawley & Lessard, 2008), although the response to a single exercise session will be related to the intensity and duration of the stimulus, which in this study was considerable (i.e. 6 h of swimming exercise). Furthermore, another important methodological consideration is the nature of the insulin stimulus, with Pauli *et al.* providing the rats with a supra-maximal insulin dose through the portal vein, as

opposed to an intraperitoneal injection or *in vitro* stimulation.

(2) It provides additional evidence that S-nitrosation of insulin signalling intermediates can impinge upon activation of this pathway and hence insulin sensitivity. It should be noted that other targets that can be regulated by S-nitrosation include receptor tyrosine kinases, ion channels, metabolic enzymes, mitochondrial electron transport chain complexes and a number of transcription factors. Whether alterations in S-nitrosation of these other targets, or changes in other parameters of nitrosative stress also play a role in high-fat diet-induced insulin resistance requires investigation. A further consideration is that the data implicating S-nitrosation in insulin resistance has so far been generated in rodent studies only. As there are several metabolic differences between rodent and human skeletal muscle (e.g. substrate preference), an important extension to this research will be to determine if S-nitrosation of insulin signalling intermediates plays any role in insulin-resistant human skeletal muscle, particularly as signalling defects are not always apparent.

(3) It demonstrates that reductions in S-nitrosation of insulin signalling proteins are a major mechanism by which exercise acutely ameliorates insulin resistance. Whether the improved insulin sensitivity observed after chronic training also involves adaptations in this pathway (e.g. reduced steady-state iNOS expression) requires further investigation.

In summary Pauli *et al.* provide new insight into the mechanism by which acute exercise restores insulin sensitivity, highlighting an important role for S-nitrosation in the regulation of insulin signalling proteins in skeletal muscle. The challenge for future studies in this area is to determine whether

therapeutic interventions targeting this pathway may have potential in the treatment of insulin resistance and type 2 diabetes.

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