CROSSTALK

CrossTalk opposing view: Intramyocellular ceramide accumulation does not modulate insulin resistance

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The hypothesis that dysregulated lipid metabolism contributes to insulin resistance in muscle is now over a half-century old (Randle *et al.* 1963). Although early work implicated reciprocal allosteric regulation of glucose and fat oxidation as a mechanism for lipid-induced impairments in glucose metabolism (Randle *et al.* 1963), more recent studies propose that distinct lipid species with signalling properties directly interfere with the insulin signalling cascade to produce insulin resistance (Shulman, 2014). Of the many lipid metabolites proposed to cause insulin resistance, the ceramides have received particular attention.

Early studies demonstrating a relationship between intracellular ceramide levels and insulin resistance were performed in cell culture; the addition of palmitate or cell-permeant short-chain ceramides to the culture medium, often at supraphysiological concentrations, was repeatedly shown to impair insulin action (Summers *et al.* 1998; Zhou *et al.* 1998; Schmitz-Peiffer *et al.* 1999; Chavez & Summers, 2003). Rodent studies in which ceramide levels were modulated pharmacologically or genetically in various models of insulin resistance also demonstrated an inverse relationship between intramyocellular ceramide levels and insulin action, although the interventions often produced pleiotropic physiological effects and non-specific, potentially confounding effects on the lipidome (Holland *et al.* 2007; Bruce et al., 2012, 2013). Palmitate is thought to modulate intramyocellular ceramide levels by two major pathways. First, palmitate provides substrate for ceramide biosynthesis, the initial step of which is the condensation of serine and palmitate-derived palmitoyl-CoA. Palmitate also activates Toll-like receptor 4 signalling, which transcriptionally upregulates ceramide biosynthetic enzymes. Because unsaturated fatty acids (USFAs) have neither of these functions, preferential induction of insulin resistance by palmitate rather than USFAs would support a role for ceramides in insulin resistance. Indeed, palmitate is capable of impairing Akt S473 phosphorylation to a greater extent than USFAs. Importantly, however, when insulin action is evaluated functionally (e.g. glycogen synthesis or glucose transport), palmitate and USFAs induce insulin resistance to a similar extent (Schmitz-Peiffer *et al.* 1999; Chavez & Summers, 2003; Holland *et al.* 2007). USFA treatment does not increase ceramide content in myocytes, indicating that ceramides are not necessary for lipid-induced insulin resistance (Chavez & Summers, 2003).

Another major challenge for the ceramide-induced insulin resistance hypothesis concerns the molecular mechanism by which ceramides impair insulin signalling. Although there was early disagreement regarding the target(s) of ceramide-induced insulin resistance, accumulating evidence eventually implicated Akt, downstream of proximal insulin receptor signalling, as the culprit (Summers *et al.* 1998; Zhou *et al.* 1998; Schmitz-Peiffer *et al.* 1999). The mechanism for ceramide-induced Akt inhibition is proposed to involve both increased protein phosphatase 2A activity (Salinas *et al.* 2000; Schubert *et al.* 2000; Stratford *et al.* 2004) and impaired insulin-stimulated Akt translocation secondary to activation of atypical protein kinase Cζ (Powell *et al.* 2003; Stratford *et al.* 2004) (Fig. 1, left panel). If ceramides mediate obesity-associated intramyocellular insulin resistance by this mechanism, it would follow that proximal insulin signalling would be unaffected in insulin resistant muscle: all insulin signalling defects should lie downstream of Akt. Although defective Akt S473 phosphorylation is certainly observed in insulin resistant muscle (Adams *et al.* 2004), proximal insulin signalling is also impaired (Caro *et al.* 1987; Cusi *et al.* 2000). Specifically, skeletal muscle from obese insulin resistant humans displays impairments in insulin receptor tyrosine phosphorylation and activity, insulin receptor substrate-1 (IRS1) tyrosine phosphorylation, and IRS-associated phosphatidylinositol-3-kinase activity (Caro *et al.* 1987; Cusi *et al.* 2000) (Fig. 1, right panel). Defective proximal insulin signalling in insulin resistant muscle challenges the hypothesis that ceramide-induced Akt inhibition is a central defect in insulin resistance. In fact, a primary impairment of Akt phosphorylation might well enhance proximal insulin signalling owing to the loss of Akt-mediated negative

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DOI: 10.1113/JP271677

The Journal of Physiology

feedback mechanisms for insulin receptor signalling, such as stabilization of growth factor receptor-bound protein 10 (Hsu *et al.* 2011; Yu *et al.* 2011). Thus, ceramide signalling as currently understood cannot fully account for the commonly observed signalling defects in obesity-associated muscle insulin resistance.

As ceramides gained prominence as putative mediators of muscle insulin resistance, an increasing number of human studies determined skeletal muscle ceramide content in parallel with measures of whole-body glucose disposal or insulin sensitivity. Though not entirely unequivocal, available data suggest that changes in skeletal muscle insulin sensitivity and ceramide content can be dissociated under multiple conditions and in diverse patient populations. One well-cited study of the relationship between muscle ceramide levels and insulin sensitivity in humans compared lean and obese subjects using a hyperinsulinaemic–euglycaemic clamp, with muscle biopsies taken at baseline and during the infusion (Adams *et al.* 2004). As expected, obese subjects displayed reduced whole-body insulin sensitivity and, interestingly, elevated muscle ceramide levels at baseline (though not during the clamp). However, there was no significant association between muscle ceramide content and muscle insulin sensitivity (Adams *et al.* 2004). Several similar comparative studies examining the relationship between intramyocellular ceramide content and insulin sensitivity in humans sometimes report a negative relationship (Straczkowski *et al.* 2007; Coen *et al.* 2010; Amati *et al.* 2011), but just as frequently report no relationship at all (Serlie *et al.* 2007; Skovbro *et al.* 2008; Vistisen *et al.* 2008; Amati *et al.* 2011). Comparing these studies is difficult due to differences in study groups and the methods used for measuring insulin sensitivity and ceramide content, highlighting the need for interventional approaches or studies with randomized crossover design.

In one such study, older obese subjects were studied by hyperinsulinaemic–euglycaemic clamp and muscle biopsy before and after a 16-week dietary intervention that produced a 10% weight loss (Dubé *et al.* 2011). Despite marked improvements in insulin-stimulated glucose uptake, there was no effect of weight loss on intramyocellular ceramide levels (Dubé *et al.* 2011). Another study used a randomized cross-over approach in which men and women ate diets that were either palmitate-rich or oleate-rich (Kien *et al.* 2013). After 3 weeks on each diet, muscle biopsies were collected for lipid profiling and frequently sampled intravenous glucose tolerance tests were performed to assess insulin sensitivity. In men, the palmitate-rich diet increased muscle ceramide levels by > 20%, but did not affect insulin sensitivity (Kien *et al.* 2013). Interestingly, although insulin sensitivity was impaired in women fed the palmitate-rich diet compared with the oleate-rich diet, and a non-significant increase in muscle ceramide content during palmitate consumption in women was reported, there was no overall association between insulin sensitivity and intramyocellular ceramides (Kien *et al.*

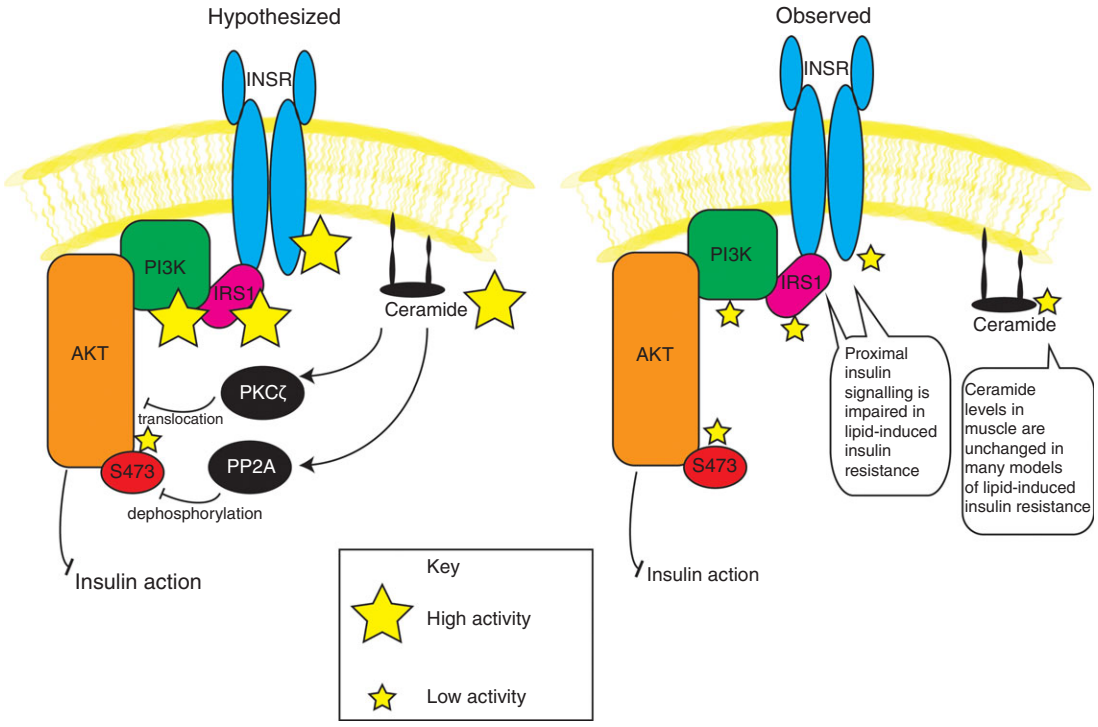

Figure 1. Hypothesized changes in intramyocellular ceramide content and insulin signalling do not reflect observed changes in either of these parameters
Left, the hypothesized increase in myocellular ceramide levels is proposed to inhibit Akt phosphorylation downstream of proximal insulin signalling. Right, changes in myocellular ceramide levels are frequently unchanged in insulin resistant muscle and proximal defects in insulin signalling are readily detected in lipid-induced insulin resistance. Abbreviations: INSR, insulin receptor; IRS1, insulin receptor substrate-1; PI3K, phosphoinositide 3-kinase.

2013). These data argue against a role for ceramide-induced insulin resistance in response to short-term changes in dietary lipids in man.

A common model of acute muscle insulin resistance employs intravenous lipid infusion to raise plasma fatty acids during a hyperinsulinaemic–euglycaemic clamp. Using this approach, several groups observed impaired insulin-stimulated glucose disposal with unchanged muscle ceramide levels in lean, overweight and obese men and women (Itani *et al.* 2002; Vistisen *et al.* 2008; Hoeks *et al.* 2012; Nowotny *et al.* 2013). Only one study, performed in lean male subjects, reported increased muscle ceramide content under similar conditions (Straczkowski *et al.* 2004). Additional acute interventions that reduced insulin-stimulated glucose uptake, including oral lipid challenge or intravenous lipopolysaccharide infusion, likewise produced no effect on muscle ceramide content (Nowotny *et al.* 2013). Finally, increasing and decreasing plasma fatty acid levels over 6 h in lean, overweight and obese subjects with a Intralipid infusion or a hypolipidaemic agent did not affect muscle ceramide levels (Serlie *et al.* 2007). Taken together, these studies demonstrate that acute lipid-induced insulin resistance occurs without concomitant changes in intramyocellular ceramide, dissociating the two phenomena.

Conclusion

Pharmacological doses of non-physiological, short-chain ceramides are sufficient to induce insulin resistance in cultured myocytes, and various genetic and pharmacological perturbations of ceramide metabolism, often accompanied by broad changes in lipid metabolism, can alter insulin sensitivity in rodents. However, the data implicating ceramide signalling in typical obesity-associated muscle insulin resistance in humans are weak. Some of the strongest evidence against a role for ceramides in mediating insulin resistance comes from human studies, in which changes in skeletal muscle ceramide levels are frequently dissociated from changes in insulin sensitivity. Further, the proposed mechanism for ceramide-induced insulin resistance – Akt inhibition – is inconsistent with the signalling abnormalities observed in insulin-resistant skeletal muscle. Overall, available data do not support a model in which increased myocellular ceramides are either necessary or sufficient for skeletal muscle insulin resistance.

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Additional information

Competing interests

None.

Author contributions

Both authors have approved the final version of the manuscript and agree to be accountable for all aspects of the work. All persons designated as authors qualify for authorship, and all those who qualify for authorship are listed.

Funding

This work was supported by NIH/NIDDK grant K01DK099402 (to M.J.J.). and F30DK104596 (to M.C.P.)