Circulating ceramide, inflammation and insulin resistance

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Obesity is associated with an increased risk of developing insulin resistance, a condition that precedes the development of type 2 diabetes. Although this relationship is well recognised, the mechanisms linking obesity to insulin resistance remain unresolved. One explanation is that obesity is associated with an 'oversupply' of lipids, which leads to the storage of lipids in tissues such as the liver and skeletal muscle that in turn cause insulin resistance. Another is that obesity is accompanied by low-grade inflammation that negatively impacts on insulin signal transduction. Ceramide accumulation in skeletal muscle is associated with the development of insulin resistance and pharmacological blockade of ceramide ameliorates lipid-induced insulin resistance in obesity (Holland *et al.*, 2007). Ceramides are also known to circulate in plasma, even though they are insoluble in aqueous environments. While plasma ceramides are increased in type 2 diabetes patients (Haus *et al.*, 2009), the mode of ceramide transport *in vivo* and the role of circulating ceramide in the development of insulin resistance and inflammation remains unresolved.

We first assessed ceramide levels in human plasma which was fractionated by fast performance liquid chromatography. The eluted fractions containing lipoproteins were identified by analysis of the ultraviolet absorption spectrum and confirmed by measuring cholesterol in each fraction. Ceramide was transported exclusively by very low density lipoproteins (VLDL, ~10%), low density lipoproteins (LDL, ~50%) and high density lipoproteins (HDL, ~40%). Total plasma ceramide content was increased in obese, type 2 diabetes (T2DM) patients compared with lean, insulin sensitive aged-matched individuals (Lean: 13.8 ± 1.1 *vs* T2DM: 17.4 ± 1.3 μM). Ceramides were higher in LDL (55%, $P = 0.006$) of T2DM, but not in VLDL ($P = 0.76$) or HDL ($P = 0.16$). LDL-ceramide was positively associated with insulin resistance (HOMA-IR), fasting insulin and glucose but not body mass index (a marker of adiposity), triglyceride or cholesterol. Thus, LDL-ceramide is associated with insulin resistance in humans.

To assess circulating ceramide function in cell culture, a novel approach was used to replicate the *in vivo* environment by creating LDL containing ceramides. This method depletes the LDL core of neutral lipids which allows for the introduction of ceramide into an intact LDL. LDL-ceramide (either C16:0 or C24:0 ceramide) mildly decreased insulin-stimulated glucose uptake in L6-GLUT4myc myotubes after 24 h treatment (~30%), whereas no effect was observed at 6 h. The reduction in insulin responsiveness was not associated with an impairment in Akt phosphorylation, suggesting no direct effect on distal insulin signalling. Interestingly, intracellular ceramide was increased with LDL-ceramide, although we are unable to provide definitive evidence for receptor-mediated uptake. LDL-ceramide activated pro-inflammatory signalling in RAW 264.7 macrophages, resulting in c-Jun terminal kinase activation and increased TNF-α secretion, but surprisingly, not IL-6 secretion . The conditioned media from LDL-ceramide treated macrophages decreased insulin-stimulated glucose uptake in L6-GLUT4myc myotubes, whereas no effect was observed with LDL conditioned media.

Overall, these results show that ceramides are elevated in the plasma of obese, type 2 diabetes patients and that this is related to insulin resistance, and not adiposity or generalised dyslipidemia. Furthermore, LDL-ceramide can cause insulin resistance in skeletal myotubes, albeit mildly, and activate pro-inflammatory signalling in macrophages, that in turn creates a milieu that decreases insulin sensitivity.

Haus JM, Kashyap SR, Kasumov T, Zhang R, Kelly KR, Defronzo RA & Kirwan JP. (2009). Plasma ceramides are elevated in obese subjects with type 2 diabetes and correlate with the severity of insulin resistance. *Diabetes* **58**, 337-343.

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