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Ceramides: a new player in the inflammation-insulin resistance paradigm?

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Ceramides are a family of lipid molecules comprising sphingosine bound to a fatty acid. They form an integral part of cell membranes and, according to cosmetics manufacturers, their inclusion in hair and skin care products can help you to maintain a 'youthful glow'. In addition to their simple structural role, it has become clear that ceramides also act as signalling molecules with multiple roles and, of interest to the diabetes field, a potential role for ceramides in the pathogenesis of insulin resistance has become evident over recent years. Increased concentrations of ceramides have been reported in skeletal muscle of obese insulin-resistant compared to lean insulin sensitive men [1] and strong correlations have been observed between muscle ceramide concentrations and insulin sensitivity across a wide cross-section of insulin sensitivity values [2,3]. However, this finding is not unequivocal with another study reporting no systematic differences in skeletal muscle ceramide concentrations between endurance trained, lean but untrained, obese and type 2 diabetic men, although a weak correlation between ceramide concentration and insulin resistance was observed [4]. Ceramides may provide a key link between lipid oversupply, inflammation and insulin resistance, as a major determinant of ceramide synthesis is the availability of long-chain saturated fatty acids [5] and, in addition, a number of inflammatory cytokines, particularly TNF-α and IL-1, have been implicated in the regulation of ceramide production [5,6]. *In vitro* studies indicate that ceramides inhibit insulin signalling in muscle cells, by decreasing insulin-stimulated activation of Akt/PKB [5,7], and by facilitating inflammatory signalling pathways known to impair insulin signalling [5,6]. Furthermore, studies in human muscle cells [8] and rodents [9] indicate that blocking ceramide synthesis can prevent the development of insulin resistance. Thus, there is accumulating evidence that ceramides in skeletal muscle may be causally implicated in the pathogenesis of insulin resistance.

In this issue of *Diabetologia*, de Mello and colleagues [10] report a strong correlation between total plasma ceramide (measured by liquid chromatography / mass spectrometry) and serum IL-6 concentrations in a cross-sectional study of 33 patients with cardiovascular disease. A modest, borderline significant correlation ($r = 0.33$, $p = 0.06$) between plasma ceramides and HOMA-estimated insulin resistance was also observed. Ceramide concentrations explained almost 35% of the variance in circulating IL-6 and this

relationship was independent of serum TNF-α and HOMA-IR in multivariate analysis. Somewhat surprisingly, a significant correlation between plasma ceramides and serum TNF-α was not observed, although this may be a consequence of low statistical power to detect a relationship due to the relatively small sample size and the relatively high co-efficient of variation in the TNF-α assay. The authors suggest that their findings may indicate a role for ceramides in the induction of inflammation associated with the insulin resistance that is often evident in patients with CHD, although of course whether inflammation is causally related to type 2 diabetes (or indeed CHD) remains to be fully established.

Nevertheless, these findings are in broad agreement with a report published earlier this year by Haus and co-workers, who reported strong correlations between plasma concentrations of ceramide sub-species and both clamp-derived insulin sensitivity and plasma TNF-α concentrations in adults with and without type 2 diabetes [11]. IL-6 was not measured in that study, so it is not known whether a relationship between circulating IL-6 and ceramides was evident, but nevertheless, two studies, in different populations, have now demonstrated relationships between circulating ceramides, inflammation and insulin resistance.

These findings raise a number of important issues and questions:

- Firstly, the de Mello *et al* study was cross-sectional in nature, so it is not possible to determine the direction of causality in the association between circulating IL-6 and ceramide concentrations. As inflammatory factors influence ceramide production and ceramides themselves induce inflammation, either direction of effect is potentially plausible. In this respect, it would be of interest to determine whether individuals with autoimmune conditions have raised plasma ceramide levels and, in particular, whether such levels are influenced by TNF-α and IL-6 blocking therapies. Similarly, it would be interesting to establish whether novel anti-inflammatory agents (e.g. anakinra, a IL-1 receptor antagonist [12]), demonstrated to improve glucose control in diabetes, induce parallel decreases in plasma ceramide concentrations.

- Secondly, the biological relevance of plasma ceramides is unknown. Are they directly implicated in the pathogenesis of insulin resistance, possibly via interactions with circulating immune cells? Or do they simply serve as a marker of tissue ceramide concentrations, which themselves play a direct role in pathogenesis? The relationship between plasma and tissue ceramide concentrations in humans is not currently known. Similarly, little is known about potential links between dietary and lifestyle factors and plasma ceramide concentrations. Further study to evaluate these issues is needed to put the interesting, but preliminary, findings from the present study into a broader context. Of course, should plasma ceramide concentrations adequately reflect tissue levels at key sites, this could accelerate studies examining their potential pathophysiological relevance to diabetes and related conditions.
- In addition, the importance of plasma ceramides in terms of clinical endpoints is not known. Prospective studies are needed to ascertain whether elevated plasma ceramides predict type 2 diabetes, coronary heart disease or mortality. Such data are required to evaluate whether plasma ceramides could conceivably be used an effective biomarker to predict risk or evaluate disease severity. However, caution is advised here for two reasons. Firstly, the methodology used for the determination of plasma ceramide concentrations is complex, and likely costly, and in its present form unlikely to be feasible for routine clinical practice. Secondly, a useful biomarker must add predictive power over and above factors currently measured routinely [13], and as yet few, if any, novel biomarkers have demonstrated a consistent ability to enhance risk prediction for either CVD or diabetes beyond established predictors. Indeed, even common markers of inflammation such as CRP, which are predictive of diabetes *per se,* do not to enhance risk prediction for diabetes beyond simple clinical predictors (i.e. fasting glucose, triglycerides, HDL cholesterol, blood pressure, BMI and family history) [14]. Thus, the benchmark for any new biomarker is set high.

Thus, the study of de Mello and colleagues [10], particularly when taken together with the earlier study from Haus et al [11] provide exciting preliminary clinical data on the emerging topic of ceramides, inflammation and insulin resistance. Nevertheless, considerable further work, as outlined above, is needed

to determine whether these recent findings offer new fruitful avenues in the investigation of diabetes pathogenesis.

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