AGEs induce alterations of sphingolipids metabolism in the liver of genetically- and diet-induced diabetic mice

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Background and aims: High plasma levels of the sphingolipid metabolism intermediates ceramide (Cer) and sphingosine-1-phosphate (S1P) have been found in obese and type 2 diabetic patients. Cer and S1P are reported to induce the inflammatory response leading to impaired insulin signaling in peripheral tissues. Emerging evidence suggests that accumulating Advanced Glycation End-products (AGEs) can alter the sphingolipids metabolism equilibrium. We, thus, investigated whether AGEs accumulation can affect the sphingolipids metabolism in animal models of insulin resistance.
Materials and methods: To study the direct contribution of AGEs to sphingolipids metabolism and the putative role of the AGE-receptor RAGE therein, HepG2 cells were incubated with 0.5 μM control-albumin and modified N^{ε}-carboxymethyllysine (CML)-albumin, pre-incubated or not with RAGE antibody. To study the role of AGEs on *in vivo* sphingolipid metabolism, C57Bl/6J (WT), and LeptrDb-/- (DbDb) mice were fed a standard diet (SD), while a group of C57Bl/6J was fed a 60% trans-fat diet (HFD). Two subgroups of SD and HFD mice were supplemented with the anti-AGEs compound pyridoxamine. AGEs levels were evaluated in the liver by LC-MSMS and Cer and S1P by GC-MS. The expressions of RAGE and of the enzymes involved in sphingolipid metabolism were assessed by RT-PCR and western blotting.
Results: High levels of AGEs and RAGE were detected in the liver of both DbDb and HFD mice in comparison to WT controls. Moreover, the expression of the sphingolipid metabolism enzymes ceramide synthase 2 and 5 and sphingosine kinase 1 was increased, while those of neutral ceramidase and S1P phosphatase was reduced, accompanied by increased levels of Cer and S1P. In addition, pyridoxamine supplementation to HFD mice diminished hepatic AGEs accumulation and prevented sphingolipids metabolism and insulin signaling alterations. In line, CML administration to HepG2 cells evoked alterations similar to those observed *in vivo*, and blocking antibodies against RAGE reverted some of the altered enzyme expressions.
Conclusion: The sphingolipid metabolism is affected in different models of diabetes. The modulation of the enzymes responsible for maintenance of the sphingolipid metabolism equilibrium in CML-incubated HepG2 cells indicate the direct involvement of AGEs and their receptor RAGE. The role of AGEs was confirmed by the *in vivo* action of pyridoxamine. These results suggest that the modulation of sphingolipids metabolism through the prevention of AGEs accumulation may reduce insulin resistance development.