CERAMIDES AND DIET-INDUCED INSULIN RESISTANCE IN RATS: NEW INSIGHTS FROM ESI-MS

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Sphingolipid metabolism, in particular the regulation of ceramide levels, appears to be linked with the development of insulin resistance. This is based on the findings that i) ceramide content is elevated in skeletal muscle of obese insulin-resistant humans,[1] ii) ceramides inhibit the insulin-stimulated protein kinase B pathway,[2] and iii) the insulin-sensitizing drug, troglitazone decreases ceramide content in rat skeletal muscle.[3] While this data provides a compelling argument there is little data describing if these effects are the result of increases in total ceramide levels or the influence of individual molecular species. Recent developments in the field of 'lipidomics', driven by HPLC and ESI-MS methodologies now provide researchers with the ability to rapidly identify and quantify these signaling lipids at a molecular level. In the current project fourteen male Sprague-Dawley rats were randomly divided into three groups. The first group was fed a standard rat chow, the second a diet high in saturated fat and the third a diet high in polyunsaturated omega 3 fat (fish oil). Total-body insulin sensitivity of each animal was assessed using a euglycemic euinsulinemic clamp. Animals were euthanised, the red gastrocnemius muscle removed and total lipids extracted. Lipid classes where then separated by normal phase HPLC and ceramides analysed by electrospray ionisation mass spectrometry on a quadrupole time-of-flight instrument equipped with a robotic nanoflow ion source. The animals on the high saturated fat, but not the high polyunsaturated fat diet had reduced insulin stimulated glucose uptake compared to the chow fed controls. Diet was also found to have an influence on several ceramides, in particular the animals on the fish oil diet had a higher percentage of ceramide-18:2 and lower percentage of ceramide-18:0 than the animals on the other diets ($P<0.05$). In addition, there was a decrease observed in the percentage of ceramide-19:0 in the animals fed a high saturated fat diet ($P<0.05$) that also correlates with insulin-stimulated glucose uptake ($r^2 = 0.72$, $P<0.05$). These findings indicate that diet can influence the profile of ceramides in rat skeletal muscle and that changes in specific ceramide molecular species may be associated with insulin action.

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