it is triggered by a complex relationship between multiple factors including genes and the environment. Here we used metabolomics combined with computational methods to identify factors that classified insulin resistance across individual mice derived from three different mouse strains fed two different diets. Three inbred ILSXISS strains were fed high fat or chow diets and subjected to metabolic phenotyping and metabolomics analysis of skeletal muscle. There was significant metabolic heterogeneity between strains, diet and individual animals. Distinct metabolites were changed with insulin resistance, diet and between strains. Computational analysis revealed 113 metabolites that were correlated with metabolic phenotypes. Using these 113 metabolites, combined with machine learning to segregate mice based on insulin sensitivity we identified C22:1-CoA, C2-carnitine and C16-ceramide as the best classifiers. Strikingly, when these three metabolites were combined into one signature, they classified mice based on insulin sensitivity more accurately than each metabolite on its own or other published metabolic signatures. Furthermore, C22:1-CoA, was 2.3-fold higher in insulin resistant mice and correlated significantly with insulin resistance. We have identified a metabolomic signature comprised of three functionally unrelated metabolites that accurately predicts whole body insulin sensitivity across three mouse strains. These data indicate the power of simultaneous analysis of individual, genetic and environmental variance in mice for identifying novel factors that accurately predict metabolic phenotypes like whole body insulin sensitivity.

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Diet and environment: some "Inconvenient Truths" about obesity-related insulin resistance

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Recent studies using different strains of mice have highlighted that genetic makeup has a significant impact on how animals respond to dietary interventions important for understanding the development of obesity, insulin resistance and type 2 diabetes in humans. However, variations in environmental conditions (diet, temperature, diurnal rhythm) can also have a major influence on the outcome, and interpretation, of experiments, although not necessarily in the way expected. Our recent investigations in chow and high fat fed mice have determined that housing temperature significantly alters metabolic rate as well as glucose and lipid metabolism in certain tissues, but this appears to have little impact on whole body glucose homeostasis and fat deposition. Obesity in humans is not always associated with reduced insulin action (Diabetologia 2013, 56:875). A similar dissociation between obesity and insulin resistance can also be demonstrated in mice made obese by feeding a high-starch diet compared to mice made obese by feeding a high-fat diet suggesting that insulin resistance is not a simple correlate of excess adipose tissue. Assessing the insulin signalling pathway and glucose uptake in muscle over the diurnal cycle of feeding and fasting revealed that the compensatory hyperinsulinemia seen in insulin resistant fat-fed rats resulted in similar phosphorylation levels of key insulin signalling proteins in muscle as chow-fed insulin sensitive animals. Despite this similar phosphorylation state, glucose uptake remained lower in muscle of fat-fed rats across the feeding period. These observations suggest that obesity-related insulin resistance is more complex than a simple relationship with excess fat deposition or reduced insulin signalling in insulin sensitive tissues. A more holistic rather than candidate approach may be required to fully understand all the mechanisms involved in this importance predictor of metabolic disease.

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The role of Ap2a2 in PPARα-mediated regulation of lipolysis in adipose tissue

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Adipose tissue plays a major role in the regulation of systemic metabolic homeostasis, and defects in insulin action and lipolysis have the capacity to impact systemic glycaemic control. In this respect, the AP2 adaptor complex plays an important role in clathrin-mediated endocytosis of various cell surface receptors in adipose tissue, including the glucose transporter GLUT4, the insulin receptor and beta-adrenergic receptors. The AP2 complex is a hetero-tetramer (α, β, μ and σ subunits), with the alpha-subunit being important for attachment of the AP2 complex to the membrane and for cargo internalization. The alpha-subunit exists in two isoforms, Ap2a1 and Ap2a2. Of interest, Ap2a2 (but not Ap2a1) has recently been identified as a peroxisome proliferator-activated receptor alpha (PPARα) target gene. The effects of PPARα on the AP2 adaptor complex and clathrin-mediated endocytosis in adipose tissue are not well described.

We have generated adipose tissue-specific Ap2a2 knockout mice and investigated metabolic alterations on a standard chow diet, in the presence of lipid overload (8 weeks high-fat feeding) as well as with dietary supplementation with the PPARα-agonist pirinixic acid (WY-14643). Supplementation with WY was required to drive expression of Ap2a2 in adipose tissue of wild-type mice and produce genotype-specific effects. Deletion of Ap2a2 led to minor improvements on systemic level, and did not affect basal or insulin-stimulated glucose uptake, or fatty acid metabolism. However, deletion of Ap2a2 had a substantial impact on beta-adrenergic activation of lipolysis. Adipose tissue of Ap2a2-KO mice lost its ability to respond to beta-adrenergic stimuli, as evidenced by a lack in increases in cAMP, PKA activation and glycerol release. These differences were not due to differential expression of beta-adrenergic receptors, but more likely to defects in AP2-mediated receptor endocytosis and recycling.

This study indicates a novel role for PPARα in beta-adrenergic regulation of lipolysis in adipose tissue.

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