Abstract

Increased lipid availability is proposed to reduce insulin sensitivity, a proposal which is primarily derived from studies in rodents and muscle cells, and from lipid infusion studies in humans. Though it is well documented that lipid infusion reduces whole-body insulin sensitivity by 30-60%, the responsible mechanisms that are induced by excess lipid availability remain elusive. There is, at the same time, evidence to suggest that eucaloric intake of high-fat diets for up to three weeks does not negatively regulate insulin sensitivity in healthy and non-obese humans. The adaptive mechanisms that are induced by high fat intake are still unexplored.

Compared to lipid infusion, a more physiological way to increase fatty acid (FA) availability is by dietary manipulation. Therefore, a short period of increased dietary FA intake was applied to investigate the impact on insulin sensitivity and molecular metabolism in skeletal muscle. The high-fat diet was primarily comprised of unsaturated FA. We demonstrated in lean, healthy and moderately trained men that three days' intake of 78 E% dietary fat coupled with 75% energy excess was sufficient to reduce whole-body insulin sensitivity by 17% and insulin-stimulated leg glucose uptake by 20%. The muscle content of *sn*-1,3 DAG was increased, while insulin signaling via Akt and TBC1D4 was unchanged, and the *sn*-1,3 DAG accumulation did not therefore seem to be involved in the obtained insulin resistance. Rather, decreased PDH-E1α protein content and increased inhibitory PDH-E1α Ser^{300} phosphorylation obtained concomitantly with increased post-absorptive FA oxidation are suggested mechanisms in handling the excess dietary FAs. In study I, a three-day hypercaloric (75%) diet with 80 E% carbohydrates was also provided. This diet increased whole-body insulin sensitivity by 41% and insulin-stimulated leg glucose uptake by 26%, concomitantly with decreased muscle PDH-E1α Ser^{300} phosphorylation. Together, the findings point towards a link between PDH-mediated pyruvate conversion to acetyl-CoA and insulin-stimulated glucose disposal in muscle.

In study II, the effect of six weeks' exposure to 64 E% high-fat diets enriched in either polyunsaturated (PUFA) or saturated FAs (SFA) were investigated under eucaloric conditions in healthy, slightly overweight and untrained men. The post-absorptive FA oxidation was increased after both high-fat diets, which seemed to be an important mechanism in the handling of the high

dietary FA load. The capacity for trans-sarcolemmal FA uptake into skeletal muscle was increased via an increased content of lipid binding proteins as FAT/CD36, FATP1 and FATP4 in skeletal muscle after both high-fat diets, potentially assisted by lower TBC1D1 protein content. The PDHE1α Ser^{300} phosphorylation was increased after both high-fat diets, as observed in study I, and the covalent inhibition of PDH-E1α thus seemed to be the regulatory point in the switch to FA oxidation. As indicated by a four-fold increase in plasma 3-hydroxybutyrate concentration after both PUFA and SFA, there was an increased hepatic beta-oxidation, and from the lower occurrence of C16:1n-7 in plasma, there was also a downregulation of hepatic *de novo* lipogenesis. Whole-body insulin sensitivity and insulin-stimulated leg glucose uptake were unchanged after both the PUFA and SFA intervention. This was observed despite the fact that that oxidative glucose disposal was decreased during the clamp. A compensatory increase in non-oxidative glucose disposal therefore seemed to explain the maintained insulin sensitivity, likely facilitated by a decreased muscle glycogen content after both high-fat diet interventions.

After both three days' hypercaloric high fat intake (study I) and six weeks' eucaloric high fat intake (study II), the basal hepatic glucose production was decreased. At the same time, the post-absorptive plasma TG concentration was markedly reduced after the high-fat diets in both study I and II. Several indices of improved hepatic metabolism and function were observed after the high-fat interventions. In contrast, the three-day hypercaloric carbohydrate-rich diet (study I) increased basal and insulin-stimulated glucose production, increased post-absorptive plasma TG concentration and the C16:1n-7 marker of *de novo* lipogenesis, pointing to adverse effect of carbohydrate excess in the liver. In study II, the human PUFA and SFA diets were fed to mice under eucaloric conditions, to enable analyses of molecular adaptations in the liver to high fat intake. Downregulation of proteins involved in gluconeogenesis and *de novo* lipogenesis confirmed the human observations after the six weeks high-fat diets.

The hypercaloric carbohydrate-rich diet was found to increase the post-absorptive plasma FGF21 concentration by eight-fold (paper III), while none of the high-fat diets significantly altered FGF21 levels. The carbohydrate-mediated increase in plasma FGF21 concentration was observed together with indices of inhibited subcutaneous adipose tissue lipolysis, and was found to

correlate with the increased glucose production, the increased *de novo* lipogenesis and an increased basal peripheral glucose disposal after the carbohydrate-rich diet. Thereby, the induction of FGF21 could be speculated to diminish further substrate excess in the liver.

Taken together, it seems that healthy non-obese humans well tolerate a high-fat content in the diet, due to several adaptations in substrate metabolism in skeletal muscle and in the liver. A period of high fat (and low carbohydrate) intake may furthermore change hepatic glucoregulation and metabolism in a non-diabetic direction. Insulin´s action on glucose uptake in skeletal muscle seems, however, to be impaired when a high fat intake was coupled with a high degree of energy surplus. The attenuation of insulin-stimulated glucose uptake during excess FA and energy intake was related to covalent PDH-E1α mediated downregulation of glucose oxidation. This substrate switch-induced lowering of glucose disposal did, presumably, reflect a sensible response to increased FA availability in skeletal muscle of non-obese healthy subjects, which can be considered an appropriate adaptation rather than a pathologic lipid-induced insulin resistance.

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