Activation of Snail via Reactive Oxygen Species Mediates Alcathelyde-Induced Disruption of Tight Junctions in CACO-2 Cell Monolayer

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Background: Exposure to alcathelyde is associated with intestinal barrier dysfunction and risk of malignant transformation in the gastro-intestinal tract. Epithelial-mesenchymal transition (EMT) is a developmental program shown to play a role in loss of epithelial integrity, cancer progression and metastases. EMT is induced by its transcription factor Snail, which downregulates E-cadherin expression. Recently, activation of EMT Snail has been found to be involved in alcoholic-induced intestinal permeability. As alcoholic drink is an even higher potency to induce barrier dysfunction and is mutagenic, we hypothesized that alcathelyde disrupts epithelial intestinal integrity by inducing oxidant-dependent Snail activation. Aims: To investigate the role of oxidative stress and activation of Snail in alcathelyde-induced intestinal barrier disruption in an in vitro model of intestinal permeability. Methods: Caco-2 monolayers were exposed from the luminal side to 25 μM alcathelyde +/- 100 μM L-cysteine to inhibit ROS generation. Intestinal epithelial permeability, localization and expression of ZO-1, occludin, E-cadherin, β-Catenin were examined using TEER and FITC-D4 fluxes, immunofluorescence and Western Blot analysis. ROS activation was assessed by ELISA and immunofluorescense. Involvement of Snail was further addressed by inhibiting Snail using small interfering RNA (siRNA): Results: Exposure to alcathelyde 25 μM significantly increased the paracellular permeability (60% decrease in TEER and 34% increase in FITC-D4 flux vs. medium only-treated controls, both P < 0.0001) in association with redistribution and decrease of tight junction (TJ) and adherens junction (AJ) protein levels. Alcathelyde increased ROS generation by 40% and Snail phosphorylation by 30% (both P < 0.001 vs. medium only-treated controls). These effects were attenuated by L-Cysteine (P < 0.0001 vs. alcathelyde only-treated monolayers). Knockdown of Snail by siRNA significantly attenuated alcathelyde-induced changes and decrease in TJ and AJ proteins, improved TEER and decreased FITC-D4 permeation (all P < 0.05 vs. non-specific Snail-transfected cells). Conclusions: Our data demonstrate that oxidative stress-mediated Snail phosphorylation is likely a novel mechanism by which alcathelyde affects cell junction function. Identification of mechanisms involved in alcathelyde-induced barrier dysfunction may provide new therapeutic targets for prevention of alcohol-related gut leakiness with subsequent development of liver disease and colon carcinogenesis.

Tu1747

MRNAs for Ucp1 and PRDM16 Are Detectable in Visceral Adipose of Obese Subjects Indicating Therapeutic Trans-Differentiation Potential

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Background and Aim: Several lines of research have suggested brown adipose tissue (BAT) that may play a role in diet induced obesity. Additionally, we profiled the levels of KCNRG which encodes a potassium current suppressor and PRDM16 genes within the visceral adipose tissue depots in a cohort of obese individuals. Methods: Obese patients undergoing bariatric surgery with clinical data, serum and adipose tissue samples were included. Visceral adipose tissue was collected at the time of bariatric surgery and snap frozen in liquid nitrogen. RNA was extracted from frozen adipose samples using the AURUM Total RNA Fatty and Fibrous tissue Kits (Biorad) and cDNA was synthesized from 1 μg of the total RNA using RT2 first strand kit (QIAGEN). The expression level of KCNRG isoform A (r=0.636, p=0.026) and PRDM16 genes within the visceral adipose tissue depots in a cohort of obese individuals. Results: The expression level of KCNRG isoform A (r=0.636, p=0.026) was increased in the visceral adipose tissue depots in a cohort of obese individuals. The expression level of PRDM16 genes within the visceral adipose tissue depots in a cohort of obese individuals. Conclusion: The data indicates that KCNRG and PRDM16 genes within the visceral adipose tissue depots in a cohort of obese individuals. This is of interest since these genes potentially have a potential for trans-differentiation into brown adipocytes. Further studies in larger cohorts is warranted to better elucidate this possibility.

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Inflammation and Oxidative Stress in the Small Intestine Are Associated With Insulin Resistance and Alterations in Lipid Homeostasis in Obese Subjects

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Intestinal chronic inflammation and oxidative stress are commonly associated with inflammatory bowel diseases. However, much less is known about their contribution to additional disorders such as metabolic syndrome and diabetic dyslipidemia. This is of interest since chronic low-grade inflammation and oxidative stress have been previously shown to contribute to the development of insulin resistance and several metabolic alterations in the liver, muscle and adipose tissue. Objective: To reveal the presence of inflammation and oxidative stress markers in small intestine and adipose tissue of obese subjects. Methods: Small intestine and adipose tissue of obese subjects was obtained by intestinal endoscopy to obtain mucosal biopsies from the duodenum at baseline and 30 min after a duodenal glucose infusion. Leucocytes were isolated from plasma (TRIT2) and glucose transporter (SGLT-1 and GLUT2) transcript were quantified by qPCR. Intestinal glucose absorption reduced intestinal expression of SGLT-1 by 26% (P<0.01) in the morbidly obese but not in healthy subjects. In contrast, intestinal expression of T1R2 and GLUT2 was increased by acute glucose infusion in both groups. Glucose infusion reduced intestinal expression of SGLT-1 by 26% (P<0.01) in the morbidly obese but not in healthy subjects. In contrast, intestinal expression of T1R2 and GLUT2 was increased by acute glucose infusion in both groups. Glucose infusion reduced intestinal expression of SGLT-1 by 26% (P<0.01) in the morbidly obese but not in healthy subjects. In contrast, intestinal expression of T1R2 and GLUT2 was increased by acute glucose infusion in both groups. Glucose infusion reduced intestinal expression of SGLT-1 by 26% (P<0.01) in the morbidly obese but not in healthy subjects. In contrast, intestinal expression of T1R2 and GLUT2 was increased by acute glucose infusion in both groups. Glucose infusion reduced intestinal expression of SGLT-1 by 26% (P<0.01) in the morbidly obese but not in healthy subjects. In contrast, intestinal expression of T1R2 and GLUT2 was increased by acute glucose infusion in both groups. Glucose infusion reduced intestinal expression of SGLT-1 by 26% (P<0.01) in the morbidly obese but not in healthy subjects. In contrast, intestinal expression of T1R2 and GLUT2 was increased by acute glucose infusion in both groups. Glucose infusion reduced intestinal expression of SGLT-1 by 26% (P<0.01) in the morbidly obese but not in healthy subjects. In contrast, intestinal expression of T1R2 and GLUT2 was increased by acute glucose infusion in both groups. Glucose infusion reduced intestinal expression of SGLT-1 by 26% (P<0.01) in the morbidly obese but not in healthy subjects. In contrast, intestinal expression of T1R2 and GLUT2 was increased by acute glucose infusion in both groups. Glucose infusion reduced intestinal expression of SGLT-1 by 26% (P<0.01) in the morbidly obese but not in healthy subjects. In contrast, intestinal expression of T1R2 and GLUT2 was increased by acute glucose infusion in both groups. Glucose infusion reduced intestinal expression of SGLT-1 by 26% (P<0.01) in the morbidly obese but not in healthy subjects. In contrast, intestinal expression of T1R2 and GLUT2 was increased by acute glucose infusion in both groups. Glucose infusion reduced intestinal expression of SGLT-1 by 26% (P<0.01) in the morbidly obese but not in healthy subjects. In contrast, intestinal expression of T1R2 and GLUT2 was increased by acute glucose infusion in both groups. Glucose infusion reduced intestinal expression of SGLT-1 by 26% (P<0.01) in the morbidly obese but not in healthy subjects. In contrast, intestinal expression of T1R2 and GLUT2 was increased by acute glucose infusion in both groups. Glucose infusion reduced intestinal expression of SGLT-1 by 26% (P<0.01) in the morbidly obese but not in healthy subjects. In contrast, intestinal expression of T1R2 and GLUT2 was increased by acute glucose infusion in both groups. Glucose infusion reduced intestinal expression of SGLT-1 by 26% (P<0.01) in the morbidly obese but not in healthy subjects. In contrast, intestinal expression of T1R2 and GLUT2 was increased by acute glucose infusion in both groups. Glucose infusion reduced intestinal expression of SGLT-1 by 26% (P<0.01) in the morbidly obese but not in healthy subjects. In contrast, intestinal expression of T1R2 and GLUT2 was increased by acute glucose infusion in both groups. Glucose infusion reduced intestinal expression of SGLT-1 by 26% (P<0.01) in the morbidly obese but not in healthy subjects. In contrast, intestinal expression of T1R2 and GLUT2 was increased by acute glucose infusion in both groups. Glucose infusion reduced intestinal expression of SGLT-1 by 26% (P<0.01) in the morbidly obese but not in healthy subjects. In contrast, intestinal expression of T1R2 and GLUT2 was increased by acute glucose infusion in both groups.