Role of NPC1L1 and cell cholesterol in adipocyte differentiation and cytokine secretion

Trevor Whitwell, David Hui, Philip Howles

Background – NPC1L1 (Niemann-Pick C1-like 1) is a protein reported to be involved in cholesterol transport across the plasma membrane of cells. It is highly expressed in intestinal enterocytes and has a major role in the absorption of dietary cholesterol. Zetia (ezetimibe) is a cholesterol lowering drug which blocks cholesterol absorption by direct and/or indirect inhibition of NPC1L1 function. In addition to reduced cholesterol absorption, it has been shown that Zetia-treated mice and NPC1L1 knockout mice are resistant to diet induced obesity, and also that dietary fat metabolism seems altered (3). The role of cholesterol and NPC1L1 in adipocyte differentiation and metabolism is not known. It is not known whether the effects of NPC1L1 and Zetia are the result of decreased intracellular cholesterol or altered cholesterol/lipid uptake and transportation. Also, the effects of NCP1L1 and Zetia on cytokine production by adipocytes has not been studied.
Hypothesis – Adipocyte differentiation under condition of low cellular cholesterol will result in mature adipocytes that have altered lipid metabolism and that secret inflammatory cytokines.
Methods – Primary mature adipocytes were cultured from knockout and wildtype mice from three different fat pads. Mature adipocytes were treated with radiolabeled oleic acid (3H) and glucose(14C) with or without zetia. To measure effects on differentiation, 3T3L1 cells were cultured under five combinations of differentiation media, statin, or zetia treatment. Triglyceride and total cholesterol content of 3T3L1 cells and primary adipocytes were measured via colormetric kits. ELISA kits were used to measure cytokines, MCP-1, IL-6 and adiponectin secretion by 3T3L1 cells and adipocytes.
Results – No significant differences were found in radiolabeled oleate and glucose uptake in any mature adipocyte groups. Cholesterol/triglyceride ratios in knockout and wildtype mature adipocytes were statistically different in all fat pads with retroperitoneal having a higher ratio in wildtypes ($p = 2.59E-06$) while subcutaneous and ovarian ratios were higher in knockouts ($p=0.015$ and 0.0009 respectively). Cholesterol/Triglyceride ratio in zetia treated wildtype ovarian adipocytes were lower than control treated cells ($p=.0304$). In 3T3L1 cultured cells, there was significantly higher cholesterol and triglyceride content in zetia differentiated cells than statin treated cells. MCP-1 production was increased in zetia differentiated cells when compared to standard differentiated cells ($p = 0.00037$)
Conclusions – Comparing a pooling of all fat pads of knockout vs. wildtype did not produce significant differences. However, when comparing individual fat pads there were significant differences between knockout and wildtype groups. The differences between cholesterol/triglyceride ratio in the different fat pads suggest that each fat pad behaves differently to altered cholesterol availability. This data suggests the ovarian and retroperitoneal fat are more suspetable to lipid droplet changes due to altered cholesterol than subcutaneous fat. Treating preadipocytes with zetia promotes differentiation while statin treatments prevent differentiation. Also, zetia-induced differentiation increased the production of the pro-inflammatory cytokine MCP-1, which is characteristic of inflammatory visceral fat. Future research should be directed to determine the specific responses of different fat pads to altered cholesterol availability as well as the specific role of cholesterol in the differentiation of preadipocytes.

References

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