The effects of ketones bodies on human adipocytes in vitro, a preliminary implication on ketogenic diet

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Background

Ketogenic or low-carbohydrate and high fat diet (LCHF) has increasingly gained recognition as an alternative to the conventional low-fat diet (LFD) in achieving effective weight loss and managing obesity. Ketone bodies are vital alternative metabolic fuel source during fasting, starvation and adherence to low carbohydrate diets; hence the use of ingestible ketone body precursors to increase and maintain ketosis is an alternative to ketogenic diets. White adipose tissue (WAT) browning is hypothesized to confer numerous metabolic benefits by decreasing adiposity and increasing energy expenditure. The mechanisms on how the exogenous ketone bodies contribute to the overall lipid utilization and metabolism in the fat cells and specifically WAT browning remained uncertain.

Objectives

- To determine the effects of ketone bodies (beta-hydroxybutyrate and acetoacetate) on WAT browning markers in differentiated adipocytes
- To determine the lipolysis effects of ketone bodies in differentiated adipocytes.

Methods

- Human primary human white preadipocytes (PromoCell, Germany) were first maintained in preadipocyte growth medium (PromoCell, Germany) and subsequently differentiated to adipocytes.
- Oil Red O staining was carried out prior to cell treatment.
- Adipocytes were treated with beta-hydroxybutyrate, acetoacetate (ketone bodies) and control vehicle for 24h.
- Total RNA were isolated from treated cells using QIAshredder™ and RNeasy® Mini Kit (Qiagen, Germany) according to the manufacturer’s protocol.
- RT-qPCR reactions were performed using iScript™ one-step RT-PCR kit with SYBR® Green (Bio-Rad Laboratories, USA) in CFX96™ Real Time PCR detection system (Bio-Rad Laboratories, USA) using optimized conditions.
- Total cellular protein was isolated using ProteoJET™ mammalian cell lysis reagent (Fermentas, Canada) separated by SDS-PAGE and electrophoretically transferred to the PVDF membrane and probed with the indicated primary antibodies (anti-UCP-1; anti-PDRM16; at optimized dilution ratio) using standard procedures.
- Free fatty acid (FFA) were quantified after 3 h of ketone bodies treatment using Cultured Human Adipocyte Lipolysis Assay Kit for Non-Esterified Fatty Acids Detection (Zen-Bio, USA) according to manufacturer’s instructions.
- All experiments were carried out in three independent experiments and data were represented as mean ± SD of three independent experiments.

Results

A. Pre-adipocytes stained with oil red O stain showing lack of oil red O stain in these cells (Representative image; Magnification 200X)
B. Fully differentiated adipocytes stained with oil red O stain showing highly concentrated staining indicating an increased content of lipid (Representative image; Magnification 200X)

There were no significant differences in the mRNA expression of the WAT browning markers in undifferentiated preadipocytes and differentiated adipocytes.

WAT browning marker such as UCP-1 and PDRM16 mRNA were expressed significantly in the adipocytes when treated with either 10 µM or 100 µM beta-hydroxybutyrate as compared with control. * p<0.01

The relative densitometric protein expression of UCP-1 and PDRM16 correlated with the mRNA expression

Beta-hydroxybutyrate did not significantly affect the lipolysis of adipocytes at all concentrations. However, acetoacetate significantly induces FFA release at concentrations 1 and 10 µM * p<0.01

Conclusion

- Beta-hydroxybutyrate upregulates WAT browning markers such as UCP-1 and PDRM16
- Acetoacetate increases FFA release of adipocytes
- Ketone bodies appears to promote WAT browning and lipolysis, hence may suggest possible enhancement of energy expenditure and breaking down of fats

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