

Effects of Fatty Acids from Avocados on Expression of the HMG1 Gene in *Tetrahymena thermophila*

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Experiment Type: Reverse transcriptase (RT) PCR
Feature: TTHERM.00691180
Gene: HMG1: Putative Hydroxymethylglutaryl-CoA reductase

1 Introduction

The enzyme 3-Hydroxy-3-Methyl-Glutaryl-CoA Reductase (HMG) HMG catalyzes the rate-limiting step in the biosynthesis of cholesterol and is a common drug target for cholesterol reduction. Previous studies in rats have shown that avocado oil reduces cholesterol levels. We were interested in determining whether the healthy fatty acids in avocado oil would have an effect on the expression of HMG.

In this study, *Tetrahymena thermophila* were treated with avocado oil to determine the effect of fatty acids on expression of the HMG1 gene that encodes HMG. Expression of HMG1 was monitored using reverse transcription and semi-quantitative PCR. We predicted that expression of HMG1 would be down regulated in *Tetrahymena* cultures that were treated with avocado oil.

2 Methods

Primer synthesis: Primers for HMG1 were designed using the *Tetrahymena* Genome Database and Integrated DNA Technologies Oligoanalyzer. The sequences of the forward and reverse primers used to amplify HMG1 are as follows: TGGTTCCATGGTTGCTGATGT (Forward) and CTAGCAAGCTGTTTTGGTAGGCTT (Reverse). The sequences of the control Btu1 primers are as follows: CCCAGAGCTATCTTGATGGACTTA (Forward) and TAACACCAGACATGGCA (Reverse).

Culturing Tetrahymena: *T. thermophila* were cultured in NEFF media and then transferred into nutrient-rich SSP media at the time of the experiment (Cassidy-Handley, 2012). For the experiment, control Tetrahymena cultures were maintained for 24 hours in SSP media supplemented with 5% vegetable oil, while experimental cultures were maintained in SSP media supplemented with 5% avocado oil.

RNA extraction: RNA was extracted from control and experimental *T. thermophila* cultures following the experiment using Qiagen's RNeasy Mini Kit as per the manufacturer's instructions. All cultures were centrifuged for three minutes at 3,000rpm and washed with 5mL of 10mM Tris (pH 7.4) before processing.

RT-PCR: cDNA was synthesized using the RevertAid RT kit (ThermoScientific) and following the manufacturer's protocol. PCR was performed using GoTaq Green PCR Master Mix (Promega) using the manufacturer's protocol. PCR amplification of BTU1 cDNA was used as a positive control for the Tetrahymena RT-PCRs while Gapdh was used as a positive control for the entire RT-PCR experiment as the reagents for this sample were provided in the RevertAid kit. The No Template Control (NTC) reactions contained all reagents for the RT-PCR reaction, except nuclease-free water was added in place of RNA template.

Gel electrophoresis: The RT-PCR reactions were electrophoresed on a 1% agarose gel (BioRad) in 1xTBE (VWR). The ImageJ gel analysis program was used to determine the relative intensities of the PCR products for semi-quantitative analysis.

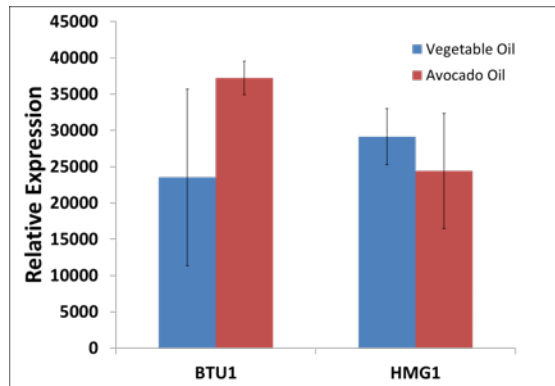
3 Results

The semi-quantitative RT-PCR results demonstrate that exposure of Tetrahymena cells to avocado oil over the course of 24 hours had no significant effect on the expression of HMG1 compared to control cells, as indicated in Figure 1 ($p = 0.348$).

Future studies should focus on varying the concentrations of oils used to supplement the Tetrahymena growth media, as well as increasing the number of replicates. Furthermore, a more quantitative measure of gene expression should be used to better determine whether exposure to avocado oil affects HMG1 expression.

4 Figures

4.1 Relative Expression of HMG1 in *Tetrahymena thermophila* Cultures Supplemented with Avocado Oil



The relative expression of HMG1 compared to the control gene BTU1 was measured using semi-quantitative RT-PCR across four control (vegetable oil) and four experimental (avocado oil) *Tetrahymena* cultures after 24 hours. The error bars represent the standard error of the means for each condition. A two-tailed t-test assuming unequal variance was performed to determine the significance of changes in gene expression between the control and experimental cultures, with $p=0.348$ for HMG1 and $p=0.629$ for BTU1.

5 References

Cassidy-Hanley DM. *Tetrahymena* in the laboratory: strain resources, methods for culture, maintenance, and storage. *Methods Cell Biol.* 2012;109: 237-76. doi: 10.1016/B978-0-12-385967-9.00008-6.

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