Increase in Triglyceride and G3PDH Assay

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Tradename: AC Southernwood Plump BG PF

Code: 20419PF

CAS #: 107-88-0 & 89957-58-4 & 7732-18-5

Test Request Form #: 2202

Lot #: NC160308-A

Sponsor: Active Concepts, LLC; 107 Technology Drive Lincolnton, NC 28092
Study Director: *Maureen Danaher*
Principle Investigator: *Jennifer Goodman*

Introduction

The purpose of this study is to determine the effect of **AC Southernwood Plump BG PF** on triglyceride and G3PDH synthesis. Triglycerides are among the primary components of adipose tissue. G3PDH (glycerol-3-phosphate dehydrogenase) is an enzyme that is involved in the storage of fat.

Materials

A. Kit:	Triglyceride Colorimeter Assay Kit (Cayman Chemical, 10010303) G3PDH Colorimeter Assay Kit (Abcam, 174095)
B. Incubation Conditions:	37°C at 5% CO_2 and 95% relative humidity (RH)
C. Equipment:	Forma humidified incubator; ESCO biosafety laminar flow hood; Synergy H1 Microplate reader; Pipettes
D. Cell Line:	Normal Human Primary Subcutaneous Pre-adipocytes (ATCC-PCS-210-010)
E. Media/Buffers:	Dulbecco's Modified Eagle Medium (DMEM); Penicillin-Streptomycin (50U-50mg/mL); Fetal Bovine Serum (FBS); Phosphate Buffered Saline (PBS); Triglyceride Kit: Sodium Phosphate Assay Buffer (50mM) G3PDH Kit: GPDH Assay Buffer;
F. Culture Plate:	Falcon Flat Bottom 96-Well Tissue Culture Treated Plates
G. Reagents:	Triglyceride Kit: Standard Diluent Assay Reagent (5X); Triglyceride Standard; G3PDH Kit: GPDH Positive Control; GPDH Probe; GPDH Substrate; NADH Standard
H. Other:	Sterile Disposable Pipette Tips

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Methods

Normal Human Primary Subcutaneous Pre-adipocytes were seeded into 96-well tissue culture plates and allowed to grow to confluency in complete DMEM. A 0.5% concentration of **AC Southernwood Plump BG PF** was added to complete DMEM and incubated for 72 hours.

For the triglyceride assay, a standard ladder was prepared through the process of serial dilution in concentrations ranging from 200mg/dL to 0mg/dL. 10 µL of the Triglyceride Standard in its varying concentrations and 10 µL of each sample were added to each respective standard or sample well. 150 µL of the diluted Enzyme Buffer solution was added to each well. The plate was gently shaken, covered, and allowed to incubate for 15 minutes at room temperature (25°C). Absorbance was read from 530-550 nm using the microplate reader. A standard curve was created by reducing the data and generating a linear curve fit. The standard ladder and each sample were run in duplicates and the average was taken.

For the G3PDH assay, a standard ladder was prepared in concentrations ranging from 12.5nmol/well to 0nmol/well. 50µL of each standard dilution, positive control, and samples were added to each respective well. 50 µL of the Reaction Mix containing GPDH Assay Buffer were added to each well. 50 µL of Background Control Mix was added to 50 µL of Background Control wells. Each well was mixed and allowed to incubate for 20-60 minutes at 37°C. Absorbance was read at 450nm. A standard curve was created by reducing the data and generating a linear curve fit. The standard ladder and each sample were run in duplicate and the average was taken.

Results

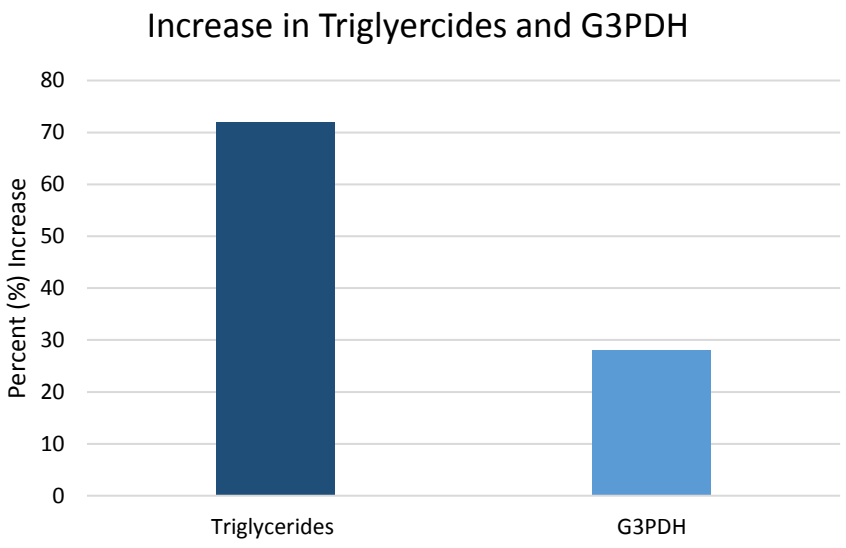

Figure 1. Percent increase in triglycerides when using **AC Southernwood Plump BG PF.**

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Discussion

The results indicate the **AC Southernwood Plump BG PF** is capable of increasing triglycerides by 72%, while also increasing G3PDH synthesis by 28%. These findings suggest that **AC Southernwood Plump BG PF** may be effective at increasing the synthesis and storage of adipose tissue.