IL-6 ELISA Analysis

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Tradename: AC Yogurt Hydrolysate SF

Code: 20531

CAS #: 9015-54-7

Test Request Form #: 5633

Lot #: 69421P

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

Test Performed:
Interleukin (IL)-6 Enzyme-Linked Immunosorbent Assay (ELISA)

Introduction
Interleukin-6 is a proinflammatory cytokine known to play an active role in inflammation, immunology, bone metabolism, reproduction, arthritis, neoplasia, and aging. IL-6 signals through the nuclear factor-kappa B (NF-κB) pathway that results in the transcription of inflammatory mediators, including matrix metalloproteinase-1 (MMP-1). MMP's are responsible for breaking down the extracellular matrix and collagen in the skin leading to wrinkles, fine lines, and loss of skin elasticity. Reducing the level of IL-6 and other inflammatory mediators is believed to slow down degradation of the skin matrix and, possibly, stimulate its replenishment.

Accordingly, an interleukin-6 ELISA was conducted to assess the in vitro effect of **AC Yogurt Hydrolysate SF** to elicit changes in IL-6 levels in dermal fibroblasts.

Assay Principle
This ELISA utilizes a colorimetric reaction employing antibodies with antigen specificity to human IL-6. Monoclonal antibodies specific for IL-6 epitopes are coated on a microtiter plate. In positive samples, IL-6 will bind to these antibodies and are tagged a second time with another IL-6-specific antibody labeled with horseradish peroxidase (HRP). The addition of the chromagen solution, containing 3,3',5,5'-tetramethylbenzidine, provides the colorimetric reaction with HRP that is quantitated through optical density (OD) readings on a microplate spectrometer. The standard curve provides a reference from the OD readings for the amount of IL-6 in each sample.

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Materials

A. Kit:	Human IL-6 ELISA Kit (Invitrogen; BMS213-2)*
B. Incubation Conditions:	37°C, 5% CO_2, and 95% relative humidity (RH)
C. Equipment:	Forma humidified incubator; ESCO biosafety laminar flow hood; Synergy HT Microplate reader; Pipettes; Light microscope
D. Cell Line:	Normal Human Dermal Fibroblasts (ATCC; PCS-201-012)*
E. Media/Buffers:	Fibroblast Basal Medium (PCS-201-030)*; Fibroblast Growth Kit (PCS-201-041)*; Phosphate Buffered Saline (PBS)
F. Reagents:	Lipopolysaccharide (LPS) (1 µg/mL); Dexamethasone (DEX) (10 µM)
G. Culture Plate:	12 Well Tissue Culture Treated Plates
H. Other:	Sterile disposable pipette tips

*Or suitable alternatives, subject to change without notice based off vendor availability

Methods

Human dermal fibroblasts were seeded into 12-well tissue culture plates and allowed to grow to confluency in complete media (CM). 0.01%, 0.1%, and 1.0% concentrations of **AC Yogurt Hydrolysate SF** were added to CM containing 1 µg/mL LPS and incubated with fibroblasts for 72 hours. LPS is utilized to create an inflammatory environment and dexamethasone (DEX) in the presence of LPS was used as a positive control to quell inflammation.

Standards were prepared in concentrations ranging from 0 pg/mL to 200 pg/mL. Prior to samples being added all wells were washed with 1X wash buffer four times. 50 µL of assay buffer was added to the sample and assay control wells. 50 µL of the controls and samples were added to appropriate wells. 100 µL of each standard concentration was added to the appropriate well. 50 µL of Biotin-conjugate were added to all wells. After a two-hour incubation at room temperature and washing, 100 µL Streptavidin-HRP was added to all wells. Following a one hour incubation and washing, 100 µL of TMB substrate solution was added for the colorimetric reaction. After a 10 minute incubation in the dark, 100 µL of stop solution was added to stop the reaction. The optical density (OD) was read at 450 nm on the Synergy HT Microplate Reader.

A standard curve was created by reducing the data and generating a linear curve fit. The IL-6 concentration of **AC Yogurt Hydrolysate SF** treated-fibroblasts was determined by extrapolation from the standard curve and expressed in pg/mL. Percent change is expressed relative to CM + LPS and calculated by the following equation:

$$Percent\ Change\ (\%) = \frac{IL\ 6\ Concentration_{Sample} - IL\ 6\ Concentration_{1\ \mu g/mL\ LPS}}{IL\ 6\ Concentration_{1\ \mu g/mL\ LPS}} \times 100$$

Results

The data obtained from this study met criteria for a valid assay and the controls performed as anticipated. Compared to untreated fibroblasts, LPS (1 µg/mL) increased IL-6 production whereas DEX (10 µM) reduced the LPS-induced inflammation. Fibroblasts treated with **AC Yogurt Hydrolysate SF** at 0.01%, 0.1%, and 1.0% demonstrated a reduction in LPS-induced IL-6 levels, comparable to the effect of DEX.

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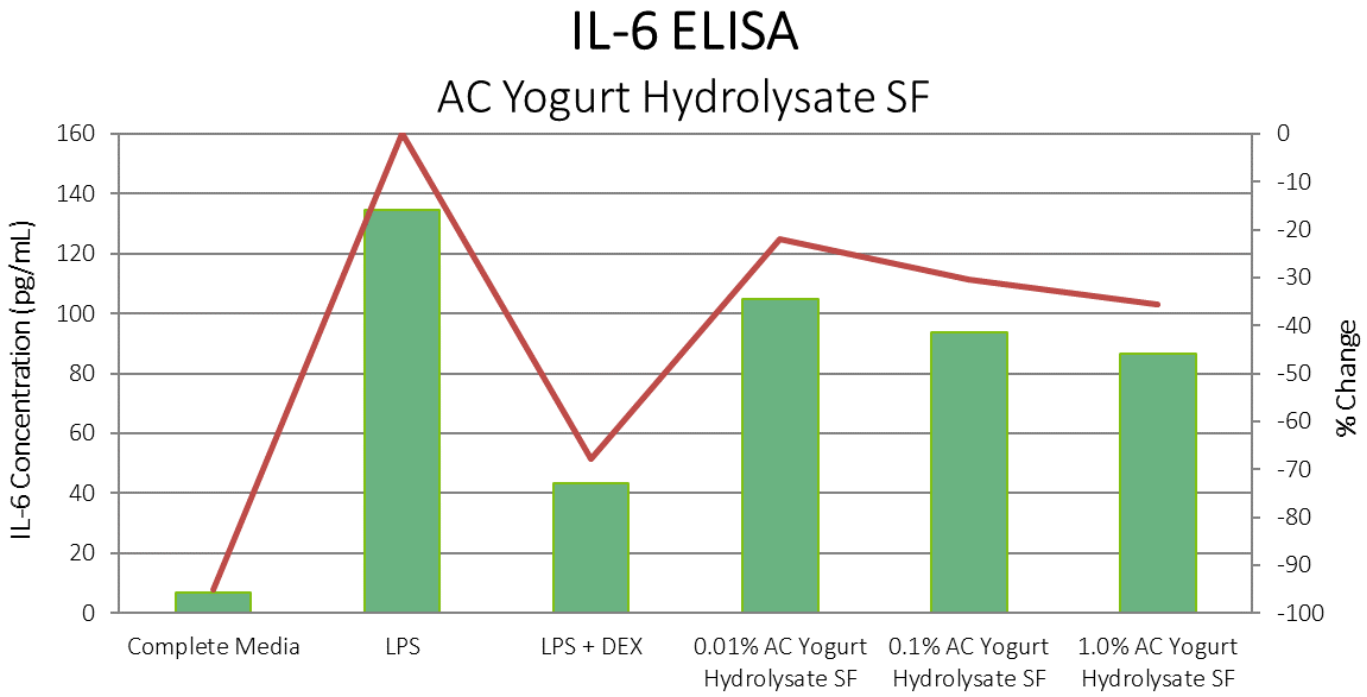

Figure 1. The effect of **AC Yogurt Hydrolysate SF** on IL-6 concentrations in fibroblasts.

Discussion

As shown in Figure 1, fibroblasts incubated with LPS, a substance known to induce an inflammatory state, exhibited a 1,912% increase in IL-6 levels compared to untreated fibroblasts. Conversely, fibroblasts exposed to LPS and DEX elicited a 68% reduction in IL-6 compared to fibroblasts treated with LPS. These data demonstrate the inflammatory environment in fibroblasts is dynamic and can be manipulated with exogenous compounds.

Similarly, fibroblasts treated with **AC Yogurt Hydrolysate SF** at 0.01%, 0.1%, and 1.0% demonstrated 22%, 30%, and 36% reductions in IL-6 levels compared to LPS-treated fibroblasts, respectively. These data demonstrate **AC Yogurt Hydrolysate SF** reduces inflammation.

Collectively, a decrease in IL-6 production indicates a reduced inflammatory environment which could diminish the signs of aging and minimize the formation of fine lines and wrinkles. These data indicate **AC Yogurt Hydrolysate SF** exerts an anti-inflammatory effect, which may help to attenuate characteristics of cellular aging.