

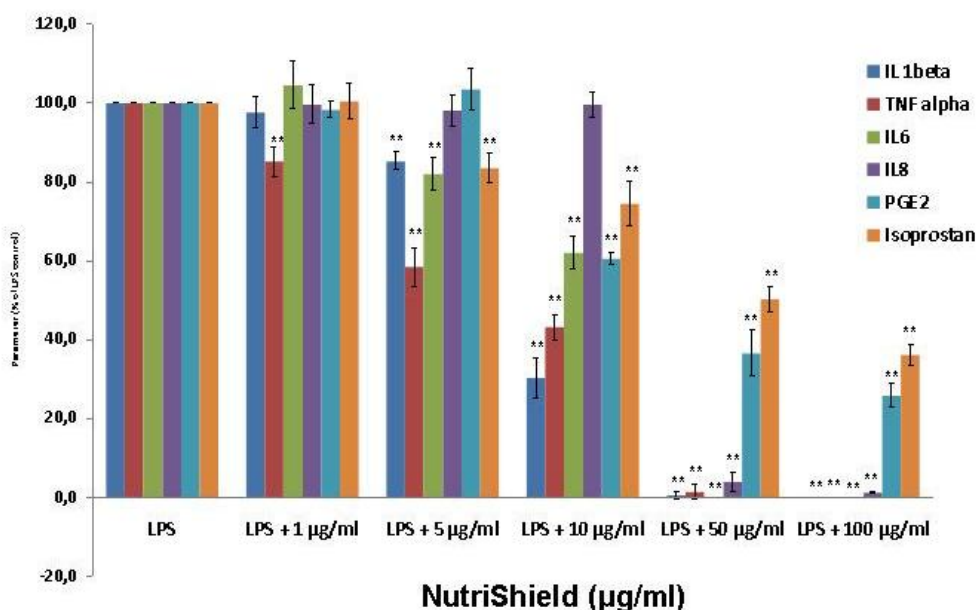
# Final Report

## Immunomodulatory effects of

### NutriShield®

#### Executive Summary

Our data clearly provide evidence that **NutriShield®** creates potent anti-inflammatory and anti-oxidant effects. **NutriShield®** is therefore a bioactive nutraceutical to be used to help prevent inflammatory disease of any kind. Our data find a synergistic effect of the several ingredients of **NutriShield®** since the combination was more effective than the summary of the single ingredients.



## **Final report**

# **Immunomodulatory effects of NutriShield®**

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## **Aims of project (objectives)**

The aim of this project was the following:

- To study the anti-inflammatory effects of **NutriShield®** on the release of LPS-induced cytokines interleukin (IL)-6, IL-8, IL-1beta and tumor necrosis factor (TNF) alpha and the release of lipopolysaccharide (LPS)-induced prostaglandin (PG) E2 and 8-Iso-PGF2alpha (8-isoprostane) in primary human monocytes.

## **Study protocol**

- **Measurement of cytokines and prostaglandin release in primary human monocytes**

Human primary monocytes were isolated from buffy coats of healthy human blood donors and seeded in 24-well-plates (approx. 500 000 cells/ml in 1 ml) for ELISA experiments.

Cells were incubated for 24 hours: (a) without (unstimulated control) or (b) with LPS (10ng/ml), obtained from *Escherichia coli*, Sigma-Aldrich.

**NutriShield®** was added 30 minutes before LPS treatment in five doses.

After 24h, supernatants were removed, centrifuged and investigated for TNFalpha, IL-6, IL-1beta and IL-8 concentrations in ELISA using manufacturer's protocol (all from Pelikine). PGE2 and 8-Iso-PGF2alpha were determined by EIA (PGE2 from AssayDesign, PGF2alpha from Cayman).

Each dose was investigated 4 times in buffy coats from two different blood donors.

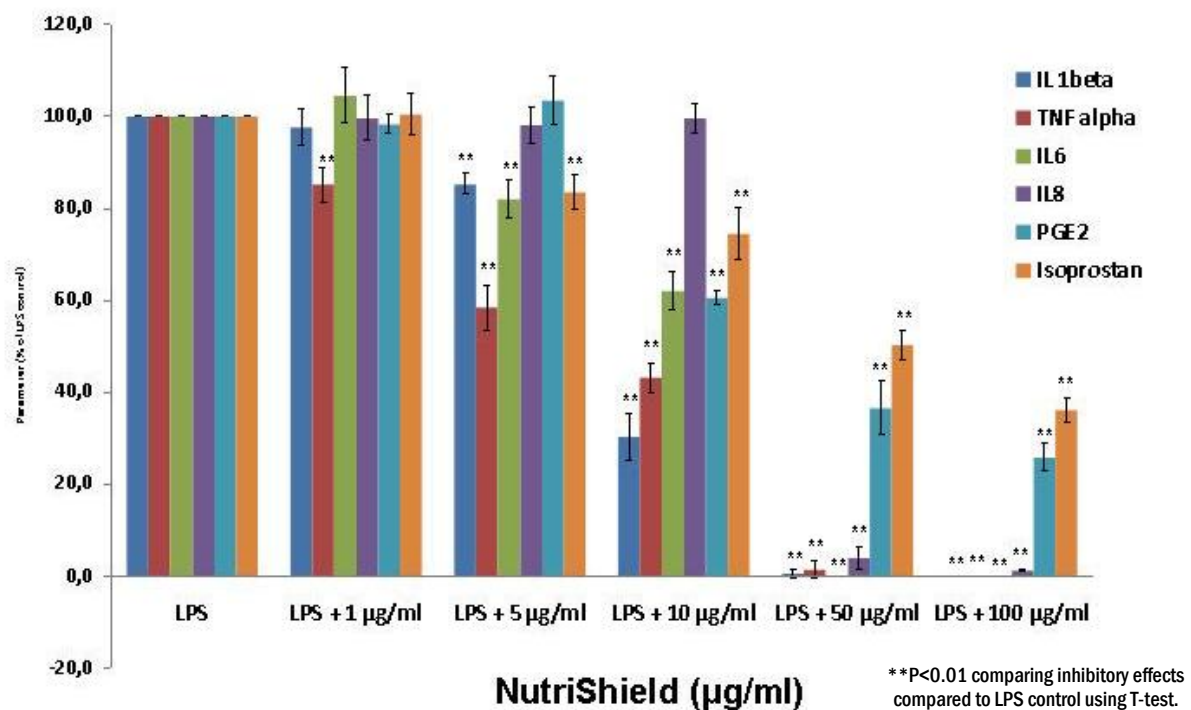
## Results and Discussion

### Anti-inflammatory effects of NutriShield®

We studied the effects of NutriShield® on cytokine and prostaglandin release in primary human monocytes.

**Fig. 1: Effects of NutriShield® on cytokine and prostaglandin release in LPS-treated human monocytes**

Values are provided as percent of control in respect to the LPS stimulated cells without NutriShield® (left column set).



- As shown in Fig. 1, **NutriShield®** potently and dose-dependently inhibited LPS-induced TNF release starting with the low dose of 1µg/ml and maximal inhibition using 50µg/ml, which completely blocked LPS-induced *TNFalpha* synthesis (red column).

- LPS-induced *IL-1beta* and *IL-6* release were significantly inhibited starting with 5µg/ml and maximal effects using 50µg/ml of **NutriShield®** (*IL-1beta*: dark blue column; *IL-6*: green column).
- Significant inhibition of *IL-8* synthesis induced by LPS was achieved by using 50µg/ml **NutriShield®**, which almost completely abolished *IL-8* levels induced by LPS (violet column).
- LPS-induced *PGE2* release was dose-dependently prevented by **NutriShield®** starting with the dose of 5µg/ml and maximal effects using 100µg/ml, which decreased LPS-induced *PGE2* levels by about 80% (light blue columns).
- Significant *8-Iso PGF2alpha* (*8-isoprostane*) inhibition was achieved with the low dose of 5µg/ml and dose-dependently further decreased with maximal effects using 100µg/ml of **NutriShield®**, which reduced PGF2alpha levels of about 60% (orange column).

Our data clearly provide evidence that **NutriShield®** is a potent anti-inflammatory nutraceutical by preventing LPS-induced cytokine and prostaglandin synthesis in primary human monocytes.

Since PGF2alpha is also known to be a marker of free radicals, our data suggest that **NutriShield®** furthermore is preventing free radical formation in immune cells.

By revealing potent anti-inflammatory and anti-oxidant effects, **NutriShield®** is a bioactive nutraceutical to be used to help prevent inflammatory disease of any kind.

Our data find a synergistic effect of the several ingredients of **NutriShield®** since the combination was more effective than the summary of the single ingredients (data from pilot study not shown here).

**Report:**  
**Immunomodulatory effects of NutriShield®**  
31.1.2012

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## Signatures

**Testing facility:**

Principal investigator:

.....31.1.2012.....

Date:



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Dr. B. L. Fiebich (CEO/CSO, VivaCell)

Submitted, Freiburg 31.1.2012

Dr. Bernd L. Fiebich

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