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Effects of *ad libitum* free - choice access to freshly squeezed domestic white asparagus juice on intestinal microbiota composition and universal biomarkers of immuno-metabolic homeostasis and general health in middle aged female and male C57BL/6 mice

Darab Ghadimi [1*], Sven Olaf Frahm [2], Christoph Röcken [3], Michael Ebsen [4], Andreas Schwiertz [5], Regina Fölster-Holst [6], Wilhelm Bockelmann [1], Knut J Heller [7]

[1] Department of Microbiology and Biotechnology, Max Rubner-Institut, Hermann-Weigmann-Str 1, D-24103 Kiel, Germany

[2] Medizinisches Versorgungszentrum (MVZ), Pathology and Laboratory Medicine Dr. Rabenhorst, Prüner Gang 7, 24103 Kiel, Germany.

[3] Institute of Pathology, Kiel University, University Hospital, Schleswig-Holstein, Arnold-Heller-Straße 3/14, D-24105 Kiel, Germany.

[4] Städtisches MVZ Kiel GmbH, Department of Pathology, Chemnitzstr.33, 24116 Kiel, Germany.

[5] MVZ Institute of Microecology, Auf den Lüppen 8, 35745 Herborn, Germany.

[6] Clinic of Dermatology, University Hospital Schleswig-Holstein, Schittenhelmstr. 7, D-24105 Kiel, Germany.

[7] Retired; formerly: Max Rubner-Institut, Federal Research Institute of Nutrition and Food, Department of Microbiology and Biotechnology; Kiel, Germany

Highlights

• Freshly cold squeezed domestic white asparagus juice (FSDWAJ) normalizes levels of SCFAs and remediates age-associated dysbiosis in middle aged female and male C57BL/6 mice.

• Both these effects lead to improved universal biomarkers of immuno-metabolic homeostasis and general health in middle aged mice.

• FSDWAJ modulates crossroads of immune-metabolic signatures of middle aged mice under changed- microbiota diversity, which imply beneficial effects of FSDWAJ in the allevaition of metainflammation.

***To whom correspondence should be addressed**:

Dr. Darab Ghadimi
Department of Microbiology and Biotechnology, Max Rubner-Institut
Hermann-Weigmann-Straße 1
24103 Kiel, Germany; Phone: +49(0)431 6092340; E-mail: darab.ghadimi@mri.bund.de

Abstract

Background and purpose: Asparagus contains different bioactive and volatile components including pyrazines, sulphur-containing compounds, and polyphenols. Asparagus juice is a new low-calorie LAB-containing natural juice product the usage of which is expanding. Pyrazines and sulphur-containing compounds are degraded by bacteria on one hand, but on the other hand, dietary polyphenols prevent human colorectal diseases as modulators of the composition and/or activity of gut microbiota. However, utility of these asparagus compounds for reversal of age-associated microbial dysbiosis and the immunometabolic disorders that dysbiosis incites body inflammatory reactions were not much explored so far. Hence, using middle aged mice, we conducted the current study to verify the effect of freshly squeezed domestic white asparagus juice on the biomarkers reflecting immuno-metabolic pathways linking age-related dysbiosis and metabolic events. ***Materials and Methods:*** Thirty-two conventional Harlan Laboratories C57BL/6 mice aged between 11-12 months were randomly divided into two groups (n=16). Mice in control group 1 received sterile tap water. Animals in group 2 had 60 days *ad libitum* free-choice access to sterile tap water supplemented with 5% (v/v) freshly squeezed domestic white asparagus juice. Clinical signs of general health, hydration, and inflammation were monitored daily. Caecal content samples were analysed by qPCR for microbial composition. Histology of relevant organs was carried out on day 60 after sacrificing the mice. Universal markers of metabolic- and liver function were determined in serum samples. Caecal SCFAs contents were measured using HPLC. ***Results:*** Overall, no significant differences in general health or clinical signs of inflammation between the two groups were observed. The liver to body weight ratio in asparagus juice-drank mice was lowered. qPCR quantification showed that asparagus juice significantly decreased the caecal *Clostridium coccoides* group, while causing an enhancement in *Clostridium leptum*, *Firmicutes* and bifidobacterial groups as well as total caecal bacterial count. Asparagus juice significantly elevated the caecal contents of SCFAs. Enhanced SCFAs (acetate, butyrate, and propionate) in mice having received asparagus juice, however, did coincide with altered lipid levels in plasma or changes in the abundance of relevant bacteria for acetate-, butyrate-, and propionate production. ***Discussion***: To the best of our knowledge, this is the first study aiming at evaluating the effect of freshly squeezed German domestic white asparagus juice on universal markers of metabolic- and liver function in middle aged mice and the role of gut microbiota in this regard. The effectiveness of asparagus juice to improve metabolism in middle aged mice was associated with alterations in intestinal microbiota, but may be also due to uptake of higher amounts of SCFAs. Hence, the key signal pathways corresponding to improved immune-metabolic homeostasis will be the important research scheme in the future.

Key words: Asparagus; dysbiosis; microbiome metabolites; short chain fatty acids (SCFA); polyphenols; bifidobacteria