

## Örebro University

#### SCHOOL OF MEDICAL SCIENCE

Nutrition-Gut-Brain Interactions Research Centre (NGBI)

### STUDY WITH TITLE:

# "Prebiotic and Anti-inflammatory Effects of a Pectin Polysaccharide"

#### STUDY PROTOCOL

#### Task 1: Evaluation of prebiotic effects RG-I

The dietary fibre fractions from RG-I will be tested for their prebiotic potential. Thus, the effects of these fibres on short chain fatty acid (SCFA) profile, microbiota composition and microbiota-associated metabolites will be investigated. In this Task, batch culture fermentation systems containing basal growth medium will be inoculated with faecal homogenates obtained from healthy subjects and RG-I. Samples from the in vitro fermentation will be obtained from different time points and will be used for analyzing microbiota composition and microbiota-associated metabolites. Subjects will complete validated questionnaires related to their dietary intake and a Gastrointestinal Symptom rating scale (GSRS-IBS) in this Task.

#### Task 2: Evaluation of the effects of RG-I fractions on intestinal permeability

By using a sigmoidoscopy procedure, colon biopsies will be collected from the subjects, where the collected biopsies will be mounted in Ussing Chambers in order to investigate the effects of the fibre fractions on intestinal permeability. Supernatants collected from Task 1 will be added to the mucosal side of the biopsy together with a stressor and two permeability markers. Samples will subsequently be collected from the serosal side and will be used to measure the permeability markers related to paracellular and transcellular permeability.

#### STATISTICAL ANALYSIS

All statistical tests will be assessed using One Way ANOVA with a nominal significance level of 5% and post hoc testing of treatment contrasts with Tukey's HSD. The primary endpoint analysis will be based on fibre effect on the intestinal barrier function in terms of FITC passage, and whether there is a difference between treatments across all healthy subjects. Secondary endpoints include HRP quantification (supernatant), TEER values (electrophysiological parameter).