

Pilot Study Protocol: Immunometabolic and Endocannabinoid Profiling in Type 1 Diabetes and At-Risk Individuals

Study Objectives

This pilot study aims to explore correlations between circulating endocannabinoid levels (AEA, 2-AG) and:

- Glycemic control (HbA1c, C-peptide)
- Inflammatory markers (CRP, IL-6, TNF- α , IL-10)
- Gut barrier integrity (zonulin, claudin-1, occludin)
- Fecal SCFA profiles (butyrate, acetate, propionate)
- Autoimmunity (GAD65 antibodies)
- Ion balance (ionogram)
- Extended lipid profile (HDL, LDL, TG, total cholesterol)
- CB1 and CB2 receptor mRNA expression (optional, see below)

This study will also investigate whether patterns in these biomarkers can distinguish between individuals with T1D and at-risk individuals (e.g., first-degree relatives with autoantibodies).

Study Design

- **Design:** Cross-sectional observational pilot study
- **Groups:**
 - 50 individuals diagnosed with Type 1 Diabetes (duration >1 year)
 - 20 at-risk individuals (positive for autoantibodies or first-degree relatives)
- **Age range:** 18–45
- **Exclusion criteria:** Chronic inflammatory diseases, active infections, recent antibiotics, immunosuppressants, cannabis use

Biosample Collection and Volume

Blood Sample Collection

Total: ~45 ml per participant, collected in multiple tubes during one session

Tube Type	Purpose	Volume per Tube	Tubes
EDTA (purple cap)	Plasma for AEA, 2-AG, cytokines	6 ml	2
Serum (red or yellow cap)	HbA1c, lipid panel, ionogram, GAD65, C-peptide	6 ml	3

Tube Type	Purpose	Volume per Tube	Tubes
PAXgene RNA tube	CB1 and CB2 mRNA expression (if included)	2.5 ml	1
Heparin (green cap)	Backup for cytokines or additional metabolic panels	6 ml	1

Optional RNA expression module adds ~2.5 ml (1 PAXgene tube). This keeps the total below **50 ml**, a standard upper limit for adult participants in single-day, non-therapeutic blood sampling protocols.

Stool Sample Collection

- One fresh stool sample in sterile container
- Divided for:
 - SCFA profiling (gas chromatography)
 - Gut permeability biomarkers (zonulin, calprotectin, etc.)
 - Microbiota analysis (optional)

Methods

Category	Analytes	Methods
Endocannabinoids	AEA, 2-AG	LC-MS/MS
Glycemic Control	HbA1c, C-peptide	Immunoassay
Inflammatory Markers	IL-6, TNF- α , IL-10, CRP	Multiplex ELISA / Luminex
Ionogram	Na ⁺ , K ⁺ , Ca ²⁺ , Mg ²⁺ , Cl ⁻	Ion-selective electrodes
Lipid Panel	LDL, HDL, TG, TC	Standard clinical chemistry
Autoimmunity	GAD65 antibodies	ELISA
Gut Barrier	Zonulin, Occludin, Claudin-1	ELISA
Fecal SCFAs	Butyrate, acetate, propionate	GC-MS
CB1/CB2 Expression (Optional)	mRNA from blood	RT-qPCR (after RNA extraction from PAXgene tube)

Statistical Analysis

- **Primary correlations:** Spearman/Pearson coefficients between AEA/2-AG and HbA1c, C-peptide
- **Secondary analyses:** Group comparisons (T1D vs at-risk) using t-tests or Mann-Whitney U-tests
- **Multivariate modeling:** Stepwise regression or PCA to assess predictive value of EC + immune + metabolic panels

Ethics and Safety

- All participants will provide written informed consent
- Ethical approval will be obtained from a relevant institutional board
- The volume of blood collection is within safe limits for adults
- Data will be anonymized and handled in compliance with GDPR