Statistical Analysis Plan for DiAOVE

The statistical analysis will be performed according to a predefined analysis plan finalized before unblinding of the intervention groups. Analyses will be conducted using validated statistical software, such as R, SPSS, or equivalent. All statistical tests will be two-sided, and a p-value <0.05 will be considered statistically significant.

The primary analysis will be conducted according to the intention-to-treat (ITT) principle, including all randomized participants analyzed according to their assigned intervention group, regardless of adherence or protocol deviations. A per-protocol (PP) analysis will also be performed as a complementary exploratory analysis, including participants who complete the intervention without major protocol deviations and with adequate adherence.

Continuous variables will be summarized using mean and standard deviation or median and interquartile range, depending on their distribution. Categorical variables will be summarized using absolute and relative frequencies. Normality of continuous variables will be assessed using graphical methods and statistical tests such as Shapiro-Wilk or Kolmogorov-Smirnov tests.

The primary outcome will be the change in glycated haemoglobin (HbA1c) from baseline to 12 weeks. The primary analysis will compare the change in HbA1c between the intervention group and the control group using a linear mixed model for repeated measures. The model will include intervention group, time, and the group × time interaction as fixed effects, with participant included as a random effect. The model will be adjusted for clinically relevant covariates, including baseline HbA1c, sex, age, and baseline body mass index. The intervention effect will be expressed as the adjusted mean between-group difference in HbA1c change with a 95% confidence interval. As a complementary analysis, an ANCOVA model comparing final HbA1c values between groups adjusted for baseline HbA1c will be performed.

Secondary continuous outcomes, including continuous glucose monitoring-derived metrics, fasting plasma glucose, fasting insulin, HOMA-IR and HOMA2-IR indices, lipid profile, anthropometric variables, blood pressure, inflammatory markers, oxidative stress markers, neuroprotective markers, anxiety and depression scores, and cognitive test scores, will be analyzed using linear mixed models including intervention group, time, and group × time interaction as fixed effects. When appropriate, models will be adjusted for baseline values and relevant covariates. Continuous glucose monitoring data will be analyzed according to international consensus recommendations, including mean glucose, glucose management indicator, coefficient of variation, time in range, time below range, time above range, hypoglycaemic episodes, and ambulatory glucose profile.

Variables with non-normal distributions may be log-transformed before analysis. If transformation does not achieve approximate normality, appropriate non-parametric methods will be used. Exploratory outcomes, including gut microbiota, circulating microRNAs, lipidomic and glycomic profiles, and baseline psychosocial variables, will be

analyzed descriptively and exploratorily. Associations between baseline psychosocial variables and metabolic, glycaemic, neuropsychological, and adherence outcomes will be explored using regression or correlation analyses, as appropriate.

Interaction analyses will be performed to explore whether the intervention effect differs according to sex, baseline HbA1c category (<8% versus ≥8%), and degree of obesity. These analyses will be considered exploratory and interpreted with caution.

Missing data will be handled primarily within the mixed-model framework, which allows inclusion of participants with incomplete data under the missing-at-random assumption. If necessary, multiple imputation will be performed to assess the robustness of the results. Sensitivity analyses will be conducted to evaluate the potential impact of missing data and protocol deviations.

Given the exploratory nature of several secondary and mechanistic outcomes, results from multiple secondary comparisons will be interpreted cautiously. No formal adjustment for multiplicity is planned for exploratory outcomes unless specified in the final detailed analysis plan.