Statistical plan
BENEFIC STUDY

Project: Proof of Mechanism of a New Ketogenic Supplement Using Dual Tracer PET (Positron Emission Tomography) (BENEFIC)
Clinical trial number: NCT02551419
REB reference number: MP-22-2015-509
Principal Investigator: Pr. Stephen Cunnane
Promotor: Université de Sherbrooke
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Description:
6-month RCT in MCI, 2 groups (Placebo and Active), repeated measurements (2 times, PRE-POST), recruitment in two phases:

- Phase 1 (2015-2017) : N=20 Placebo et N=19 Active, completed
- Phase 2 (2017-2020) : n= 20 placebo and n=20 active, expected completers

- Final participant: Jan 2020

Statistical analysis :

Outcomes	Variables
Outcome I-a	Brain ketone metabolism (CMR_{ket} and K_{ket}) (phase 1)
Outcome I-b	Blood total ketone response (phase 1 + phase 2)
Outcome II-a	Brain glucose metabolism (CMR_{glu} and K_{glu}) (phase 1)
Outcome II-b	Regional CMR_{ket} and CMR_{glu} (phase 1)
Outcome II-c	Change in MRI-based parameters
Outcome II-d	Cognitive evaluation
Outcome II-e	Blood metabolite profile as a measure of safety
Outcome II-f	Derived Brain ketone metabolism ($dCMR_{ket}$ phase 2)
Outcome II-g	Pharmacokinetic ketone response (phase 2)

Variables for brain energy metabolism (phase 1)
- cerebral metabolic rate of total ketones (CMR_{ket})
- cerebral metabolic rate of glucose (CMR_{glu})
- regional CMR_{ket} and CMR_{glu}
- Brain ketone and glucose uptake (K)

Variables for plasma ketone concentrations and derived brain ketone uptake:
- Total ketones (mM).
- Derived cerebral metabolic rate of total ketones ($dCMR_{ket}$, µmol/100 g/min) (phase 2)
- Pharmacokinetic ketone response (AUC – 8 hour) (phase 2)
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Variables for plasma metabolites profile:
- Total cholesterol (mM).
- Triglycerides (mM).
- Glucose (mM).
- Acetoacetate (mM).
- Beta-hydroxybutyrate (mM).

Variables for cognitive evaluation:
- Raw scores for RL/RI-16, BVMT-R, Digit Symbol, Digit Span, *Trail Making Test A and B*, *Stroop Color and Word Tests*, *Verbal Fluency and Boston naming test*.
- Composite score for each of the five main cognitive domains (Z-score).

Variables for MRI-based parameters (phase 1)
- brain blood flow (ASL)
- brain activation (rs-fMRI)
- integrity of brain white matter tracts (dMRI)
- regional brain volumes (vMRI)

1) <u>Data analysis</u>

- Shapiro-Wilks Normality Test ->
  - <u>parametric statistics</u> -> ANCOVA analysis - With the POST measure as the dependent variable and the PRE as a covariate. This model will assess between-group differences in the POST means after accounting for PRE values (Dimitrov et Rumrill 2003). The model will also be controlled for age, education and APOE-4 genotype (if no interaction Groupe x covariable).
  - Non parametric statistics -> Intra-group PRE vs. POST (dependent samples): Wilcoxon Sign tests. Inter-group delta Placebo vs. delta Active: Mann–Whitney U tests

- Linear regression between plasma ketones and cerebral metabolic rate of total ketones (CMR_{ket})
- Linear regression between plasma ketones and cognitive performance.
- Linear regression between cerebral metabolic rate of total ketone (including $dCMR_{ket}$) and cognitive performance