Supplementary material

Empagliflozin improves insulin sensitivity of the hypothalamus in humans with prediabetes: a randomized, double-blind, placebo-controlled, phase 2 trial

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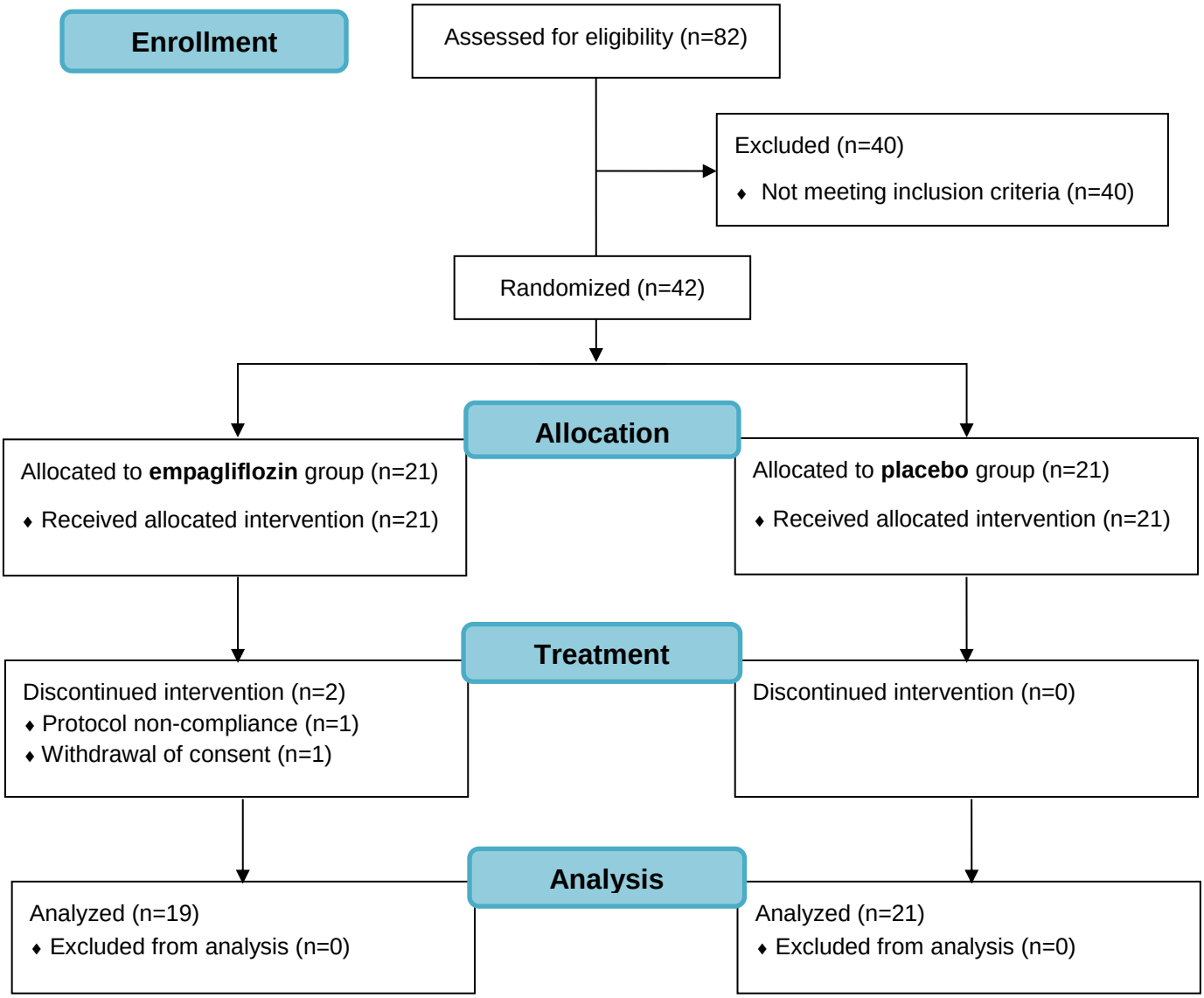
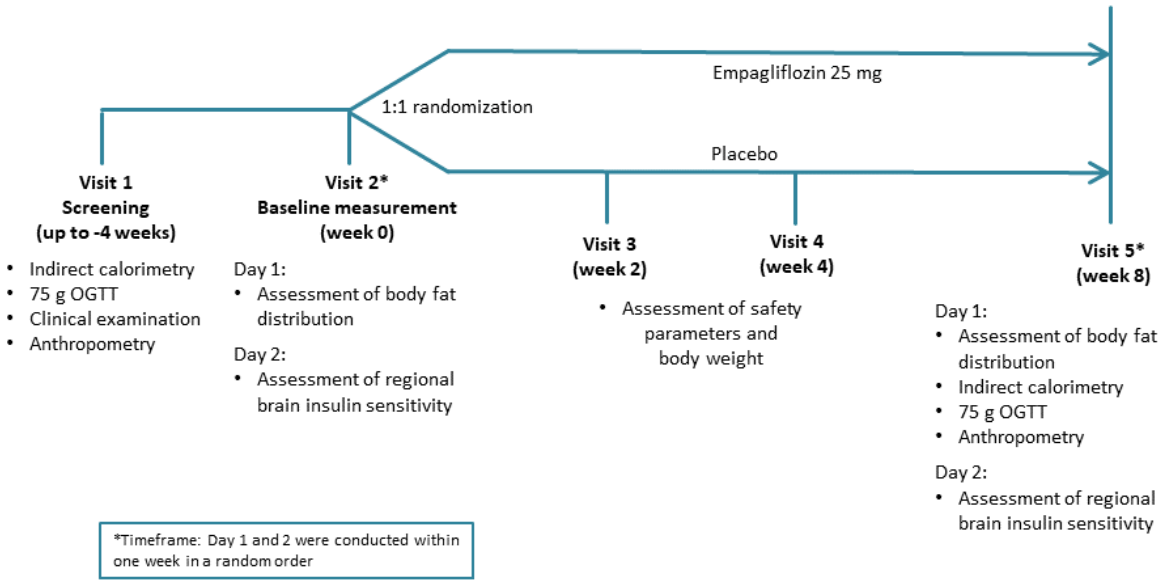
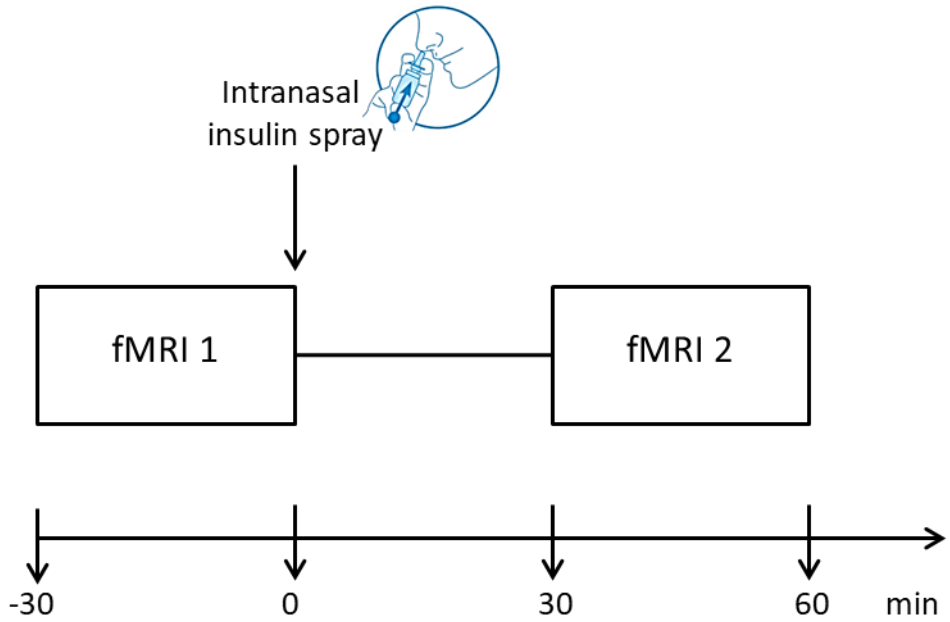
Supplementary figure 1: CONSORT flow diagram



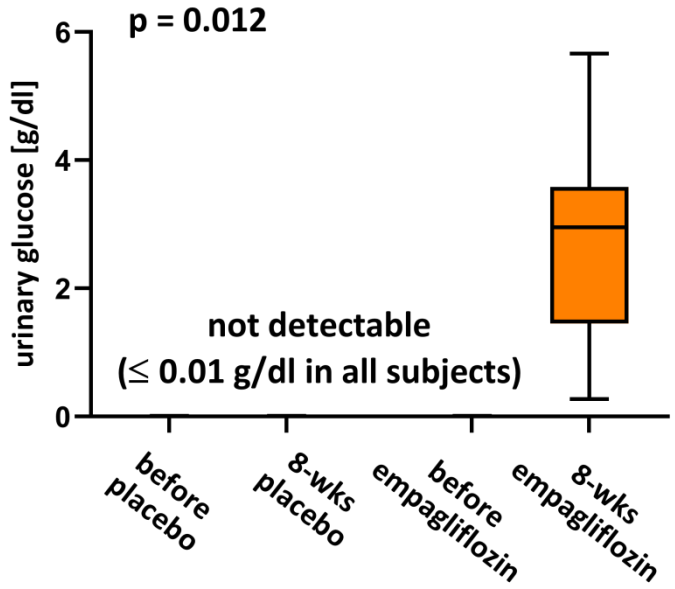
Supplementary figure 2: Study outline



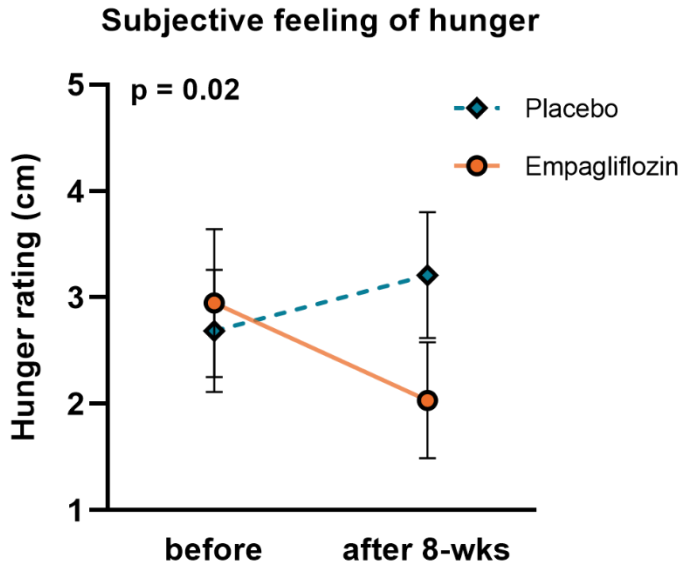
Supplementary figure 3:



Supplementary figure 4: Urinary glucose



Supplementary figure 5: Subjective feeling of hunger



Presented are hunger ratings based on the visual analogue scale (VAS) in the fasting state. There was a significant treatment by time interaction ($F(1,35)=6.4$, $p=0.02$). Presented are means ± SEM.

Supplementary table 1: Classification und number of adverse events (AE) according to MedDRA LLT coding

Adverse events (MedDRA LLT)	**Placebo** (n=21)	**Empagliflozin** (n=19)
Fall	1	1
Fibrinous bronchitis	0	1
Pruritus	0	1
Gonitis	0	1
Cold	1	2
Stomach pain	0	1
Diarrhoea	0	1
Urine leukocyte esterase positive	0	1
Pain in lumbar spine	1	0
Aching pain in hands, forearms, elbows	1	0
Creatine kinase increased	1	0
Burning micturition	0	2
Deafness left ear	1	0
Gum pain	0	1
Acute nasopharyngitis (common cold)	0	1
Rash	0	1
Muscle pain	0	1
Tooth pain	1	0
Presyncope	1	0
Urinary tract infection	2	1
Cyst of kidney	1	0
Stye	0	1
Back pain	0	1
Hematoma	1	0
Total Number of AEs	**12**	**18**

Supplementary table 2: Daily intake of nutrients in the empagliflozin and the placebo group before and at the end of treatment.

	Placebo (n=16)		**Empagliflozin** (n=17)		
	Before	**After 8 weeks**	**Before**	**After 8 weeks**	**p**$_{(Manova)}$
Caloric intake (kcal)	2342 ± 699	2220 ± 732	2078 ± 875	2165 ± 891	0.3/0.2*
Fat (g)	103 ± 37	96.7 ± 40	90 ± 39	90 ± 39	0.7
Carbohydrates (g)	233 ± 69	219 ± 80	204 ± 86	221 ± 113	0.2
Protein (g)	85 ± 26	81 ± 26	77 ± 30	82 ± 36	0.5
Fibers (g)	22.7 ± 7.1	20 ± 1.7	21.2 ± 8.8	22.2 ± 8.9	0.057

Data is given as mean ± SD. A repeated measures ANOVA was performed to investigate treatment by time interaction. *adjusted for sex. 33 subjects completed food diaries on seven consecutive days before and at the end of treatment. Diet composition was estimated with validated software, using four days with complete data out of the seven-day diary (DGE-PC 3.0; Deutsche Gesellschaft für Ernährung, Bonn, Germany).

Supplementary table 3: ß-Hydroxybutyric acid, glucagon, and erythropoetin before and after treatment in both study arms.

	Placebo (n=21)		**Empagliflozin** (n=19)		
	Before	**After 8 weeks**	**Before**	**After 8 weeks**	**p**$_{(Manova)}$
ß-Hydroxybutyric acid (µM/l)	79.8 ± 102.8	95.7 ± 100.9	79.9 ± 87.4	75.9 ± 76.7	0.1
Fasting glucagon (pmol/l)	7.07 ± 4.59	6.90 ± 4.28	6.88 ± 3.09	6.42 ± 3.72	0.7
Area under the glucagon curve during the OGTT (pmol/l)	7.29 ± 3.13[a]	8.31 ± 4.46[b]	6.34 ± 2.21[c]	6.66 ± 2.28[d]	0.3
Erythropoetin (mU/ml)	8.85 ± 5.40[c]	8.17 ± 3.41	10.84 ± 7.74[d]	12.17 ± 8.78	0.8

Data are given as mean ± SD. A repeated measures ANOVA was performed to investigate treatment by time interactions. a: n=14, b: n=15, c: n=16, d: n=18. OGTT: oral glucose tolerance test.

Supplementary table 4: Clinical chemistry

	Placebo (n=21)		**Empagiflozin** (n=19)		
	Before	**After 8 weeks**	**Before**	**After 8 weeks**	**p**(Manova)
Liver enzymes					
Aspartate aminotransferase (U/l)	22.2 ± 12.6	23.4 ±12.5	18.3 ± 8.9	16.8 ± 6.2	0.4
Alanine aminotransferase (U/l)	31.4 ± 19.5	31.3 ± 19.1	30.1 ± 16.5	27.1 ± 12.2	0.5
γ-Glutamyltransferase (U/l)	30.3 ± 21.0	29.8 ± 21.0	33.7 ± 19.0	31.0 ± 19.0	0.4
Alkaline phosphatase (U/l)	70.7 ± 17.9[a]	66.6 ± 16.0	70.2 ± 17.1	72.6 ± 16.6	**0.025**
Serum lipids					
Fasting non-esterified fatty acids (µmol/l)	591 ± 239	571 ± 217	532 ± 162	605 ± 187	0.1
Area under the curve of non-esterified fatty acid during OGTT (µmol/l)	504 ± 170	437 ± 140	439 ± 120[b]	520 ± 167	**0.004**
Triglycerides (mg/dl)	125.0 ± 52.9	126.9 ± 59.8	113.4 ± 60.6	118.8 ± 74.2	0.8
Cholesterol (mg/dl)	210 ± 40	204 ± 44	211 ± 40	212 ± 46	0.4
LDL-Cholesterol (mg/dl)	139 ± 35	137 ± 43	141 ± 43	144 ± 46	0.5
HDL-Cholesterol (mg/dl)	52 ± 14	52 ± 16	55 ± 12	56 ± 16	0.9
Kidney and urine					
Plasma creatinine (mg/dl)	0.80 ± 0.17	0.80 ± 0.13	0.70 ± 0.13	0.70 ± 0.14	0.6
GFR-MDRD (ml/min/1.73 m^2)	90.56 ± 24.45	87.59 ± 16.82	97.13 ± 19.80	98.38 ± 24.92	0.6
GFR-CKD-EPI (ml/min/1.73 m^2)	85.52 ± 5.19	85.57 ± 6.58	89.11 ± 2.81	88.89 ± 3.70	0.9
Urinary protein-to-creatinine ratio (mg/g)	83.3 ± 67.6[c]	80.6 ± 60.2	75.1 ± 46.6[b]	120.5 ± 104.5[b]	0.4
Urinary albumin-to-creatinine ratio (mg/g)	14.6 ± 14.1[c]	15.4 ± 12.2	15.1 ± 13.7[b]	22.2 ± 20.5[b]	0.3
Urinary urea-to-creatinine ratio (g/g)	14.3 ± 3.9[c]	15.2 ± 3.4	14.2 ± 4.8[b]	15.9 ± 6.1[b]	0.9
Urinary glucose (g/dl)	0.01 ± 0.0[d]	0.01 ± 0.0	0.01 ± 0.0a	2.6 ± 1.6	0.011
Electrolytes					
Potassium (mmol/l)	4.08 ± 0.29	4.11 ± 0.33	4.02 ± 0.23	4.11 ± 0.18	0.5
Sodium (mmol/l)	140.43 ± 1.91	140.38 ± 1.77	139.95 ± 2.17	140.32 ± 1.67	0.5
Chloride (mmol/l)	106.48 ± 2.27	106.86 ± 2.06	106.74 ± 2.64	107.42 ± 2.36	0.7

Magnesium (mmol/l)	0.86 ± 0.06	0.84 ± 0.05	0.83 ± 0.06	0.88 ± 0.05	**0.0002**
Phosphorus (mmol/l)	1.07 ± 0.22	1.07 ± 0.20	1.06 ± 0.14	1.08 ± 0.15	0.6
Further blood parameters					
C-reactive protein (mg/dl)	0.34 ± 0.52	0.19 ± 0.25	0.47 ± 0.49	0.62 ± 0.75	**0.009**
Uric acid (mg/dl)	6.8 ± 1.2	6.6 ± 1.1	5.4 ± 0.9	4.1 ± 0.9	**0.0095**
Morning cortisol, serum (nmol/l)	371 ± 115	348 ± 93	434 ± 137	383 ± 137	0.7
Fasting insulin (pmol/l)	114 ± 62	100 ± 58	111 ± 51	103 ± 50	0.6
Fasting C-Peptide (pmol/l)	708 ± 270	698 ± 282	727 ± 206	685 ± 173	0.5

Data are given as mean ± SD. A repeated measures ANOVA was performed to investigate treatment by time interactions. a: n=17, b: n=18, c: n=20, d: n=15. OGTT: oral glucose tolerance test, LDL: low-density lipoprotein cholesterol, HDL: high-density lipoprotein cholesterol, GFR-MDRD: glomerular filtration rate-modification of diet in renal disease, GFR-CKD-EPI: glomerular filtration rate-chronic kidney disease epidemiology collaboration.

Supplementary table 5: Inclusion and exclusion criteria

Inclusion Criteria	Subjects meeting all of the following criteria will be considered for admission to the trial: • Must be between 30 and 75 years at the time of signing the informed consent. • Fasting blood glucose between 100 and 125 mg/dl and/or 2-hour post load glucose between 140 and 199 mg/dl during a 75 g oral glucose tolerance test (ADA criteria for prediabetes). • Body mass index (BMI) between 25 and 40 kg/m². • Understand and voluntarily sign an informed consent document prior to any study related assessments/procedures. • Ability to adhere to the study visit schedule and other protocol requirements. • Females of childbearing potential (FCBP[1]) must agree o to utilize two reliable forms of contraception simultaneously or practice complete abstinence from heterosexual contact for at least 28 days before starting study drug, while participating in the study (including dose interruptions), and for at least 28 days after study treatment discontinuation and must agree to pregnancy testing during this timeframe o to abstain from breastfeeding during study participation and 28 days after study drug discontinuation. • Males must agree o to use a latex condom during any sexual contact with FCBP while participating in the study and for 28 days following discontinuation from this study, even if he has undergone a successful vasectomy o to refrain from donating semen or sperm while participating in this study and for 28 days after discontinuation from this study treatment. • All subjects must agree to refrain from donating blood while on study drug and for 28 days after discontinuation from this study treatment. • All subjects must agree not to share medication.

	[1]A female of childbearing potential is a sexually mature woman who: 1) has not undergone a hysterectomy or bilateral oophorectomy; or 2) has not been naturally postmenopausal for at least 24 consecutive months (i.e., who has had menses at any time in the preceding 24 consecutive months).
Exclusion Criteria	Subjects presenting with any of the following criteria will not be included in the trial: • Women during pregnancy and lactation. • History of hypersensitivity to the investigational medicinal product or to any drug with similar chemical structure or to any excipient present in the pharmaceutical form of the investigational medicinal products. This includes empagliflozin, placebo and human insulin. • Participation in other clinical trials or observation period of competing trials up to 30 days prior to this study. • Diabetes mellitus • Known malformation of the central nervous system • Persons working nightshift • Treatment with glucose lowering drugs, drugs with central nervous actions or systemic steroid therapy • Any relevant (according to investigator's judgment) cardiovascular disease, e.g. myocardial infarction, acute coronary syndrome, unstable angina pectoris, PTCA, heart failure (NYHA II-IV), stroke or transient ischemic attack (TIA), within 12 months prior to screening. • Indication of liver disease, as per medical history or defined by serum levels of either Alanine Aminotransferase (ALT [SGPT]), Aspartate Aminotransferase (AST [SGOT]), or alkaline phosphatase above 3 x upper limit of normal (ULN) as determined during screening. • Alcohol abuse, defined as more than 20 gr/day • Impaired renal function, defined as estimated Glomerular Filtration Rate (eGFR) ≤ 60 ml/min (MDRD formula) as determined during screening. • Known structural and functional urogenital abnormalities, that predispose for urogenital infections. • Subjects with a haemoglobin (Hb) ≤ 11.5 g/dl (for males) and Hb ≤ 10.5 g/dl (for females) at screening. • Bariatric surgery within the past two years and other gastrointestinal surgeries that induce chronic malabsorption within the last 5 years.

	• Medical history of cancer (except for basal cell carcinoma) and/or treatment for cancer within the last 5 years. • Treatment with anti-obesity drugs 3 months prior to informed consent or any other treatment at the time of screening (i.e. surgery, aggressive diet regimen, etc.) leading to unstable body weight. • Known autoimmune disease (except autoimmune disease of the thyroid gland) or chronic inflammatory condition. • Claustrophobia • Any other clinically significant major organ system disease at screening such as relevant gastrointestinal, neurologic, psychiatric, endocrine (i.e. pancreatic), hematologic, malignant, infection or other major systemic diseases making implementation of the protocol or interpretation of the study results difficult. • Presence of any contraindication for the conduct of an MRI investigation, such as cardiac pacemakers, ferromagnetic haemostatic clips in the central nervous system, metallic splinters in the eye, ferromagnetic or electronically operated active devices like automatic cardioverter defibrillators, cochlear implants, insulin pumps and nerve stimulators, prosthetic heart valves etc. • Refusal to get informed of unexpected detected pathological MRI findings • Any other clinical condition that would jeopardize subjects' safety while participating in this clinical trial.

Supplementary material 6: Sample size and power considerations

We designed the trial to study a continuous response variable (brain response to intranasal insulin). Based on previous studies with fMRI and intranasal insulin delivery, we hypothesize that the response will be normally distributed with a standard deviation of 20%. If the true difference is 20%, we will need to study 17 subjects for each treatment group to be able to reject the null hypothesis that the follow-up versus baseline measurements are equal with probability (power) 0.8. The Type I error probability associated with this test of this null hypothesis is 0.05. We plan to include three more subjects in each group who could replace possible dropouts. Thus, 40 subjects are needed.