Turku PET Centre
Turku University Central Hospital

CLINICAL STUDY PROTOCOL

Date: 24.5.2023

Version: **1.3**

Study code: TOTAL

Study title: Total-body glucose utilization in obesity

Principal investigators:

Prof. Lauri Nummenmaa, PhD
Turku PET Centre
University of Turku
Kiinamyllynkatu 4-8
20520 Turku, Finland
Phone: +358 50 574 7933
Email:latanu@utu.fi

Prof. Pirjo Nuutila, MD, PhD
Turku PET Centre
University of Turku and Turku University hospital
Kiinamyllynkatu 4-8,
20520 Turku, Finland
Phone: +358 40 162 6834
Email:pirjo.nuutila@utu.fi

Responsible Physician:
Pirjo Nuutila
Turku PET Centre, University of Turku
Kiinamyllynkatu 4-8,
20520 Turku, Finland
Email:pirjo.nuutila@utu.fi

LIST OF INVESTIGATORS

University of Turku
Prof. Pirjo Nuutila, MD, MD, PhD
Prof. Lauri Nummenmaa, PhD
Assoc. Prof Kirsi Virtanen, MD, PhD
Prof. Jussi Hirvonen, MD, PhD

1 SUMMARY

Background: Brain has a central role in controlling glucose metabolism, but the central-peripheral link in energy metabolism and homeostasis have remained poorly understood. The goal of this project is to characterize the neural, psychological, and physiological mechanisms that contribute to aberrant reward processing and glucose metabolism in obesity using total-body PET imaging.

Aims: We address the central-peripheral axis in metabolic control by imaging glucose utilization and perfusion. Brain and peripheral organs (heart, kidneys, liver) will be studied simultaneously to allow network-level analysis of the metabolic integration. Functional neural and peripheral responses to emotional stimuli will be measured separately for mapping the role of the brain's reward circuit in metabolic regulation. These studies will improve the understanding of the neural and psychological mechanisms obesity. This knowledge will translate into crucial knowledge for developing novel risk factor screening procedures, and novel pharmacological and psychological treatments for common obesity.

Methods: A total of 120 male and female subjects aged 20 to 60 years are recruited into this prospective study. The subjects will undergo physical examination, body tissue composition measurement as well as PET imaging. The study will start in January 2021 and end in the beginning of 2026.

2 INTRODUCTION

Brain has a central role in controlling glucose metabolism, and glucose tolerance, insulin secretion and insulin sensitivity, are in part modulated by the brain (Chen et al., 1975; Obici et al., 2002). Whereas it is well known that insulin sensitivity and secretion are tightly coupled (Kahn et al., 1993; Utzschneider et al., 2009), the mechanisms leading to this tight coupling have not been fully elucidated, with some researchers proposing an important role of the brain (Morton et al., 2017). Central insulin administration suppresses endogenous glucose production (EGP) in mice (Obici et al., 2002). Similarly, in humans intranasal insulin suppresses EGP in lean but not in overweight subjects (Heni et al., 2017). Insulin-stimulated brain glucose uptake (BGU) associates positively with EGP in obese and overweight subjects, but not in lean controls (Latva-Rasku et al., 2017; Rebelos et al., 2018) suggesting that the brain differentially controls peripheral insulin sensitivity by degree of adiposity.

The organ-specific insulin sensitivity has been associated with subtypes of metabolic conditions, for example insulin resistance being localized to skeletal but not heart muscle in type 1 diabetes (Nuutila et al., 1993). Insulin is also a vasodilator and enhances tissue perfusion in skeletal muscle (Clark et al., 2003) and the heart in healthy subjects (Sundell & Knuuti, 2003) but these effects are poorly understood in obesity. Studies comparing insulin effects on perfusion and glucose extraction in the whole body are lacking. In line with this, the role of perfusion to insulin resistance remains unresolved. In addition, brown adipose tissue has emerged as a potential therapeutic target. Brown adipose tissue (BAT) activity has large catabolic potential, and its activity is conserved in human adults (Virtanen et al., 2009). BAT activity is negatively associated with insulin resistance and obesity (Cypess et al., 2009; Lichtenbelt et al., 2009) and furthermore BAT thermogenesis might be impaired in eating disorders (Bredella et al., 2012). These data suggest that BAT dysfunction could be an important pathway contributing to the development of obesity. Accordingly, it is imperative to quantify the links between peripheral and central glucose utilization in normal-weight and obese subjects, and their association with the potentially disrupted BAT thermogenesis.

Feeding and appetite are modulated by a complex set of brain networks involving hypothalamus, amygdala and ventral striatum. Multi-level pathophysiology of this circuit is involved in numerous conditions involved altered food intake. Several studies have reported mesoscopic alterations of this circuit in common obesity and eating disorders despite vastly different endophenotypes (Karlsson et al.,

2013; King et al., 2018; Phillipou et al., 2014), thus the underlying neurobiological mechanisms leading to these similar cerebral alterations across different phenotypes likely differ. Bodily signals influence cognition, emotion, and behaviour at multiple scales and vice versa, and electrical stimulation studies have confirmed the coupling between visceral responses and neocortex (Penfield & Faulk, 1955). Emotions promote survival by automating prompt detection beneficial harmful events, and triggering of approach versus safety-seeking behaviour (Mobbs et al., 2015). Bodily signals influence cognition, emotion, and behaviour at multiple scales and vice versa, and electrical stimulation studies have confirmed the coupling between visceral responses and neocortex. Distinct emotions are supported by discernible neural circuits (Nummenmaa & Saarimäki, 2017) and dysfunction of the emotion circuit has dramatic impact on individuals' well-being. However, the interplay between brain's emotional systems and peripheral components of the emotional circuit in allostatic control of feeding and appetite have remained poorly understood as simultaneous imaging of the central-peripheral axis has not been possible

3 OBJECTIVES AND PURPOSE

The goal of this project is to establish the biological links between brain-periphery axis in human metabolism and test how their specific alterations are linked with obesity. Using cross-sectional design, we characterise individuals with obesity using total-body PET imaging and behavioural assessment. We test three hypotheses derived from human and animal studies:

1. **Insulin-stimulated increases** in tissue specific perfusions are generally blunted in obesity and contribute to the insulin resistance
2. **Insulin-stimulated brain glucose uptake (BGU)** associates positively with EGP in obese and subjects
3. **Central emotional reactivity** is linked with myocardial perfusion and metabolism as well as subjective experience of emotion

4 STUDY DESIGN

This is a cross-sectional study investigating the relationship between the brain and physiological functions in obesity nervosa. Subjects (n= 100) will undergo a screening visit, body composition measurement and calorimetry, and PET with radioligands $H_2[^{15}]O$ and [^{18}F]FDG on two separate visits. A subset of the subjects (Branch B) will only undergo the H2[15]O activation scans.

5 PATIENT/SUBJECT SELECTION

5.1 Source population

We recruit 50 adults (males and females) with obesity from the Eating Disorder Unit at the Turku University Hospital. Their data are compared with 50 age and sex-matched healthy controls with normal body weight and no reported history of an eating disorder

5.2 Inclusion criteria for the obese group

1) Age 20-60 years
2) BMI > 30 kg/m^2

5.3 Inclusion criteria for the control group

1) Age 20-60 years
2) BMI 18.5-25 kg/m^2
3) No lifetime history of obesity, eating disorders or type 2 diabetes

5.4 Exclusion criteria

1) Any chronic disease or medication that could affect glucose metabolism or neurotransmission, with the exception of previously diagnosed type 2 diabetes and use of metformin, DPP-IV-inhibitors or SGLT2-inhibitors in the obese group
2) Any current psychiatric disorder
3) History of severe psychiatric disorders
4) Smoking of tobacco, taking of snuffs, or use of narcotics
5) Abusive use of alcohol
6) Any other condition that in the opinion of the investigator could create a hazard to the subject safety, endanger the study procedures or interfere with the interpretation of study results

6 ASSESSMENTS

6.1 General study outline

The baseline measurements will be performed on three separate visits (a screening visit and a two PET scanning visit). The interval between the screening visit and the first scanning visit is 2-28 days, scanning visits are done at maximum 28 days apart.

1. **Screening visit**

- Physical examination
- Anthropometry
- ECG
- Blood samples, including OGTT
- Body fat mass measurement with bioimpedance
- Body fat and composition measurement with BodPod
- Questionnaire measurements

2. **PETscanning visit 1**

- **Branch A**: Sensitivity of brain's emotion circuits will be measured using activation protocol with $H_2[^{15}]O$
- **Branch B**: Total-body perfusion changes during cold exposure will be measured using activation protocol with H2[15]O. Subjects participating in Branch B will not participate in the glucose utilization scan (visit 2)
-

3. **PET Scanning visit 2**

- Central and peripheral perfusion will be measured with radioligand $H_2[^{15}]O$
- Skeletal muscle microvascular perfusion will be measured with contrast-enhanced ultrasound
- Carotid artery intima media thickness will be measured with ultrasound
- Central and peripheral glucose utilization measured with radioligand $[^{18}F]FDG$.
- Whole-body insulin resistance will be measured with euglycemic hyperinsulinemic clamp technique

6.2 Perfusion imaging with $H_2[15]O$

Total-body PET imaging is performed with Siemens Biograph Vision Quadra scanner (Siemens Healthineers) at Turku PET Centre. The system consists of four consecutive digital detector rings and in comparison with conventional PET-CT, it provides four times wider FOV and 40 times higher SNR in whole-body imaging and 10 times higher SNR for single-organ (e.g. brain) imaging. Spatial resolution is $3mm^3$ and the FOV (106 cm) covers brain and all internal organs and skeletal muscles down to the quadriceps. Total-body perfusion scans are taken three times (**Figure 1B**): At the beginning of the

session, at baseline, after intravenous insulin priming (at and after 50 minutes of the insulin clamp. Additionally, we measure cerebral and peripheral responses to audiovisual emotional stimuli using ultrafast perfusion imaging. For each scan, $H_2[^{15}]O$ (400 ± 10 MBq) is injected intravenously, and radioactivity is followed for 5 minutes. In the brain, regions of interest (ROIs) will be delineated using the AAL atlas (Tzourio-Mazoyer et al., 2002) after spatially normalizing the brain data to functional PET template image in MNI space. For peripheral data, ROIs will be defined for myocardium, lungs, skeletal muscle (upper thigh / biceps), liver, pancreas, kidneys, and gut.

6.3 Measuring total-body glucose utilization with PET

We measure whole-body glucose utilization with radiotracer [^{18}F]FDG. Whole-body low-dose CT scan and MRIs are acquired for anatomical reference. Euglycemic hyperinsulinemic clamp technique is used as previously described (DeFronzo et al., 1979). Briefly, plasma glucose concentration is kept constant at 5 mmol/l by a variable glucose infusion using a negative feedback principle. Under steady-state euglycemic condition, the glucose infusion rate equals glucose uptake by all the tissues in the body and is therefore a measure of tissue sensitivity to exogenous insulin. The rate of insulin infusion is set at 40 mU m^{-2} $min^{-1.}$ During hyperinsulinemia, euglycemia is maintained by infusing 20 % glucose intravenously. The rate of glucose infusion is adjusted according to plasma glucose concentrations measured every 5-10 min from arterialized blood. At the time point 100+-10 minutes of euglycemic hyperinsulinemic clamp, [^{18}F]FDG (100 ± 9 MBq) is injected intravenously over 40 second and PET data is acquired from the 106 cm of the field of view including the brain, neck area (BAT), heart, liver, abdomen and skeletal muscle regions. Arterialized venous blood samples are drawn for radioactivity analysis. A GE Advance PET scanner (General Electric Medical Systems, Milwaukee, WI, USA) with resolution of 4.25 mm was used for PET studies as previously described (Kaisti et al., 2003; Kaisti et al., 2002). [^{18}F]FDG is synthesized as previously described (Hamacher et al., 1986). Plasma radioactivity is measured with an automatic gamma counter (Wizard 1480 3", Wallac, Turku, Finland). Cerebral glucose uptake rate is measured for each voxel separately from dynamic PET scans as described previously (Kaisti et al., 2003; Kaisti et al., 2002). Urine samples are collected following the PET scan and the amount of radiotracer lost into urine is measured using isotope dose calibrator (Model VDC-205, Comecer Netherlands, Joure, Netherlands). Endogenous glucose production (EGP) is assessed by subtracting glucose infusion rate from rate of glucose disposal derived from 18F-FDG consumption (Iozzo et al., 2007). After PET, insulin infusion is stopped. Glucose infusion is continued until a stable level of plasma glucose is achieved (healthy individuals: 6.5 mmol/l).

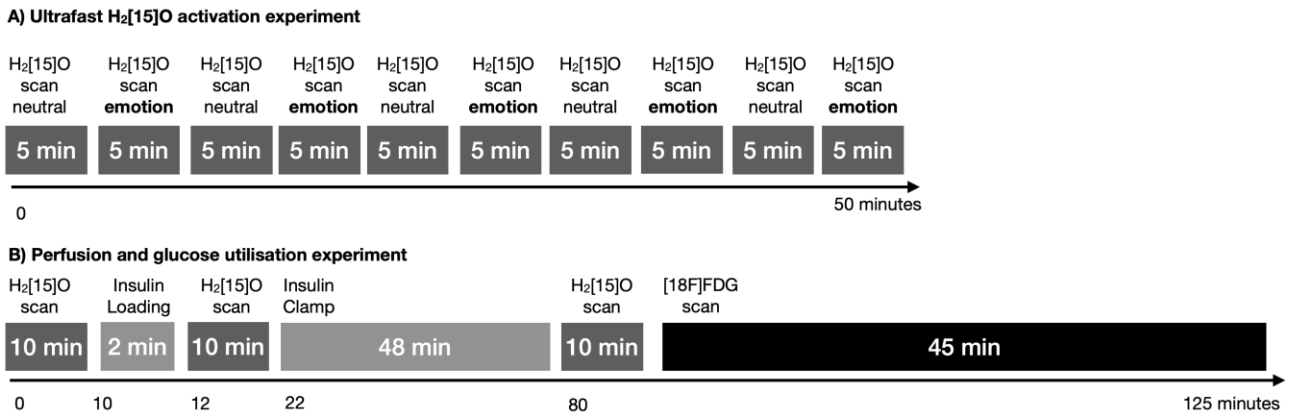

Figure 1. Structure of the PET scanning sessions completed on two separate visits.

6.4 Mapping cerebral and peripheral emotion circuits with H_2[15]O activation protocol

Conventional brain perfusion imaging with H_2[15]O is based on approximately 60-second activation blocks with bolus radiowater injections intserspreded by > 14 minute (seven half-lives) breaks allowing radiotracer decay (Jonides et al., 1993). Increased sensitivity of the total-body system (Tan et al., 2020). Coupled with recent developments in dynamic PET data modelling at our centre however allow us to perform functional activation studies with H_2[15]O with significantly higher stimulation cycle frequencies (Watabe et al., submitted). The technique is based on accounting for the residual radioactivity from the previous scans for each subsequent bolus injection. Simulations and primate data suggest that interscan intervals below 5 minutes are feasible, with the prospects of bringing the interval below two minutes (Watabe et al., submitted). This significantly increases the rCBF signal acquired per minute and coupled with the high-SNR-low-dose capabilities of total-body PET, allows simultaneous high-SNR activation studies of the brain and the peripheral organs. For the activation experiment in **Branch A** we use previously validated paradigm (Saarimäki et al., 2016) where emotional videos are shown to participants in blocks, with single block duration matching the peak of the H_2[15]O activity in tissue (**Figure 1A**). Fast phasic bolus injection protocol will be used. Two different stimulus categories will be used – emotional and neutral. Five blocks per emotion condition will be run. Subjects are asked to empathize with the movies as strongly as possible. Central and organ-specific peripheral perfusion will be modelled, and network-level interactions between the central and peripheral activation will be measured. For Branch B, we used the previously validated cold pressor test (UDin et al., 2016; Walsh et al., 1989). Briefly, subjects foot is immersed into cold water for the duration of each of the PET scans. This induces sympathetic activation, vasoconstriction, and unpleasant feelings. Subjective responses to the cold stimulus are measured using VAS scales. Five blocks with cold stimulation and five blocks with neutral stimulation (no cold) are run; otherwise scanning is similar as in **Branch A.**

6.4b Dosage estimation experiment

To estimate the lowest possible dose for the new total-body PET camera, for a subset of subjects the H_2[15]O activity study will be run without external stimulation and with variable H_2[15]O doses (set 1: 0.05, 10, 100, 300, 1000 MBq, set2: 50,100,150,200,300 MBq, set 3: 100,200,400, 800 MBq). Subjects participating in the dosage estimation experiment (n=20) will not undergo the FDG scan.

6.5 Assessment of skeletal muscle microvascular perfusion with ultrasound

On the insulin clamp day, skeletal muscle microvascular perfusion will be assessed by using contrast enhanced ultrasound (CEUS). For the study, a steady flow of contrast agent (Sonovue, Bracco) will be administered, and after reaching a steady tissue concentration (app. 2 min), a high-intensity ultrasound pulse is used to destroy the contrast agent microbubbles. After this, four 30 sec cine-loop recordings are stored for further analysis to obtain a replenishment curve, which will represent tissue microcirculation.

Vascular health will also be evaluated with carotid intima media thickness measured with ultrasound. For the measurement, 4 cine loops of 5 cardiac cycles will be recorded from the carotid bifurcation area.

6.6 Laboratory measurements

Physicians will evaluate the general physical condition of the subjects, involving weight, height, blood pressure, medical history, and current medication. Adiposity is determined by BMI and BodPod device as described earlier (Kantonen et al., 2021). Screening laboratory measurements also include oral glucose tolerance test (OGTT). A zero time (baseline) intravenous blood sample is drawn prior to the OGTT. 75g glucose mixed in 2 dl of water is administered orally up to 5 minutes (WHO recommendation). During OGTT blood samples will be taken at time points 0 (baseline sample), 15, 30, 60, 90, 120 min for glucose, C-peptide and insulin analysis and calculation of AUC. Fasting blood samples include blood count, glucose, insulin, liver enzymes (ALT, AST, AFOS, GT), potassium, sodium, creatinine, albumin, magnesium, chloride, phosphate, high-sensitive CRP, TSH, free T4, free T3, troponin T and NT-

proBNP. Drug screen from the urine include: amphetamine, cannabis, cocaine, opiate, methadone, bentsodiazepine, buprenorphine and creatinine. Lipids, lipoprotein subclasses, amino acids and metabolites of glucose, fatty acid and amino acid metabolism are measured using quantitative nuclear magnetic resonance and mass spectrometry metabolomics technique. Chronic stress responses will be measured using hair cortisol samples. Serum progesterone and estradiol samples will be collected on screening visit to determine the phase of menstrual cycle. An urine sample, a stool sample and blood samples (plasma and serum) will also be collected and stored at a freezer to be used in further analyses. All personal identification information will be removed from these samples, and they will be anonymized before storing.

6.7 Questionnaires and computer-based tasks

All the participants will complete the following self-administered questionnaires before the study: UCLA-LS (Russell et al., 1978), MAIA-2 (Mehling et al., 2012), SDT (Furnham et al., 2013), DASS-21 (Lovibond & Lovibond, 1995), TIPI (Gosling et al., 2003) and (van Strien et al., 1986). Subjects will also evaluate a short standardized set of emotional pictures to assess affective reactivity (Lang et al., 2005). Emotional state will be evaluated using VAS scales at the beginning of the experiment and after each PET scan. Measurements will be done using the Gorilla.sc toolbox.

7 ADVERSE EVENTS

7.1 Definition of Adverse Events (AEs)

An AE is the development of an undesirable medical condition or the deterioration of a pre-existing medical condition following or during exposure to a pharmaceutical product, whether or not considered causally related to the product. An undesirable medical condition can be symptoms (e.g. nausea), signs (e.g. tachycardia) or the abnormal results of an investigation (e.g. laboratory findings).

A serious adverse event (SAE) or serious adverse drug reaction (SADR) is any event that is fatal, life-threatening, results in hospitalisation, prolongs hospitalisation, results in persistent or significant injury or inability to conduct normal life functions, causes a congenital anomaly or a birth-defect or is an important medical event that may jeopardize the subject or may require intervention to prevent one of the outcomes listed above. A suspected unexpected serious adverse reaction (SUSAR) is a suspected serious adverse event that is also unexpected.

7.2 Reporting of Adverse events (AEs)

All adverse events will be registered, and when necessary, the ethical committee and the Finnish Medicines Agency will be notified. All adverse events will be fully documented and followed in order to determine the final outcome (until the event has subsided or the condition as stabilised).

7.3 Expedited Reporting

According to Finnish national regulation, fatal or life-threatening unexpected adverse events will be reported as soon as possible but at least within 7 days. All other SAEs, SADRs and SUSARs will be reported within 15 days.

7.4 Emergencies

Emergencies may occur during the experiments. A medical doctor will be available during all the measurements in the study. Vital functions will be monitored, and materials and drugs needed for first aid/resuscitation are readily available.

8 ETHICS

8.1 Ethical considerations

The studies will be performed using standard procedures that have been in clinical or research routine use for years in the Turku PET Centre. This study will be conducted according to Good Clinical Practice and the Declaration of Helsinki. Written informed consent for the study will be obtained from all subjects- before the beginning of the study. Subjects will be informed of their right to withdraw from the study at any time. At least one medical doctor will be available at all times during the study.

The radiation dose for the study will be 9.02-9.72 mSv depending on the number of whole-body CT scans required for the studies. The higher dose, 9.72 mSv corresponds to 1 year and 8 months of background radiation in Finland, so the risk for radiation related adverse events is low when subjects with significant previous radiation burden will be excluded. For Branch B activation studies the total radiation dose will be 5,1 mSv, which corresponds to 11 months of background radiation.

8.2 Ethical Review

Study protocol, patient information, and informed consent form will be submitted to the Ethical Committee of the Hospital District of the South-Western Finland (EC). Studies will not start before getting ethical permission. The EC will be informed by the Investigator of all subsequent protocol amendments and of serious or unexpected adverse experiences occurring during the study, which are likely to affect the safety of the subjects or the conduct of the study.

8.3 Subject information and informed consent

Information about the study procedures and design will be given in both oral and written form to the study subjects and their parents. All necessary investigator contact information will be provided along with a description of the study. Written informed consent for the study will be obtained from all subjects before the beginning of the study. The principles of informed consent in the current edition of the Declaration of Helsinki will be implemented. Subjects will be informed of their right to withdraw from the study at any time.

9 DATA COLLECTION AND MANAGEMENT

9.1 Case Report Forms

One set of case report forms will be filled during the initial clinical examination at screening visit in order to collect subjects' personal information and their medical status and history relevant to the aims and exclusion criteria of this study.

9.2 Electronic data collection

Data from the case report forms, clinical observations (such as adverse effects or changes in medical status) as well as results from the study (laboratory analyses and PET imaging data) will be gathered in electrical form.

9.3 Data management

All data collected is strictly confidential. It will be archived on paper or electronically at least for fifteen years.

10 DATA ANALYSIS

Brain-PET data (perfusion and glucose uptake) will be analyzed with SPM12 and in-house MAGIA toolbox (Karjalainen et al., 2020). After motion correction and normalization to MNI space, data will be analyzed using conventional random effects model, where task events are modeled as boxcar

functions. Peripheral PET data will be analyzed as described previously (Rebelos et al., 2019). Structural equation modelling and network analyses will be used for quantifying the relationship between regional cerebral and peripheral glucose uptake and perfusion.

11 STATISTICS

11.1 Sample size

The sample size was calculated based on comparable experiments on brain structure and obesity. In these experiments statistically significant differences in brain structure could be established between lean (n = 22) and obese individuals (n = 23). A priori power calculations with the G*Power software suggest that the predicted effects will be observed with a power of 0.95 with sample sizes exceeding 30 in both groups. The design is thus sufficiently powered.

11.2 Statistical analyses

Appropriate statistical analyses will be performed corresponding to current standards.

11.3 Possible interim analyses and stopping rules

No interim analysis is intended.

12 QUALITY ASSURANCE

12.1 Information of study personnel and training

All the personnel involved in the study will be informed about the aims and the practical issues of the study. The measurements are routinely performed at the Turku PET Centre and have been applied for years.

12.2 Protocol amendments

According to Finnish regulations, protocol amendments can be made if all investigators agree. They are presented in a written form and dated. They include the original chapter of the study protocol and the amended chapter with and explanation of this change. All protocol amendments will be submitted for review to the ethical committee.

13 STUDY SCHEDULE

The study will start in September 2022 and end in beginning of 2030. Analysis and reporting of the data will be started as soon as possible and will be continued until all the results have been reported.

14 CRITERIA FOR PREMATURE STUDY TERMINATION

No interim analysis is intended.

15 FINANCING

The studies in PET Centre will be financed with project grants and VTR-funding of prof. Lauri Nummenmaa and prof. Pirjo Nuutila. The study has neither a straight nor indirect financing from the industry. The financing of the study is governed through the Turku University Hospital and University of Turku.

Expense	Per subject	Total for 100 subjects
OGTT	60	6000
TYKSLAB analyses	122	12200
PET-imaging	300	30000
Pharmacy services	30	3000
Travel expenses	50	5000
Subject compensations	340	34000
Total		**90200**

16 INSURANCE

The subjects are covered by the patient insurance. No extra insurance will be taken.

17 STUDY REPORT AND PUBLICATION(S)

The results will be reported in international peer-review journals.

18 ARCHIVING

All data collected will be strictly confidential. It will be archived on paper and electronically for at least fifteen years.

19 REFERENCES

References

Bredella, M. A., et al. (2012). Young Women with Cold-Activated Brown Adipose Tissue Have Higher Bone Mineral Density and Lower Pref-1 Than Women without Brown Adipose Tissue: A Study in Women with Anorexia Nervosa, Women Recovered from Anorexia Nervosa, and Normal-Weight Women. *J Clin Endocrinol Metab, 97*, E584-E590.

Chen, M., Woods, S. C., & Porte, D., Jr. (1975). Effect of Cerebral Intraventricular Insulin on Pancreatic Insulin Secretion in the Dog. *Diabetes, 24*, 910-914.

Clark, M. G., et al. (2003). Blood Flow and Muscle Metabolism: A Focus on Insulin Action. *Am J Physiol-Endocrinol Metab, 284*, E241-E258.

Cypess, A. M., et al. (2009). Identification and Importance of Brown Adipose Tissue in Adult Humans. *N Engl J Med, 360*, 1509-1517.

Defronzo, R. A., Tobin, J. D., & Andres, R. (1979). Glucose Clamp Technique: A Method for Quantifying Insulin Secretion and Resistance. *AmJPhysiol, 237*, E214-E223.

Furnham, A., Richards, S. C., & Paulhus, D. L. (2013). The Dark Triad of Personality: A 10 Year Review. *Soc Personal Psychol Compass, 7*, 199-216.

Gosling, S. D., Rentfrow, P. J., & Swann, W. B. (2003). A Very Brief Measure of the Big-Five Personality Domains. *Journal of Research in Personality, 37*, 504-528.

Hamacher, K., Coenen, H. H., & Stocklin, G. (1986). Efficient Stereospecific Synthesis of No-Carrier-Added 2-[F-18]-Fluoro-2-Deoxy-D-Glucose Using Aminopolyether Supported Nucleophilic-Substitution. *J Nucl Med, 27*, 235-238.

Heni, M., et al. (2017). Hypothalamic and Striatal Insulin Action Suppresses Endogenous Glucose Production and May Stimulate Glucose Uptake During Hyperinsulinemia in Lean but Not in Overweight Men. *Diabetes*.

Iozzo, P., et al. (2007). Quantification of Liver Glucose Metabolism by Positron Emission Tomography: Validation Study in Pigs. *Gastroenterology, 132*, 531-542.

Jonides, J., et al. (1993). Spatial Working Memory in Humans as Revealed by Pet. *Nature, 363*, 623-625.

Kahn, S. E., et al. (1993). Quantification of the Relationship between Insulin Sensitivity and Beta-Cell Function in Human Subjects. Evidence for a Hyperbolic Function. *Diabetes, 42*, 1663-1672.

Kaisti, K. K., et al. (2003). Effects of Sevoflurane, Propofol and Adjunct Nitrous Oxide on Regional Cerebral Blood Flow, Oxygen Consumption, and Blood Volume in Humans. *Anesthesiology, 99*, 603-613.

Kaisti, K. K., et al. (2002). Effects of Surgical Levels of Propofol and Sevoflurane Anesthesia on Cerebral Blood Flow in Healthy Subjects Studied with Positron Emission Tomography. *Anesthesiology, 96*, 1358-1370.

Kantonen, T., et al. (2021). Obesity Risk Is Associated with Altered Cerebral Glucose Metabolism and Decreased Mu-Opioid and Cb1-Receptor Availability *International Journal of Obesity*.

Karjalainen, T., et al. (2020). Magia: Robust Automated Modeling and Image Processing Toolbox for Pet Neuroinformatics. *Frontiers in Neuroinformatics*, 604835.

Karlsson, H. K., et al. (2013). Obesity Is Associated with White Matter Atrophy: A Combined Diffusion Tensor Imaging and Voxel-Based Morphometric Study. *Obesity, 21*, 2530-2537.

King, J. A., Frank, G. K. W., Thompson, P. M., & Ehrlich, S. (2018). Structural Neuroimaging of Anorexia Nervosa: Future Directions in the Quest for Mechanisms Underlying Dynamic Alterations. *Biological psychiatry, 83*, 224-234.

Lang, P. J., Bradley, M. M., & Cuthbert, B. N. (2005). *International Affective Picture System (Iaps): Digitized Photographs, Instruction Man- Ual and Affective Ratings (Tech. Rep. No. A-6)*. Retrieved from

Latva-Rasku, A., et al. (2017). A Partial Loss-of-Function Variant in Akt2 Is Associated with Reduced Insulin-Mediated Glucose Uptake in Multiple Insulin Sensitive Tissues: A Genotype-Based Callback Positron Emission Tomography Study. *Diabetes*.

Lichtenbelt, W. D. V., et al. (2009). Cold-Activated Brown Adipose Tissue in Healthy Men. *N Engl J Med, 360*, 1500-1508.

Lovibond, S. H., & Lovibond, P. F. (1995). *Manual for the Depression Anxiety Stress Scales.* Sydney: Psychology Foundation.

Mehling, W. E., et al. (2012). The Multidimensional Assessment of Interoceptive Awareness (Maia). *PLoS One, 7*, e48230.

Mobbs, D., et al. (2015). The Ecology of Human Fear: Survival Optimization and the Nervous System. *Frontiers in Neuroscience, 9*.

Morton, G. J., et al. (2017). Evidence That the Sympathetic Nervous System Elicits Rapid, Coordinated, and Reciprocal Adjustments of Insulin Secretion and Insulin Sensitivity During Cold Exposure. *Diabetes, 66*, 823-834.

Nummenmaa, L., & Saarimäki, H. (2017). Emotions as Discrete Patterns of Systemic Activity. *Neuroscience Letters*.

Nuutila, P., et al. (1993). Insulin Resistance Is Localized to Skeletal but Not Heart-Muscle in Type-1 Diabetes. *Am J Physiol, 264*, E756-E762.

Obici, S., Zhang, B. B., Karkanias, G., & Rossetti, L. (2002). Hypothalamic Insulin Signaling Is Required for Inhibition of Glucose Production. *Nature medicine, 8*, 1376-1382.

Penfield, W., & Faulk, M. E. (1955). The Insula: Further Observations on Its Function. *Brain, 78*, 445-470.

Phillipou, A., Rossell, S. L., & Castle, D. J. (2014). The Neurobiology of Anorexia Nervosa: A Systematic Review. *Aust N Z J Psych, 48*, 128-152.

Rebelos, E., Immonen, H., & Bucci, M. (2018). Brain Glucose Uptake Is Associated with Endogenous Glucose Production in Obese Patients before and after Bariatric Surgery and Predicts Metabolic Outcome at Follow-Up.

Rebelos, E., et al. (2019). Brain Glucose Uptake Is Associated with Endogenous Glucose Production in Obese Patients before and after Bariatric Surgery and Predicts Metabolic Outcome at Follow-Up. *Diabetes, Obesity and Metabolism, 21*, 218-226.

Russell, D., Peplau, L. A., & Ferguson, M. L. (1978). Developing a Measure of Loneliness. *J Pers Assess, 42*, 290-294.

Saarimäki, H., et al. (2016). Discrete Neural Signatures of Basic Emotions. *Cereb Cortex, 6*, 2563-2573.

Sundell, J., & Knuuti, J. (2003). Insulin and Myocardial Blood Flow. *CARDIOVASCULAR RESEARCH, 57*, 312-319.

Tan, H., et al. (2020). Total-Body Pet/Ct: Current Applications and Future Perspectives. *American Journal of Roentgenology, 215*, 325-337.

Tzourio-Mazoyer, N., et al. (2002). Automated Anatomical Labeling of Activations in Spm Using a Macroscopic Anatomical Parcellation of the Mni Mri Single-Subject Brain. *Neuroimage, 15*, 273-289.

Utzschneider, K. M., et al. (2009). Oral Disposition Index Predicts the Development of Future Diabetes above and Beyond Fasting and 2-H Glucose Levels. *Diabetes Care, 32*, 335-341.

Van Strien, T., Frijters, J. E. R., Bergers, G. P. A., & Defares, P. B. (1986). The Dutch Eating Behavior Questionnaire (Debq) for Assessment of Restrained, Emotional, and External Eating Behavior. *Int J Eating Disord, 5*, 295-315.

Virtanen, K. A., et al. (2009). Brief Report: Functional Brown Adipose Tissue in Healthy Adults. *N Engl J Med, 360*, 1518-1525.

Watabe, H., et al. (submitted). Shortening Rcbf Measurement Interval in O-15 Water Pet.