Exercise training reverses the obesity-induced increase in resting state brain activity but not insulin-stimulated glucose uptake in monozygotic twin pairs discordant for BMI

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ABSTRACT

Introduction. Insulin-stimulated brain glucose uptake (BGU) is increased in obese and insulin resistant individuals but the underlying mechanisms are unknown. We hypothesized that brain inflammation drives the obesity-induced increase in BGU and that this can be reversed by regular exercise training.

Materials and methods. Twelve monozygotic twin pairs (40.4±4.5 years) discordant for BMI (leaner 29.1±6.3, heavier 36.7±7.0 $kg \cdot m^{-2}$) performed a six month-long exercise intervention and insulin-stimulated BGU was studied by [^{18}F]FDG-PET, brain inflammation using [^{11}C]PK11195-PET (translocator protein (TSPO) availability) and resting state brain activity using fMRI. Cognitive function was assessed by online survey.

Results. At baseline, heavier co-twins had worse aerobic fitness (VO_{2peak}) and whole-body insulin sensitivity (M-value) ($p<0.01$) compared to their leaner co-twins, but there was no difference in cognitive function. Exercise improved VO_{2peak} and M-value similarly in both groups ($p<0.05$) but had no effect on BMI. At baseline, heavier co-twins had higher BGU in parietal cortex and caudatus and higher resting state brain activity ($p<0.05$), while leaner co-twins had higher TSPO availability in white matter and hippocampus ($p<0.05$). Training improved cognitive function similarly in both groups and reversed obesity-induced increase in resting state brain activity (both, $p<0.05$) but had no effect on BGU or brain TSPO availability.

Conclusions. This study suggests that independent of genetic factors, obesity increases insulin-stimulated BGU in specific brain regions accompanied with increased resting state brain activity. Training reversed the obesity-induced increase in resting state brain activity and improved cognitive function but had no effect on insulin-stimulated BGU nor TSPO availability independent of BMI.

INTRODUCTION

Obesity and physical inactivity are major risk factors for the development of insulin resistance (IR) and type 2 diabetes (T2D) (Hruby and Hu 2015; Chatterjee et al. 2017). Insulin resistance in peripheral tissues including skeletal muscle, adipose tissue and liver followed by impaired pancreatic β-cell function play a key role in the development of T2D (James et al. 2021; Reed et al. 2021). During the past years, several studies have highlighted the possible involvement of the brain in the development of IR/T2D. (Obici et al. 2002; Tschritter et al. 2006; Parton et al. 2007; Reno et al. 2017).

Previous studies from our (Hirvonen et al. 2011; Tuulari et al. 2013; Rebelos et al. 2021; Pekkarinen et al. 2022) and others' laboratory (Boersma et al. 2018; Eriksson et al. 2021) using [^{18}F]FDG positron emission tomography (PET) imaging have shown that brain glucose uptake (BGU) is increased upon insulin stimulation in obese humans but not in normal weight healthy controls. However, the cause, effect and the underlying mechanisms of the increased insulin-stimulated BGU are not currently understood.

Intriguingly, preclinical studies suggest that high fat diet induces brain inflammation especially in hypothalamus which can impair normal brain function. This may subsequently contribute to the disrupted whole-body glucose homeostasis (De Souza et al. 2005; Posey et al. 2009). In humans, hypothalamic inflammation has been mainly assessed from MRI-scan based analysis. Some (Thaler et al. 2012; Puig et al. 2015; Schur et al. 2015) but not all (Rebelos et al. 2020) of these studies suggest that obesity is associated with hypothalamic inflammation.

Exercise has numerous health benefits such as improved whole-body insulin sensitivity, which is mainly attributed to improved insulin-stimulated glucose uptake in skeletal muscle (Eskelinen et al. 2015; Sjöros et al. 2018; Ruegsegger and Booth 2018). We have also shown that only two weeks of high intensity interval training decreased insulin-stimulated BGU which was accompanied by improvement in whole-body insulin sensitivity in sedentary insulin resistant humans (Honkala et al. 2018). Furthermore, previous studies have shown that in morbidly obese patients, a bariatric surgery-induced weight loss improves whole-body insulin sensitivity and decreases insulin-stimulated BGU (Tuulari et al. 2013; Rebelos et al. 2019). Hence, in obese populations with impaired glucose tolerance/IR, increased BGU can be reversed with different interventions that improve whole-body insulin sensitivity.

Using functional MRI (fMRI) it is possible to study brain activity and temporal synchronisation between different brain regions and networks (Barkhof et al. 2014). The default mode network (DMN) is one of the most studied resting state networks and is activated at rest i.e. in a task free environment when individual reflects his/her inner state (Buckner et al. 2008). Previous studies measuring resting state brain activity by fMRI have shown body adiposity-induced increases in brain regions related to DMN (Tregellas et al. 2011; Kullmann et al. 2012; Doucet et al. 2018). Furthermore, ineffective suppression of the DMN was observed in obese individuals during an attention requiring task, which was associated with worse cognitive performance (Syan et al. 2019). Interestingly, six months of aerobic exercise was shown to decrease the activity of precuneus (McFadden et al. 2013) that is an essential node of the DMN (Cavanna and Trimble 2006). This suggests that exercise training can reverse the obesity-induced increase in resting state brain activity in regions related to DMN.

The purpose of this study was to investigate whether increased BGU in obesity associates with increased brain inflammation, impaired cognitive function and altered resting state brain activity. To control for confounding by genetic factors, we studied MZ twins discordant for body weight. We hypothesized that obesity-induced increase in BGU is associated with brain inflammation, altered resting state brain activity as well as impaired cognitive function and these changes are reversed by regular exercise training.

MATERIALS AND METHODS

Ethics

This study is part of a larger clinical exercise training intervention entitled "Systemic cross-talk between brain, gut, and peripheral tissues in glucose homeostasis: effects of exercise training (CROSSYS, NCT03730610)".

The study protocol, patient information and informed consent were approved by the Ethical committee of the Hospital district of Southern Western Finland (100/1801/2018/438§). All the participants signed a written consent. The study was conducted according to the good clinical practices and the Declaration of Helsinki.

Study participants and study design

The participants were monozygotic (MZ) twin pairs discordant for BMI that were recruited from three population-based longitudinal twin cohort studies. (Heiskanen et al. 2021) Of the 12 recruited twin pairs that fulfilled the inclusion criteria 10 complete pairs finalised the exercise intervention period and participated in the post measurements (Figure 1). Both co-twin groups were classified as normal according to American Diabetes Association (ADA) guidelines. However, of the leaner co-twins, five met the criteria for impaired fasting glucose (IFG) and two for impaired glucose tolerance (IGT).

On a screening visit day, anthropometric measurements, a thorough health examination and an oral glucose tolerance test (OGTT) were performed in a fasted state (at least 10 h). In addition, physical performance tests at Paavo Nurmi Centre were conducted. Baseline measurements were carried out after the screening visit. During the baseline measurements, functional and anatomical brain MRI scans and two positron emission tomography (PET) imaging studies ([^{11}C]-(*R*)-PK11195-PET and [^{18}F]-FDG-PET) were carried out. After the baseline measurements, twin pairs exercised for six months. After the exercise intervention period, the same measurements as at baseline were repeated (Heiskanen et al. 2021)(Figure 2).

Exercise performance tests, anthropometric measurements, and training intervention

Cardiorespiratory capacity (VO_{2peak}) was measured by a stationary bicycle ergometer test (Ergoline 800 s, VIASYS Healthcare Germany) until a volitional exhaustion and body composition (whole-body fat percentage and lean mass) by Inbody 720 (Biospace Co, Korea) at Paavo Nurmi Centre (Turku, Finland) as previously described in detail (Heiskanen et al. 2021).
Intervention consisted of six months of mixed type progressive training (Heiskanen et al. 2021). Twin pairs exercised at their place of residence four times per week and were supervised by a

personal trainer once a week. Training consisted of two endurance, one resistance, and one high intensity interval exercise session per week. Participants completed training logs and wore a heart rate monitor (Polar A370, Polar, Finland) in order to monitor training adherence and intensity, respectively. (Heiskanen et al. 2021)

Euglycaemic hyperinsulinemic clamp, [^{18}F]FDG–PET/CT scan, and T1 weighted MRI scan

Insulin-stimulated BGU was studied during euglycaemic hyperinsulinemic clamp with [^{18}F]FDG by PET/CT (Discovery MI (DMI), GE Healthcare, US) and the protocol has been previously described in detail (Heiskanen et al. 2021). The M-value was calculated as previously described (DeFronzo et al. 1979; Ojala et al. 2024). After the steady-state was achieved in the euglycemic hyperinsulinemic clamp, 150 MBq of [^{18}F]FDG was injected to the antecubital vein via a catheter, and brain scanning was immediately started for 40 minutes. Plasma radioactivity for the input function was measured from arterialised blood samples.

To achieve anatomical reference images for PET and rs-fMRI analysis, T1 weighted brain MRI-scan was performed with Siemens Magnetom Skyra fit 3 T MRI system using a Siemens Head/Neck 20 channel coil (Siemens Healthcare, Erlangen, Germany) as previously described in detail (Heiskanen et al. 2021). Visceral fat mass scan and analysis protocol has been described earlier (Heiskanen et al. 2021; Ojala et al. 2024).

[^{11}C]PK11195-PET/CT scan

TSPO availability to assess brain-specific inflammation was measured with [^{11}C]PK11195 by PET PET-scanning (60 minutes). The scanning was conducted in a supine position by the same scanner as [^{18}F]FDG-PET. Approximately 350 MBq of tracer was injected to the antecubital vein via a catheter and scanning was initiated immediately after the injection. (Heiskanen et al. 2021).

PET-image analysis and modelling

The obtained raw [^{18}F]FDG-PET and [^{11}C]PK11195-PET-images were corrected for attenuation, dead time and decay. The block sequential regularized expectation maximization algorithm with BETA factor 150 for [^{18}F]FDG and 350 for [^{11}C]PK11195 were used to reconstruct the images.

For [^{18}F]FDG data, PET images were processed using MAGIA pipeline (Karjalainen et al. 2020) in MATLAB (The Mathworks, Natick, MA), where PET data were first realigned frame-by-frame and co-registered to individual T1 weighted MRI images using SPM12 software (Wellcome Trust Centre for Neuroimaging, London, UK). Next, [^{18}F]FDG and [^{11}C]PK11195-PET kinetic modelling was carried out for ROIs (whole brain, cortical grey matter, white matter, frontal cortex, parietal

cortex, temporal cortex, occipital cortex, cingular cortex, posterior cingulate cortex, precuneus, hippocampus, putamen, thalamus and caudatus) that were parcellated using FreeSurfer software (version 6.0.0, http://freesurfer.net/). For [^{18}F]FDG-PET data, brain glucose uptake was quantified using arterial input Patlak method for 15–40 minute period, applied to regional and voxel level data.

For [^{11}C]PK11195-PET data, TSPO availability was quantified as a distribution volume ratio (DVR), estimated with Logan's method within 20–60 minutes, using pseudoreference region obtained using clustered reference algorithm (Schubert et al. 2021). In addition, parametric binding potential (BP_{ND}) images were calculated using a basis function implementation of simplified reference tissue model with 250 basis functions. The resulting parametric maps were further normalized into MNI152 space in SPM12 and smoothed with Gaussian 8 mm FWHM filter.

Resting state fMRI

Resting state functional MRI data, to measure resting brain activity from BOLD signal, was acquired as previously described (Heiskanen et al. 2021). The scan was conducted twice and the participants had eyes open during the scan. A total of 197 functional volumes were acquired per scan and the mean of two scans was used for the analysis.

We used fMRIPrep 23.1.4 to preprocess the fMRI data (Esteban et al. 2019). Anatomical T1 weighted reference images were processed with following steps: correction for intensity non-uniformity, skull-stripping, brain surface reconstruction, spatial normalization to the ICBM 152 Nonlinear Asymmetrical template version 2009c (Fonov et al. 2009) using nonlinear registration with antsRegistration (ANTs 2.2.0), and brain tissue segmentation. fMRI data were processed with following steps: co-registration to the T1 weighted reference image, slice-time correction, spatial smoothing with a 6-mm Gaussian kernel, automatic removal of motion artifacts using ICA-AROMA (Pruim et al. 2015), and resampling to the MNI152NLin2009cAsym standard space. Quality of images was assessed via the visual reports of fMRIPrep and was inspected manually in accord to the whole-brain field of view coverage, proper alignment to the anatomical images, and signal artifacts. All functional data were retained in the analysis.

Cognitive function test

Cognitive function was assessed by an online survey using Gorilla Experiment Builder (gorilla.sc) by a standard desktop computer in a quiet room (Heiskanen et al. 2021). The survey consisted of tasks that measure working memory (N-back tasks with N=1 and N=2, digit span text entry), memory encoding and retrieval with CERAD Word List Memory task type test, vigilance, simple reaction time, and fluid intelligence with *The matrix reasoning item bank (MaRs-IB)* which is a

modified open-source variant of the Raven's progressive matrices test. Emotional sensitivity was measured by asking subjects to report their feelings of valence (pleasure-displeasure) and arousal to a set of pleasant, unpleasant and neutral pictures derived from the International Affective Picture system (IAPS). The detailed protocol is described in supplementary file 3.

Statistical analysis

The normal distribution of the data was evaluated visually from Q-Q plots and histograms. Logarithmic (Log_{10}) and squareroot transformations were carried out to achieve normal distribution, when necessary. The transformations are noted by special characters in table and figure legends when appropriate. Data in figures and tables are represented as model based means and 95 % confidence intervals. Pearson's product-moment correlation coefficient was used to determine the association between the variables. Statistical tests were performed by SAS System (version 9.4 for Windows SAS Institute, Cary, NC, USA) as two-sided, and p-values less than 0.05 were considered as statistically significant. Statistical analyses were conducted using a linear mixed model for repeated time points using compound symmetry covariance structure. The model included twin as a statistical unit, time (PRE and POST intervention) and twin (leaner and heavier co-twin) as within-factors and their interaction term (time x group). Participants with the missing data points were included to the statistical analyses by using the restricted maximum likelihood estimation within the linear mixed model. If there was a significant time x group effect, same model was used to determine the within twin-group effects over time. The baseline difference between the co-twins was analysed with the same model but only using PRE –intervention data.
For the rs-fMRI data, general linear model (GLM) was used to map resting-state network features to activation *z*-scores. At the first (within-twin) level analysis, the effect of higher BMI at baseline (heavier vs. leaner co-twin at baseline) and exercise training intervention (Pre vs. Post) were involved in the model. This was done to generate contrast images for the (time x group) interaction effect as well as for the main effect of exercise training (time-effect). Furthermore, the main effect of training was assessed within heavier and leaner co-twins as there was a group x time interaction effect in BOLD activity. Thereafter, contrast images were subjected to second-level analyses.

RESULTS

Anthropometrics, physical fitness, glucose and lipid profile

At baseline, heavier co-twins had significantly higher body adiposity as well as lower cardiorespiratory fitness (VO_{2peak}) (p=0.003) and whole-body insulin sensitivity (p=0.007) (M-value) compared with their leaner co-twins (Table 1). Furthermore, heavier co-twins had worse blood glucose homeostasis profile compared with their leaner co-twins.

Exercise intervention improved cardiorespiratory fitness (p=0.001) and M-value (p=0.022) as well as lowered systolic (p=0.011) and diastolic blood pressure (p=0.017) similarly in leaner and heavier co-twins but had no effect on whole-body fat percentage (p=0.370). However, visceral fat mass tended to decrease in the whole study sample (p=0.067), while the decrease was statistically significant only within heavier co-twins (p=0.029).

Insulin-stimulated brain glucose uptake

At baseline, heavier co-twins had higher BGU globally in each ROI-set but the difference reached statistical significance in parietal cortex (p=0.032) and caudatus (p=0.043, Figures 3A & B) and there was strong tendency in precuneus (p=0.058), cortical grey matter (p=0.078), frontal cortex (p=0.081) and posterior cingulate cortex (p=0.087) (data not shown). When the difference in BGU was examined at voxel level, the increased BGU in heavier co-twins was mapped solely to left hemisphere of the brain (Supplementary file 1). Exercise had no effect on BGU.

Brain inflammation measured by TSPO availability

At baseline, opposite to our initial hypothesis, leaner co-twins had higher TSPO availability at white matter (p=0.031) and hippocampus (p=0.032), while there was no difference in other brain areas (Figures 4A and B). However, exploratory voxel-based analysis revealed that heavier co-twins had higher TSPO availability at solely on right hemisphere (Supplementary file 2). Interestingly, TSPO availability in hippocampus, white matter and whole brain correlated positively with M-value and VO_{2peak} as well as negatively with CRP, BMI and visceral fat mass (Table 2).

Resting state-functional MRI

At baseline, heavier co-twins demonstrated higher resting state brain activity (BOLD signal) in the precuneus that is a key node of brain DMN (Figure 5A). Exercise training intervention decreased resting state brain activity in medial prefrontal cortex, precuneus and insula (Figure 5B) and interestingly, the training response was different between the groups (Figure 5C) showing statistically significant decrease only in heavier co-twins (Figure 5D).

Cognitive function

At baseline, there was no difference in cognitive performance between the leaner and heavier co-twins (Supplementary file 3). Exercise training intervention improved performance in memory encoding and retrieval ($p<0.05$, Figures 6A-C) as well as in fluid intelligence tests ($p<0.05$, Figures 6D & E) similarly in leaner and heavier co-twins. When participants were shown pleasant pictures, exercise intervention shifted the emotional reaction towards agitation more in heavier co-twins ($p=0.034$) compared with their leaner co-twins (time x group: $p=0.038$, Figure 6F).

DISCUSSION

This study showed that independent of genetics, higher BMI is associated with higher insulin-stimulated BGU. This result was accompanied by increased resting brain activity. Intriguingly, this increase in brain activity observed in heavier co-twins was reversed by exercise intervention that also improved memory encoding, fluid intelligence, cardiorespiratory fitness, and whole-body insulin sensitivity but had no effect on body weight. Contrary to our initial hypothesis, the increased insulin-stimulated BGU in caudatus and parietal cortex was not reversed by exercise training. Additionally, BGU did not associate with TSPO availability which was measured to assess inflammation. Vice versa, we observed that TSPO availability in white matter and hippocampus was increased in leaner co-twins compared with their heavier co-twins.

Previous studies have shown that BGU is increased upon insulin stimulation in overweight/obese and insulin resistant populations but not in normal weight people (Hirvonen et al. 2011; Tuulari et al. 2013). Furthermore, increased insulin-stimulated BGU has been associated with worse whole-body insulin sensitivity (Boersma et al. 2018; Rebelos et al. 2021; Eriksson et al. 2021; Pekkarinen et al. 2022) but the underlying mechanism has remained unknown. The increase in insulin-stimulated BGU in previous studies has been global across the whole brain (Hirvonen et al. 2011; Tuulari et al. 2013) but there has been also regional differences. For example, Tuulari et al. found that upon insulin-stimulation the increase in BGU was highest in right caudate nucleus (Tuulari et al. 2013). In the current study, we showed that independent of genetics increased BMI resulted in higher insulin-stimulated BGU in all analysed regions, but the difference was statistically significant only in caudate nucleus and parietal cortex. This aligns to some extent with the results by Tuulari et al. and might possibly suggest the caudate nucleus being most sensitive to obesity-induced changes. The reason for not reaching statistical significance in all brain regions may be due to the lack of statistical power due to limited sample size.

Even though we saw obesity-induced region specific increase in insulin-stimulated BGU, exercise intervention did not decrease BGU in these regions in neither of the twin groups. We have previously shown that only two weeks of high intensity interval training decreases insulin-stimulated BGU in middle aged people with insulin resistance (Honkala et al. 2018). Furthermore, Tuulari et al. has shown decreased BGU in morbidly obese participants after bariatric surgery (Tuulari et al. 2013). In both of these previous studies, the intervention induced a decrease in total body fat, whereas this was not observed in the current study. Furthermore, the participants in the

previous studies had more advanced insulin resistance compared to participants in our study and thus may respond to the intervention more effectively.

Preclinical (De Souza et al. 2005; Posey et al. 2009) and some (Thaler et al. 2012; Puig et al. 2015; Schur et al. 2015) but not all (Rebelos et al. 2020) clinical studies have suggested that high fat diet and obesity induces inflammation in brain, especially in hypothalamus. Thus, we hypothesised that the increased insulin-stimulated BGU observed in previous studies (Hirvonen et al. 2011; Tuulari et al. 2013; Boersma et al. 2018; Rebelos et al. 2021; Pekkarinen et al. 2022) could be an obesity-induced neuroinflammatory response. In the present study we measured neuroinflammation with PET-imaging using radio tracer [^{11}C]PK11195 (Cagnin et al. 2007). This radioligand binds to TSPO protein that has been mainly found in microglia and macrophages but also in astrocytes in human brain. In normal non-diseased brain, TSPO expression is very low. However, upon pro-inflammatory stimuli, the expression of TSPO increases in microglia and astrocytes as they activate and change their phenotype (Chen and Guilarte 2008; Cosenza-Nashat et al. 2009). Thus, hypothetically, as brain-resident astrocytes and microglias respond to obesity-induced pro-inflammatory stimulus, their energy expenditure and abundance would increase which would be reflected as an increased BGU. This hypothesis is supported by a previous preclinical [^{18}F]FDG-PET-study showing that astrocytes also contribute to the brain [^{18}F]-FDG signal in addition to the glucose uptake by neurons (Zimmer et al. 2017). In addition, preclinical studies suggest that high fat diet induced-obesity increases the amount of astrocytes in mouse brain (Jones et al. 2021; Delle et al. 2023).

However, contrary to our initial hypothesis, we showed that leaner co-twins had higher TSPO availability in white matter and hippocampus. Furthermore, we found that whole brain TSPO availability correlated negatively with BMI, CRP and visceral fat mass and positively with whole-body insulin sensitivity and VO_{2peak}. Thus, based on this data, higher TSPO availability was associated with biomarkers of better metabolic health. This result aligns with a previous study investigating the association between TSPO availability and BMI. By combining brain scans from three different PET centres, thus yielding a study sample of n=140, the authors were able to show a significant negative correlation between BMI and TSPO availability measured with [^{11}C]PBR (Tuisku et al. 2019) that is a second generation radioligand of TSPO. Because TSPO is a mitochondrial protein with many functions such as cholesterol transport, steroid hormone synthesis and mitochondrial respiration (Lee et al. 2020), it may be speculated that obesity may decrease mitochondrial content or functional properties also in the brain. The derangement of the mitochondrial function is a common feature observed in peripheral tissues in obese people and

contribute to whole-body insulin resistance (Heinonen et al. 2020). Overall, our study combined with the previous study suggests that TSPO availability in healthy adults without brain abnormalities is linked to better metabolic health. Even though exercise training in the current study improved whole-body insulin sensitivity and cardiorespiratory fitness, it had no effect on brain TSPO availability. Whether a substantial body weight or fat loss would increase brain TSPO availability should be addressed in the future.

A recent study showed that during neuroinflammation, TSPO expression is only increased in certain rodent species including rat and mouse but not in humans (Nutma et al. 2023). This study emphasized that TSPO signal in human neuroinflammation studies should be interpreted as a change in glial cell density rather than activation of glial cells.

Interestingly, we showed that heavier co-twins had higher brain activity at resting state at baseline in DMN specific brain regions, specifically in precuneus. Insulin-stimulated BGU also tended to be higher in precuneus in heavier co-twins When we overlapped the significantly different voxels between heavier and leaner co-twins from BGU and BOLD signal analysis, we saw overlap at parietal cortex and precuneus (Supplementary file 2). Thus, this suggest that the higher BGU observed in heavier co-twins may reflect increased energy need due to higher brain activity at resting state. This hypothesis is supported by findings from Passow and colleagues who also showed positive correlation between BGU and resting state brain activity (BOLD signal measured by fMRI) (Passow et al. 2015).

The obesity-induced increase in resting state brain activity observed in the present study aligns with previous studies showing that brain activity in regions related to DMN are more active in obese humans compared to age and- sex-matched normal-weight controls (Tregellas et al. 2011; Kullmann et al. 2012; Doucet et al. 2018). Our study adds to the current literature that obesity *per se,* independent of genetics, induces increased brain activity in DMN specific brain areas. We also showed that the exercise intervention decreased brain activity at resting state more in heavier co-twins compared to their leaner co-twins which is also in line with a previous study (McFadden et al. 2013) showing that six months of exercise decreased activity in the precuneus in overweight participants.

Previous literature suggests that obesity is associated with worse cognitive function (especially executive function and working memory) (Yang et al. 2018). Even though cognitive performance did not differ between groups at baseline in the present study, exercise intervention improved memory encoding and retrieval in CERAD type word list task without difference between heavier

and leaner co-twins. In addition, the performance in the task that measured fluid intelligence was improved by exercise training. The benefits of exercise on cognitive function are previously widely shown (Northey et al. 2018). The important notion from this study is that exercise without weight loss is also beneficial for cognitive function and is not limited to older populations with mild or more severe cognitive impairment. However, we cannot rule out the learning effect in this study because we did not have a control group and the cognitive test survey was similar before and after the intervention. One possible mechanism for the improved cognitive performance induced by exercise training may be mediated by exerkine called brain derived neural factor (BDNF) that has been shown to increase post exercise (Marston et al. 2017). Upon its delivery to the brain, it induces among other functions, neurogenesis which is postulated to be beneficial for the brain and cognitive function. (Kowiański et al. 2018). Another possible explanation for the improved cognitive function after exercise training may be improved ability to switch from resting state network (e.g. from DMN) to task related/attention requiring brain network (Syan et al. 2019). To indirectly support this postulation, we showed that exercise training decreased brain activity at resting state in regions that are part of DMN (medial prefrontal cortex, precuneus and insula) and this effect was larger in heavier co-twins compared to their leaner co-twins. However, to fully confirm this hypothesis, we should have measured brain activity by fMRI while changing the test environment from rest to cognitive tasks.

The strength of this study is that we were able to study the effect of increased body weight *per se* by studying MZ-twins discordant for BMI. Furthermore, the exercise intervention was well planned which manifested as high training adherence (approximately 80 %) as well as improved cardiorespiratory fitness and whole-body insulin sensitivity. In addition, we highlight that the changes induced by the intervention are not confounded by body weight loss. We also used state of the art methods to measure non-invasively insulin stimulated BGU.

The limitation of this study is that, even though there was a substantial mean level BMI-difference (7.6 kg/m^2) between leaner and heavier co-twins, there was heterogeneity between the twin pairs which may cause confounding variability into the data. More precisely, in some pairs of twins both individuals were obese, and in one twin-pair both were close to be normal weight. In addition, on average the leaner co-twins were overweight. Ideally, in this study, the BMI of the leaner co-twins would have been < 25 kg/m^2 and that of heavier co-twins > 25 kg/m^2. Moreover, the total number of twin-pairs in this study was smaller than we aimed to, and some measurements were not successful which yielded as modest sample size. Furthermore, in the light of new evidence (Nutma

et al. 2023), the [^{11}C]PK11195 is not optimal radiotracer to measure neuroinflammation in human brain.

To conclude, this study showed that independent of genetic factors, obesity increases insulin-stimulated glucose uptake in caudatus and parietal cortex which is accompanied by increased resting state brain activity in regions that are part of default mode network. Regular exercise training reverses the obesity-induced increase in resting state brain activity and improves cognitive function, but has no effect on brain insulin-stimulated glucose uptake nor TSPO availability.

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Conflict of interest statement

The authors declare no conflicts of interest

Authors' contribution

Planning of the study design (JCH, TM, LL and JOR). Participant recruitment and data collection (JH, RO, MSL). PET-data analysis (JT and JH). Resting state-fMRI data analysis (LS and JH). Planning the cognitive function test (LN). Planning of the statistical analyses (EL). Responsible physician (RL). MRI scans (KK). Visceral fat mass analysis (HV). Radiotracer production [^{11}C]PK11195 (SH). Responsible of the twin cohort (KHP and JK). JH wrote the first manuscript which was then revised by JCH. The revised version of the manuscript was read, commented and approved by all authors.

FIGURE LEGENDS

Figure 1. Consort flow of the clinical Crossys study. MZ=monozygotic.

Figure 2. Overview of the clinical Crossys study protocol. ECG: electrocardiography, OGTT: oral glucose tolerance test, VO_{2peak}: peak oxygen uptake, ^{18}F-FDG: 2-deoxy-2-[^{18}F]fluoro-D-glucose, PET: positron emission tomography, CT: computed tomography, MRI: magnetic resonance imaging, rs-fMRI: resting state functional magnetic resonance imaging, [^{11}C]-(R)-PK11195: ^{11}C-labelled R isomer of [1-(2-chlorophenyl)-N-methyl-N-(1-methylpropyl)-3-isoquinolinecarboxamide].

Figure 3. Insulin-stimulated glucose uptake (GU) in A) caudatus and B) parietal cortex measured by [^{18}F]FDG-PET/CT during euglycaemic hyperinsulinamic clamp before (PRE) and after (POST) exercise intervention in heavier and leaner co-twins. C) Brain [^{18}F]PET-PET/CT -images of one representative twin-pair at baseline. Masks of caudatus and parietal cortex are highlighted as transparent white in the images. In A and B figures, twin pairs share the same color in dashed lines and solid black line depicts model-based mean with 95 % confidence intervals. P-values indicate statistical significance between leaner and heavier co-twins at baseline.

Figure 4. Brain translocator protein (TSPO) availability measured by [^{11}C]PK11195 distribution volume ratio (DVR) before (PRE) and after (POST) exercise intervention in heavier and leaner co-twins in A) white matter and B) hippocampus. C) Brain [^{11}C]PK11195-PET-images of one representative twin-pair at baseline. Masks of white matter and hippocampus are highlighted as transparent white in the images. In A and B figures, twin pairs share the same colour in dashed lines and solid black line depicts model based mean with 95 % confidence intervals. P-values indicate statistical significance between leaner and heavier co-twins at baseline.

Figure 5. Resting state brain activity (BOLD signal) measured by functional MRI at resting state. In A) blue voxels depict brain areas where heavier co-twins had higher brain activity compared with their leaner co-twins at baseline, B) blue voxels depict areas where brain activity was decreased post training, C) red and yellow voxels depict brain areas where training response was greater in heavier co-twins compared with their leaner co-twins, and D) blue voxels depict areas where brain activity was decreased post training within heavier co-twins. All data are FDR-corrected at $p < 0.05$.

Figure 6. Cognitive test results that showed statistically significant improvement post training. A) CERAD 10 Word list memory task type test from the third round: B & C) Fluid intelligence tests: B) degree of difficulty medium C) degree of difficulty hard. D) Word retrieval test, E) Word recognition test, F) Emotional sensitivity, pleasant pictures shown (reaction: 0=peaceful – 100=agitated).Twin pairs share the same color in dashed lines and solid black line depicts model based mean with 95 % confidence intervals. P-values for time indicate statistical significance for the change from pre to post in the whole sample.

Supplementary file 1. Voxel-based analysis of the baseline difference (FDR < 0.05) in insulin-stimulated brain glucose uptake (blue) and translocator protein (TSPO) availability shown as PK distribution volume (DVR) (red) between leaner and heavier co-twins (heavier co-twins > leaner co-twins).

Supplementary file 2. Voxel-based analysis of the baseline difference (FDR < 0.05) in insulin-stimulated brain glucose uptake (red) and resting state brain activity (BOLD signal) (green) between leaner and heavier co-twins (heavier co-twins > leaner co-twins).

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