Chapter 6

Therapeutic effects of dietary intervention on behavior, neuroinflammation and brain metabolism in a rat model of postoperative cognitive dysfunction

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Abstract

Postoperative cognitive dysfunction (POCD) is a common complication after surgery that can have long-lasting negative impact on the patient’s quality of life. Although the underlying mechanisms are still unknown, evidence suggests that neuroinflammation may mediate cognitive impairment following surgery. Here we confirmed the presence of neuroinflammation in a rat model of POCD and investigated the impact of the surgical procedure on brain metabolism. Nutrition is believed to affect cognition and brain metabolism and could modulate neuroinflammation. We therefore also evaluated the impact of a multi-nutrient supplementation diet containing anti-inflammatory ingredients on surgery-induced biochemical alterations in the brain and consequently on the symptoms of POCD.

Methods: As model of POCD, major surgery in humans was mimicked in rats by exteriorizing the gastrointestinal tract and clamping the mesenteric artery for 30 min. To assess the validity of the model, behavioral changes were evaluated in the first postoperative week. The effects of surgery on neuroinflammation and brain glucose metabolism were monitored noninvasively by positron emission tomography (PET) and confirmed by immunohistochemistry. To assess the effect of nutrition, rats were fed a control diet or investigational diet starting either 2 weeks before or immediately after the surgical intervention.

Results: Major surgery caused significant bodyweight loss, reduced exploratory behavior, increased anxiety and tends to decrease spatial memory. $[^{11}C]PK11195$-PET imaging and immunohistochemistry confirmed the presence of neuroinflammation in several brain regions after surgery. $[^{18}F]FDG$-PET imaging revealed both increased and decreased brain metabolism in distinct parts of the brain. Dietary intervention started after surgery reversed astrocyte activation in cerebellum and the periventricular zone had and decreased brain metabolism in the piriform cortex but it had no beneficial effect on anxiety and spatial memory. Dietary intervention started prior to surgery had a positive impact on recovery, resulting in faster recovery of body weight, normalization of exploratory behavior and spatial memory. This improvement was accompanied by reversal of astrocyte activation in the periventricular zone – but not in other brain regions – and normalization of brain metabolism in part of the motor cortex.
Conclusion: This study shows that major surgery can be accompanied by neuroinflammation and changes in glucose metabolism in several brain regions. Preventive intervention with a diet containing elevated amounts of anti-inflammatory nutrients can affect neuroinflammation and brain metabolism and has a positive effect on the recovery from abdominal surgery in rats.

1. Introduction

Postoperative cognitive dysfunction (POCD) is a complication after surgery that is characterized by postoperative deterioration in several cognitive domains, such as working memory, long-term memory, concentration, information processing and language comprehension [1,2]. The symptoms of POCD can be long-lasting and have a significant negative impact on the quality of life of patients [3].

Initially, POCD was described as a complication of cardiac surgery, but later it was shown to also occur after other types of major surgery [4]. Evidence indicates that multiple factors play a role in the development of POCD. The predominant risk factor for POCD is advanced age. Transient cognitive disturbances are observed in about 40% of the patients older than 60 and cognitive disturbances persisting for longer than 3 months are observed in around 10% of the elderly patients. Other risk factors include duration of surgery, pre-existing cerebral, cardiac or vascular disease, educational level, neurological diseases (e.g. Alzheimer’s disease) and the use of anticholinergic medication [5–8].

The pathophysiology behind POCD is not yet fully elucidated. However, increasing evidence suggest that neuroinflammation plays a pivotal role in mediating cognitive impairment following surgery [1]. Surgical procedures cause local inflammation at the site of the intervention, as well as systemic inflammation. This may result in the activation of the immune cells in the brain [9,10]. Neuroinflammation is a normal protective response of the brain, but when it is exaggerated and persists for a longer period of time, it may lead to cognitive impairment [3,11]. In patients, the levels of proinflammatory biomarkers correlate to the severity of cognitive decline [12]. The involvement of neuroinflammation in the development of cognitive decline has been supported by animal studies. Abdominal surgery in rats was described as a model mimicking major surgery in humans and was shown to cause impairment in learning and memory in young rats.
and an even more severe decline in cognitive performance and affective behavior in aged rats. These cognitive and behavioral effects were accompanied by an increase in central and peripheral inflammatory markers in both young and old animals [10,13,14].

Adequate treatment for POCD is not yet available. Since neuroinflammation is believed to be an important factor in the pathology of POCD, it could be a promising target for future therapies. Evidence suggests that nutrients may have an impact on the development of cognitive decline and neuroinflammation. Depletion of nutrients, such as B vitamins, vitamin A and D, and omega-3 fatty acids, has been associated with cognitive decline in animal models and in humans [15–19]. Moreover, studies in cells, animal models and in humans have demonstrated that food components, such as specific omega 3 fatty acids or vitamins, can ameliorate neuroinflammation and cognitive dysfunction [20–24]. A specific dietary intervention aiming to modulate inflammation might be a promising treatment strategy for POCD.

The goal of this study was to confirm the presence of neuroinflammation in a rat model of postoperative cognitive decline to investigate the effect of POCD on brain glucose metabolism, as surrogate marker for brain activity. Brain metabolism may provide more insight in the mechanisms underlying of POCD, as it may be a link in the interaction between neuroinflammation and behavior. Thus, rats underwent major abdominal surgery and brain metabolism and neuroinflammation were investigated non-invasively with positron emission tomography (PET), using $[{ }^{18} \text{F}]$FDG and $[{ }^{11} \text{C}]$PK11195 as the tracers, respectively. PET imaging is a powerful tool for monitoring of the brain physiology and neuroinflammation that can be applied both in animals and in humans and therefore facilitates translation to future clinical studies. In addition, we investigated whether dietary intervention, aimed to ameliorate the inflammatory response in the brain, can prevent or treat POCD. To this end, rats were subjected to an investigational diet containing elevated amounts of specific anti-inflammatory nutrients and the ability of the dietary intervention to reverse the effects of surgery on behavior, neuroinflammation and brain metabolism was determined.
2. Material and methods

2.1 Experimental animals

Animal experiments were performed in accordance with Dutch Regulations for Animal Welfare. All procedures were approved by the Institutional Animal Care and Use Committee of the University of Groningen (protocol DEC 5963E).

Male outbred Wistar-Unilever rats (10 weeks of age, n=32) were purchased from Harlan (Horst, The Netherlands) and housed in groups (2-6 animals per cage) in thermo-regulated (21 ±2 °C) and humidity-controlled rooms, under a 12-12 h light-dark cycle (lights on at 6:00 AM). After arrival, the rats were allowed to acclimatize for at least 7 days. All rats were fed with control diet from the time of arrival until the start of the experiment; food and water were available ad libitum before and during the experiment, and paper rolls were provided as a cage enrichment. From the day of surgery onward, animals were housed individually.

2.2 Diet

The investigational diet was based on the standard food for laboratory rodents: AIN93-G [25], which served as the control diet. Both diets were iso-caloric and were produced by Research Diet Services (Wijk bij Duurstede, The Netherlands). The composition of the investigational diet is listed in Table 1, and differed from the control diet by the presence of low-glycaemic index carbohydrates, dietary fibers, tryptophan, and a lipid profile that predominantly differed in docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA) content. Diets were stored at -20 °C until use, to prevent fatty acid oxidation.

2.3 Study design

The study design is depicted in figure 1. The rats were randomly divided in four groups (n=8 per group). Three groups were subjected to abdominal surgery combined with jugular vein cannulation, under sevoflurane-O\textsubscript{2} anesthesia. Each group was subjected to a different feeding protocol: the surgery control group (SC) was fed with control diet during the entire experiment; the surgery with postoperative dietary intervention group (SI-post) was fed with the investigational diet from the day of the surgery (day 0) until the end of the experiment (day 7); the preoperative dietary intervention group (SI-pre) was fed with the investigational
diet from 2 weeks before surgery (day -14) until the end of the experiment (day 7). A sham control group (CC) was not subjected to any surgical procedure or anesthesia and was fed with the control diet throughout the entire experiment.

Rats were weighed daily. Exploratory behavior, learning and memory were assessed with the open field, novel object recognition, and novel location recognition tests, on postoperative day 3. PET imaging of brain metabolism (using $^{18}$F-FDG) and neuroinflammation (using $^{11}$C-PK11195) was performed on postsurgical days 4 and 7, respectively. After the PET scan on postoperative day 7, the rats were sacrificed and the brains were collected for immunohistochemistry.

Due to surgical complications one rat did not survive the surgery on day 1 (from the SI-pre group), and one rat did not survive the $^{18}$F-FDG PET scan on day 4 (from the SI-pre group). During the $^{11}$C-PK11195 PET scan procedure, 7 rats did not survive the scan (2 from CC, 1 from SC and 4 from SI-post group) and in 2 rats the injected activity was too low for reliable measurement of the plasma input (1 from SI-post group and 1 from SI-pre group). Therefore, these animals were excluded from the analysis of $^{11}$C-PK11195 PET imaging, but were included in all other measurements.

2.4 Surgery

A rat model of abdominal surgery was used to mimic major abdominal surgery in humans. Surgery was performed as described previously [13]. Briefly, rats were anesthetized with sevoflurane (5% induction, 3% maintenance at 0.8 L/min) mixed with oxygen. Immediately following induction, rats received buprenorphine analgesia (0.01 mg/kg Temgesic s.c.). The gastrointestinal tract was exteriorized and the upper mesenteric artery was clamped for 30 min to restrict the blood flow in the mesenteric vascular bed. During this procedure, an indwelling catheter was placed in the jugular vein to allow repeated blood sampling. The control group of rats was not subjected to any surgical procedure or anesthesia. From the day of the surgery (day 0) until the end of the experiment, all rats were single housed.

2.5 Behavioral tests

All the behavioral experiments were performed during the dark phase and recorded on video for further analysis using Ethovision XT8 (Noldus Information Technology, Wageningen, The Netherlands) or Eline software.
2.5.1 Open field

To measure exploratory activity and anxiety, an open field test was performed on postoperative day 2, according to Schoemaker and Smits (1994). A square box (100x100x40 cm) divided into a center area (60x60 cm), 4 corner areas (20x20 cm), and 4 side areas (20x60 cm), as depicted in figure 3, was used and behavior was recorded for 5 min. The percentage of time spent in the center was determined using Ethovision and used as a measure for anxiety. The percentage of time the rats spent walking was scored manually using Eline software and used as measure for open field activity.

2.5.2 Novel object and novel location recognition

To test visual and spatial memory, a novel object and novel location test were performed on postoperative day 3 according to the procedure described by Hovens et al. (2014). Rats were habituated to the test box (50x50x40 cm) on day 2 for 5 min (see figure 4). The habituation was repeated on day 3 for 3 min. Two identical objects (plastic bottles or Lego cubes) were then presented to the rats. The animals were allowed to explore those objects for 3 min. The objects were then removed and, after a 45-s pause, during which the rat remained in the test box, one familiar object and one new object were presented to the rats for 3 min to test novel object recognition (NOR). After the NOR phase, the objects were removed and, after a 45-s pause, the objects were placed in the box again to test novel location recognition (NLR). The familiar object was placed in its original location, while the novel object was placed at a novel location. All objects were cleaned with 70% ethanol prior placement in the test box to remove smell cues.

The percentage of time each animal spent exploring each object was scored manually using Eline software. To measure NOR and NLR, the ratio between the time spent exploring the novel object or the relocated object and the total time spent on object exploration was calculated, respectively. Trials in which the animal did not explore the objects were removed from the analysis. Therefore 1 animal from SI-pre was removed from analysis of the novel object tests and 1 animal from SI-post and 2 from SC were removed from analysis of the novel location test.
2.6 PET imaging

$[^{11}\text{C}]$PK11195 with a specific activity of $>120$ GBq/$\mu$mol was produced as previously described [28]. The specific activity of $[^{11}\text{C}]$PK11195 did not differ significantly between the experimental groups ($p=0.8$). The radiochemical purity was always $>95\%$. $[^{18}\text{F}]$FDG and $[^{11}\text{C}]$PK11195 PET scans were performed using a dedicated small animal PET scanner (Focus 220, Siemens Medical Solutions, United States) at postoperative days 4 and 7, respectively. After each PET acquisition, a transmission scan of 10 min with a $^{57}$Co point source was performed and used during image reconstruction to correct for attenuation, scatter and random coincidences. Two rats from different experimental groups were scanned simultaneously in each scanning session. The body temperature of the rats was maintained with heating pads, eye salve was applied onto the eyes to prevent dehydration, and heart rate and blood oxygen levels were monitored with a BioVet system (M2M Imaging, United States).

On postoperative day 4, the $[^{18}\text{F}]$FDG PET scan was performed. First, the rats were anesthetized with sevoflurane mixed with medical air (5% induction and 3% maintenance, 0.8 L/min) and a cannula was inserted into the tail vein for the injection of the PET tracer. After the cannulation, the rats were positioned in the camera in a transaxial position with their heads in the center of the field of view. $[^{18}\text{F}]$FDG ($20 \pm 8$ MBq) was injected with a pump over a period of 1 min and a 60-min PET scan was started. There were no statistical differences in the injected tracer dose between the groups ($p=0.075$). After the scan, rats were allowed to recover from anesthesia in their home cages that were placed on a heating pad, for at least 2 h.

On postoperative day 7, a $[^{11}\text{C}]$PK11195 PET scan with arterial blood sampling and metabolite analysis was performed. The rats were anesthetized with sevoflurane mixed with medical air (5% induction and 3% maintenance, 0.8 L/min) and a cannula was inserted into the femoral vein for PET tracer injection and another cannula into the femoral artery to allow for arterial blood sampling. Then the rats were positioned in the camera in a transaxial position with their heads in the center of the field of view. $[^{11}\text{C}]$PK11195 ($55 \pm 17$ MBq, $0.66 \pm 0.29$ nmol) was injected with a pump over a period of 1 min. At the same time a 60-min emission scan was started. No significant differences between the injected $[^{11}\text{C}]$PK11195 dose of radioactivity and injected mass were observed between the
groups (p=0.30; p=0.353, respectively). After the scan, the rats were sacrificed and tissue samples were collected.

2.7 Arterial blood sampling and metabolite analysis

The \[^{11}C\]PK11195 PET scan was accompanied by arterial blood sampling. Blood samples of approximately 100 µl were collected at the following time points after injection: 10, 20, 30, 40, 50, 60, 80, 120, 180, 450, 600, 900, 1200 s. Additional blood samples of approximately 300 µl were collected at 300, 1800, 2400 and 3600 s for analysis of radioactive metabolites. After withdrawal of each blood sample, an equal volume of heparinized saline was injected to prevent large changes in blood pressure. A 25 µl aliquot of each blood sample was used to measure the whole blood radioactivity at each time point. The rest of the blood sample was centrifuged in Eppendorf-type centrifuge at 13000 rpm (15,996 g) for 5 min to collect plasma. Blood and plasma activity (25 µl each) was measured with a gamma counter (LKB Wallace, Finland).

Plasma samples from 4 time points were used for the analysis of radioactive metabolites. Each plasma sample was mixed with one third of its volume acetonitrile in order to precipitate proteins, followed by centrifugation at 13000 rpm (15,996 g) for 5 min. An aliquot of 250 µl of the supernatant was applied into a UPLC system to separate the parent tracer from its metabolites using an Acquity column (UPLC HSS T3 1.8 µm 3.0 x 50 mm, 35 °C) with acetonitrile and MilliQ water as eluent (flow: 0.8 mL/min, UV: 254 nm, Loop: 250 µL). The eluent was collected in 15 fractions of 30 s (retention time of metabolites was 30 s and 5 min, respectively; and 6.5 min for the intact tracer) and radioactivity in the samples was counted using a gamma counter. After correction for background radioactivity, the percentage of intact tracer was calculated as: (number of counts in fractions containing the intact tracer / total number of counts in all fractions) * 100%.

The percentage of intact tracer in plasma measured at 4 time points was fitted by a mono-exponential function. The radioactivity levels in plasma were corrected for the percentage of intact tracer at the respective time points. The metabolite-corrected plasma activity curve was used as input function for kinetic modelling.
2.8 PET image reconstruction and analysis

The list-mode data of the \(^{18}\text{F}\)FDG scans were separated into 2 frames (2x1800 sec), while the list-mode data of the \(^{11}\text{C}\)PK11195 scans were separated into 21 frames (6x10, 4x30, 2x60, 1x120, 1x180, 4x300, 3x600 s). Emission sinograms were iteratively reconstructed using OSEM2D (4 iterations and 16 subsets).

The \(^{18}\text{F}\)FDG PET scans were automatically co-registered with a tracer-specific template [29], spatially aligned with a stereotaxic T2-weighed MRI template in Paxinos space [30], using Vinci 4.26 software (Max Planck Institute for Neurological Research, Germany). A frame of 30 min, starting 30 min post injection was used to evaluate the differences between the groups. \(^{18}\text{F}\)FDG uptake images were compared using SPM voxel-based analysis, a method which is more sensitive than volume of interest-based approaches [31], since it allows detection of effects in part of an anatomical region. All the images were normalised for the whole brain \(^{18}\text{F}\)FDG uptake. A prerequisite for using normalized images for voxel-based analysis is that none of the groups showed any significant difference in the whole brain uptake. One-way ANOVA showed that this requirement was met (p=0.15).

The \(^{11}\text{C}\)PK11195 PET images were analyzed by pharmacokinetic modelling, using the whole blood curve and metabolite-corrected plasma curve as the input functions. Volumes of interest (VOIs) were determined using an MRI template of the rat brain [30]. Data were analysed with PMOD software 3.5 (PMOD Technologies, Switzerland), using the reversible two-tissue compartment model with \(K_1/k_2\) fixed to the whole cortex value [32], and a fixed blood volume of 3.6% [33]. The non-displaceable binding potential (\(BP_{ND}\)) of \(^{11}\text{C}\)PK11195 was calculated as \(k_3/k_4\) [34].

2.9 Immunohistochemistry

After the PET scan on postoperative day 7, the rats were sacrificed under deep sevoflurane anesthesia (5%) by transcardial perfusion with saline, followed by transcardial perfusion with 4% paraformaldehyde. Brains were dissected and immersion fixed with 4% paraformaldehyde for 24 h at room temperature, followed by cryopreservation in 30% sucrose in phosphate-buffered saline (PBS) for 24 h. Brains were stored at -80 °C until further processing. One hemisphere of each brain was cut into 10 µm thick sagittal sections, which were placed onto adhesive
microscope slides and stored at -20 °C until further analysis. The other hemisphere was cut into 30 µm thick sagittal sections and stored in PBS (0.01M phosphate buffered saline) containing 0.1% sodium azide at 4°C until further analysis.

To analyse microglia and astrocytes, 10 µm brain sections were blocked with 5% bovine serum albumin (BSA, Sigma Aldrich) in PBS for 30 min at room temperature. The primary antibody, mouse anti-GFAP (Sigma Aldrich) for astrocytes, was applied overnight (~16 h) in 1:400 dilution in PBS containing 1% BSA at 4°C. Next, the sections were washed 3 times with PBS and the secondary antibody, anti-mouse Cy3 (Life Technologies) in a 1:1000 dilution in PBS containing 1% BSA, was applied for 1 h. The sections were washed 3 times with PBS and the slides were analyzed with a TissueFAXS system (Tissue Gnostics).

The images were scored by an independent researcher that was blinded for the treatment of the animals (images were coded by numbers without any indication of the group). Morphology of GFAP cells was determined in randomly selected 0.0432 mm² sections of the Cornu Ammonis (CA) 1 region of the hippocampus, the region of the periventricular zone surrounding the lateral ventricle, and the preculminate fissure region of the cerebellum of 3-5 brain slices per rat using ImageJ software.

2.10 Statistical analysis

The statistical analysis was performed using IBM SPSS software Statistics 22 (SPSS Inc., United States). Bodyweight and metabolite analysis data are presented as mean ± standard error of the mean (SEM). Differences in bodyweight between groups at the start of the experiment were analyzed by one-way ANOVA. Bodyweight changes and [¹¹C]PK11195 metabolism were analyzed for the differences between time points and between groups with the generalized estimating equations (GEE) model with a Bonferroni post-hoc correction to account for multiple comparisons [35,36]. The exchangeable correlation matrix and the Wald test were used to calculate p-values. P-values <0.05 were considered statistically significant.

Results from behavioral tests, [¹¹C]PK11195 PET and immunohistochemistry are presented as dot-plots with a line representing the mean and error bars representing the SEM. In the results section, the median value and the 0.25-0.75 interquartile range (IQR) are reported. Due to the small sample size and not
normal distribution, differences between the groups were analyzed with non-parametric tests. First, the Kruskal-Wallis H test was used to test for a main group effect and if statistical significance was reached, the Mann Whitney U test was used for comparisons between the experimental groups.

[\(^{18}\text{F}\)]FDG PET uptake images were normalized for the whole brain [\(^{18}\text{F}\)]FDG uptake and analyzed using SPM voxel-based analysis to allow detection of effects in part of an anatomical region. [\(^{18}\text{F}\)]FDG PET results are presented as T-maps obtained from voxel-based analysis. Voxel-based analysis was performed using the SPM12 software package (Wellcome Trust Centre for Neuroimaging, United Kingdom) and the SAMIT toolbox [37]. PET images were smoothed with a 1.2 mm isotropic Gaussian kernel, global uptake differences between the rats were normalized for the mean whole brain uptake as described previously [38,39]. Mean values for the whole brain uptake were not statistically different across the groups (p=0.145). An ANOVA test was performed followed by a two-sample t-test comparison between groups to evaluate the effect of surgery and the effect of diet (CC vs. SC, SC vs. SI-post, SC vs SI-pre). The T-map data were interrogated at \(p<0.005\) (uncorrected) and a minimum cluster size threshold of 200 voxels. Only clusters with \(p<0.05\) corrected for family-wise error were considered statistically significant.

3. Results

3.1.1 The effects of surgery on bodyweight

Before surgery, the average bodyweight was 392 ±49 g and was not statistically different between groups (p=0.89), indicating that the diet by itself did not affect bodyweight. The bodyweight following surgery was calculated relative to the bodyweight of the animal on the day of surgery (experimental day 0). Bodyweight was significantly reduced (p≤0.001) during the postoperative period in SC group as compared to the control group (fig 2).

3.1.2 The effects of investigational diet on bodyweight following surgery

Bodyweight changes were affected by dietary intervention (fig 2). There were no significant differences between SC and SI-pre. However, on the last two days of the
experiment, the average bodyweight in the SI-pre group was not significantly different anymore from that in the untreated control group (CC vs SI-pre: p=0.23 at day 6; p=1.0 at day 7), while the body weight for SI-post and SC groups were significantly reduced (p≤0.001) as compared to CC during the whole postoperative period. This suggests that the SI-pre group recovered faster from the surgery. The relative body weight in the SI-post group, on the other hand, was also significantly lower than in the SC group (p≤0.037) at each time point after surgery.

3.2.1 The effects of surgery on exploratory behavior and anxiety

To investigate the impact of surgery on postoperative behavior, we used the open field test as an indication for mobility and anxiety-related behavior on postoperative day 2 (fig 3 A-C). The time spent walking (mobility) differed significantly between the experimental groups (p<0.001, fig 3B). The SC group spent significantly less time walking as compared to the CC group (CC: 36.0 s, IQR 33.8-38.7, SC: 25.9 s, IQR 20.8-26.57, U<0.001, Z=-3.258, p<0.001).

The time spent in the center of the open field (anxiety) also differed significantly between groups (p=0.016, fig 3C). Posthoc analysis revealed that surgery caused a significant reduction in the time spent in the center of the open field arena (CC: 65 s IQR, 43-82, SC: 21 s, IQR: 17-36, U=6.0, Z=-2.73, p=0.005).

3.2.2 The effects of investigational diet on exploratory behavior and anxiety following surgery

As depicted on figure 3B, postsurgical intervention with an investigational diet affected the time spent on walking. The preoperative diet significantly reduced the effect of surgery on the time spent walking (SI-pre vs. SC group, U<0.001, Z=-3.255, p<0.001). In fact, the time spent walking by the SI-pre group was completely normalized and did not differ significantly anymore from the CC group (SI-pre vs. CC, U=19.0, Z=-0.705, p=0.5). In contrast, the postoperative diet intervention was not able to reverse these effects of surgery (SI-post vs. SC group, U=15.5, Z=-1.751, p=0.083).

The pre-surgical intervention with investigational diet did not have effect surgery-induced anxiety related behavior (SI-pre 19 s. IQR 12-38, SI-pre vs. SC: U=24.0 Z=-0.46 p=0.69). The SI-post group spent more time in the center of the area than the SC group, although this difference was not statistically significant (U=26.5 Z=-
0.58 \( p=0.57 \). As a result, however, the time spent in the center of the arena by the SI-post group was not significantly different from the control group (24 s. IQR 18-61, SI-post vs. CC: \( U=16.0, Z=-1.68, p=0.11 \)), suggesting a potential trend towards a positive impact of postoperative dietary intervention on anxiety-related behavior.

In conclusion, we observed decreased mobility and increased anxiety following surgery. The decreased mobility was normalized only by pre-surgical intervention, while anxiety was not significantly changed by none of the treatment with the investigational diet.

### 3.3.1 The effects of surgery on learning and memory

On postoperative day 3, postoperative memory performance was measured using the NOR and NLR tests, in order to measure the effect of surgery on short-term visual memory and spatial memory, respectively. As depicted in figure 3D-E, there were no differences in object preferences between groups during the exploratory phase. During the tests, there were no significant differences between the groups in the NOR phase, but a significant main effect was observed in the NLR phase (\( p=0.038 \)). Post-hoc analysis revealed that the SC group showed a trend towards lower location recognition, as compared to the CC group, as this effect almost reached statistical significance (time spent exploring relocated object; CC: 75\%, IQR 63-84; SC: 63\%, IQR 50-67, \( U=9.00, Z=-1.94, p=0.06 \)).

### 3.3.2 The effects of investigational diet on learning and memory following surgery

The SI-post group did not show any beneficial effect on surgery-induced impaired spatial memory (SI-post vs. SC: \( U=11.0, Z=-1.43, p=0.18 \)), and still had a significantly lower preference for the relocated object as compared to the CC group (SI-post: 51\%, IQR 46-61, \( U=4.0, Z=-2.777, p=0.004 \)). The SI-pre group was not significantly different from the CC group (SI-pre: 62\%, IQR 58-85, \( U=24.00, Z=-0.46, p=0.69 \)) or the SC group (\( U=18.00, Z=-0.43, p=0.73 \)). No significant differences between the different dietary regimens were observed.

In conclusion, we did not observe changes in novel object recognition following surgery, while there was a trend towards reduced novel location recognition in the SC control group which reached significance in the SI-post group, but such changes were not observed in the SI-pre group.
3.4.1 The effects of surgery on neuroinflammation

Neuroinflammation was measured by PET with $[{\^{11}}C]PK11195$, a tracer targeting TSPO receptors that are expressed by microglia and astrocytes during inflammation. A statistically significant group effect in the $[{\^{11}}C]PK11195$ binding potential was found for the whole cortex ($p=0.009$), cerebellum ($p=0.025$), whereas a trend was found in hippocampus ($p=0.056$).

As depicted in figure 4, post-hoc analysis revealed that the binding potential in the cortex (CC: 0.57, IQR 0.49-1.24; SC: 2.42, IQR 1.49-3.36, $U=0.001$, $Z=-2.739$, $p=0.004$) and cerebellum (CC: 1.07, IQR 0.75-1.5, SC: 3.44, IQR 1.84-3.53, $U=1.0$, $Z=-2.402$, $p=0.016$) was significantly increased in the SC group as compared to CC. The binding potential in the hippocampus only showed a trend towards increased tracer uptake in the SC group, as compared to CC (CC: 0.4, IQR 0.21-0.68, SC: BP 1.8, IQR 0.59-1.93, $U=3.0$, $Z=-1.984$, $p=0.056$). These results indicate that surgery increased TSPO receptors density in some brain regions.

3.4.2 The effects of investigational diet on neuroinflammation following surgery

As depicted in figure 4, no differences in $[{\^{11}}C]PK11195$ binding potentials in the cortex were observed between the animals subjected to surgery alone and surgery combined with a pre-surgery dietary regimen SC vs. SI-pre ($U=7.0$, $Z=-0.516$, $p=0.71$). The post-surgery dietary intervention reduced the $[{\^{11}}C]PK11195$ binding potential in the cortex by approximately 40%, but this effect did not reach statistical significance yet (SC vs. SI-post, $U=3.0$, $Z=-1.55$, $p=0.17$).

The$[{\^{11}}C]PK11195$ binding potential in the hippocampus was not affected by either dietary intervention, as there was no significant difference between the SC group and either the SI-post group or the SI-pre group (SC vs. SI-post, $U=6.0$, $Z=-0.447$, $p=0.79$; SC vs. SI-pre, $U=6.0$, $Z=-0.447$, $p=0.79$). Dietary intervention also did not have any effect on the $[{\^{11}}C]PK11195$ binding potential in cerebellum (SC vs. SI-post, $U=5.0$, $Z=-0.745$, $p=0.57$; SC vs. SI-pre, $U=6.0$, $Z=-0.447$, $p=0.79$). Taken together, these results indicate that no significant effects of either investigational diet on the surgery-induced increased TSPO receptors density could be detected by $[{\^{11}}C]PK11195$ PET imaging.
3.5 PK11195 metabolism

In order to apply pharmacokinetic modelling for $^{11}$C]PK11195 PET imaging, it was necessary to monitor changes in $^{11}$C]PK11195 metabolism during the PET scan. Interestingly, we observed that $^{11}$C]PK11195 metabolism was significantly different between the treatment groups (p<0.001, fig 5). Tracer metabolism was significantly slower in groups subjected to the surgery than the CC group (p<0.008). Moreover, the SI-pre group displayed an even significantly slower metabolism than the SC group (p=0.035). These results indicate that both surgery and the investigational diet had a significant effect on the metabolism of the PET tracer.

3.6.1 The effects of surgery on brain astrocyte activation

The extent of astrocyte activation showed a main group effect in the hippocampus (p=0.023), cerebellum (p=0.047) and the periventricular zone (p=0.003) (fig 6), but not in the cortex (data not shown). In the hippocampus (fig 6A; SC: 230%, IQR 143-308, p=0.03) and the periventricular zone of the lateral ventricles (SC: 126%, IQR 121-129, p=0.004), the relative GFAP staining (represented as % of the optical density as compared to the CC group) was increased significantly in the SC group as compared to CC controls. In the cerebellum, surgery only induced a trend towards increased relative GFAP staining, which did not reach statistical significance (fig 6B; SC: 161%, IQR: 134-182, p=0.11).

These results demonstrate that surgery induced significant activation of astrocytes in several brain regions.

3.6.2 The effects of investigational diet on brain astrocyte activation following surgery

In the hippocampus (fig 6A), astrocyte activation was not affected by the investigational diet, as no significant differences between the SC group and the dietary intervention groups were observed (SC vs. SI-post, U=7.0, Z=-1.06, p=0.35; SC vs. SI-pre, U=11.0, Z=-0.213, p=0.91). Interestingly, the SI-post group, but not the SI-pre group, showed a significantly lower GFAP expression in cerebellum as compared to the SC group (SC vs. SI-post, U<0.001, Z=-2.309, p=0.029). In fact, the GFAP levels could be completely normalized to the level of
CC controls by the postoperative dietary intervention (CC vs. Sip-post, U=9.0, Z=-0.245, p=0.905).

In contrast, both dietary interventions significantly reversed the effect of surgery on GFAP expression in periventricular zone of the lateral ventricles (fig 6C; SC vs. SI-post, U<0.001, Z=-2.334, p=0.024; SC vs. SI-pre, U<0.001, Z=-2.745, p=0.004), indicating that dietary intervention had normalized the surgery-induced activation of astrocytes in the periventricular zone.

3.7.1 The effects of surgery on brain metabolism

Surgery caused significant changes in $^{[18]}$FDG uptake in different brain regions on postoperative day 4. As shown in fig 7 A, increased $^{[18]}$FDG uptake after surgery was mainly observed in a part of the motor cortex (peak at Paxinos coordinates: 1.7, 3.6, -3.2), pons (peaks at coordinates: -4.3, -10.8, -7.4 and 2.3, -11.0, -7.6) and amygdala (peak at coordinates: -1.5, -1.8, -7.6) of the SC group. On the other hand, a large cluster of voxels with decreased $^{[18]}$FDG uptake with a peak at Paxinos coordinates -4.9, 0.8, -1.8 was detected after surgery (fig 7 B). This cluster included part of the cortex (mainly motor cortex and somatosensory cortex) and striatum.

3.7.2 The effects of investigational diet on brain metabolism following surgery

As shown in figure 8A and B, postoperative dietary intervention did not increase glucose metabolism in any brain region as compared to the SC group, but it induced a focal decrease in $^{[18]}$FDG uptake in the cortex, mainly in the piriform cortex (peak coordinates -0.5, 2.2, 7.6). Pre-operative dietary intervention (fig 8C and D) partly reversed the effect of surgery on brain metabolism, in particular it increased glucose metabolism in the motor cortex (peak coordinates -4.3, 4.4, -11 and 4.3, 4.0, -2.4), the region in which glucose metabolism was decreased due to the surgery. Moreover, Pre-operative dietary intervention decreased $^{[18]}$FDG uptake in cerebellum (peak coordinates -3.9, -10.6, -6.6 and 2.9, -13.8, -4.0) as compared to the SC group. The data on the number of voxels affected by surgery and dietary interventions are listed in the supplementary section (supplementary data, Table 1). These results indicate that the investigational diet might partly ameliorate the effects of surgery on brain metabolism in the motor cortex and cerebellum.
4. Discussion

In this study, a rat model of postoperative cognitive decline was investigated using non-invasive PET imaging techniques in order 1) to confirm previous data on the presence of neuroinflammation and behavioral abnormalities, and 2) to investigate how brain glucose metabolism is affected. Furthermore, we assessed the potential of a dietary intervention to influence POCD via modulation of neuroinflammation in this model. For this purpose, we monitored the effects of abdominal surgery on brain glucose metabolism, neuroinflammation and behavior in rats receiving different diet regimens: 1) control diet, 2) investigational diet started immediately after the surgery and 3) investigational diet started 14 days before surgery. The diet investigated in this study was iso-caloric to the control diet and designed to target neuroinflammation, as it contains elevated amounts of components which have been described to exert an anti-inflammatory effect.

A rat model of abdominal surgery was used to mimic major abdominal surgery in humans that can lead to POCD. In accordance with previous studies [10,13], we observed that surgery induced loss of body weight, reduction of motor activity and increased anxiety. While previous studies in this model showed reduced spatial memory performance, we only observed a strong trend. This may be due to the relatively small sample size for behavioral experiments in this study.

Abdominal surgery was previously shown to induce microglia activation in rats, using immunohistochemistry [10]. In contrast to these studies, we used $^{11}$C]PK11195 PET imaging to monitor neuroinflammation in this POCD model. This allowed us to explore the inflammatory response throughout the entire brain in a non-invasive manner, rather than only in selected regions of the post-mortem brain. The PET tracer $^{11}$C]PK11195 binds to TSPO receptors which are overexpressed on the outer membrane of the mitochondria of activated microglia, astrocytes and infiltrating macrophages [40,41]. $^{11}$C]PK11195 PET confirmed that surgery induced an increase in TSPO receptors density in the cortex and cerebellum, whereas in the hippocampus a trend towards increased TSPO expression was observed. This increased hippocampal neuroinflammation is in line with previous findings from immunohistochemistry in the POCD model [10]. Neuroinflammation has not been investigated in the cortex and cerebellum before. These regions are involved in spatial memory and movement. The activity of the cerebellum is classically related to motor functions, although recent findings
indicate that it is also involved in memory and anxiety [42]. Therefore, neuroinflammation in these brain regions appears to be correlated to the observed changes in behavior.

Immunohistochemical staining of GFAP also revealed increased astrocyte activation in the hippocampus and periventricular zone after surgery, as well as a trend towards an increase in cerebellum. Iba-1 staining did not reveal any significant changes between the groups (data not shown). Therefore, $[^{11}C]PK11195$ PET imaging appears to reflect activation of astrocytes more than microglia/macrophage activation. Astrocytes play a crucial role in brain functions like memory, and the maintenance of neurons. Some studies showed that increased spatial learning performance in rats is correlated to an increased number of astrocytes in the hippocampus [43,44]. Other studies indicate a role for astrocyte activation in the pathology of brain diseases (including cognitive decline) and suggest chronic astrogliosis to be a possible target for the treatment for these disorders [45–47]. Chronic astrocyte activation has already been demonstrated in a mouse model of POCD, more than 30 days after 70% hepatectomy [48].

In this study, we also monitored glucose metabolism with $[^{18}F]FDG$ PET imaging, as this parameter has been shown to be changed in humans affected by cognitive decline [49,50] and could provide a link between neuroinflammation and behavior. Reductions in brain metabolism are often related to brain impairment, such as decreased motor activity or cognitive decline [51]. On the other hand, compensation mechanisms may occur that could lead to increased metabolism in other brain regions. In this study, $[^{18}F]FDG$ PET revealed regions with increased as well as with decreased glucose metabolism after surgery. The main effect of surgery was a decrease in $[^{18}F]FDG$ uptake in the cortex, mainly in the somatosensory and motor cortex, and to a lesser extent, in a part of striatum. Hypometabolism in the motor cortex is associated with altered motor behavior [49,52], which could be in line with the decrease in mobility observed in the open-field test. The striatum is involved in memory [53] and therefore hypometabolism in part of this brain region seems to be related to the memory impairment observed in the NLR tests. Some brain regions showed an increase in $[^{18}F]FDG$ uptake following surgery, including part of the pons, amygdala and motor cortex. The latter has a complex reaction to the surgery as in some parts of the motor cortex $[^{18}F]FDG$ uptake was increased while it was decreased in other parts. Unfortunately, the resolution of the PET camera does not allow a reliable discrimination of the individual parts of the motor
cortex involved, which hampers a more detailed interpretation of these results. The increased $[^{18}\text{F}]$FDG uptake in the pons might be correlated to the decreased activity demonstrated in the open field test. In patients with neurodegenerative diseases, increased metabolism in the pons was also correlated with altered motor behavior [51]. The amygdala is involved in spatial memory impairment under stress [54], which is in agreement with the poor performance of the SC group in the NLR test. Increased metabolism in the amygdala has also been correlated to depressive symptoms [51] which can be caused by neuroinflammation [55]. In our study, however, not all brain regions in which neuroinflammation was detected (e.g. hippocampus and cerebellum) showed surgery-induced changes in glucose metabolism, which suggests that neuroinflammation and brain glucose metabolism are not directly connected. Possibly, overall changes in brain glucose metabolism might be more correlated to the surgery-induced behavioral changes than neuroinflammation.

Several studies have shown that specific nutrients can affect neuroinflammation, brain activity and behavior. Therefore, we also investigated whether the effects of surgery observed in our model of POCD could be prevented or reversed by preoperative and postoperative intervention with investigational diet, respectively. Preoperative nutritional intervention reduced bodyweight loss and restored mobility, both indicators of faster recovery as a result of the dietary intervention. In contrast, postoperative nutritional intervention almost did not have an effect on brain glucose metabolism and behavior, whereas it even increased bodyweight loss. The latter might indicate that animals need time to adapt to a change in diet. We observed a similar drop in bodyweight for the SI-pre group, when the diet was changed on day -14 (data not shown). In this group, bodyweight normalized again within one week. Since surgery did not significantly affect memory parameters in this study, it is difficult to draw conclusions on the restorative effects of either diet on cognition. This parameter might need further investigation in a larger sample size study.

Preoperative nutritional intervention not only beneficially affected bodyweight and mobility, but also partly prevented the effects of surgery on brain metabolism in the motor cortex. This observation is consistent with the normalization of mobility of the SI-pre group, as observed in the open-field test. Postoperative dietary intervention, on the other hand, did not reverse the surgery-induced changes in brain glucose metabolism. Overall, these data suggest that administration of the
investigational diet before the surgical intervention could partly reverse the effects of surgery on bodyweight, mobility and brain activity, whereas dietary treatment started immediately after surgery did not have an effect.

When investigating the effect of dietary intervention with $^{11}$C$\text{PK11195}$ PET, we observed a significant reduction in $^{11}$C$\text{PK11195}$ metabolism as a result of surgery. This effect reduction of tracer metabolism was further enhanced by preoperative dietary intervention. $^{11}$C$\text{PK11195}$ is metabolized in the liver. This suggests that liver enzymes are affected by both surgery and dietary intervention. Blood sampling during the PET scan followed by pharmacokinetic modelling of the PET data allowed us to correct for confounders, such as changed tracer metabolism. Despite the correction for tracer metabolism, $^{11}$C$\text{PK11195}$ PET imaging could not detect any reversal of the effect of surgery on TSPO receptor density by the pre- or postoperative dietary intervention, suggesting that the beneficial effects of the diet were not due to direct inhibition of glial cell activation/macrophage infiltration.

In contrast to $^{11}$C$\text{PK11195}$ PET, GFAP staining showed that the postoperative dietary intervention completely reversed the effect of surgery on astrocyte activation in cerebellum and the periventricular zone, whereas the preoperative dietary intervention reversed the surgery-induced increase in GFAP staining only in the periventricular zone surrounding the lateral ventricles. No effects of dietary intervention on glucose metabolism were observed in these brain regions and consequently there does not seem to be a direct link between glial activation and local changes in brain glucose metabolism. Thus, diet-induced changes in brain glucose metabolism (brain activity) appear to be secondary effects resulting from changes in behavior, which seem to be caused by neuroinflammation.

Our immunohistochemistry results are in line with other studies that showed reversal or prevention of cognitive decline and astrocyte activation by treatment with anti-inflammatory nutrients [56–59]. Still it remains to be determined whether it is beneficial to inhibit astrocyte activation in the acute phase, since astrocytes are important mediators of restoring homeostasis. On the other hand, chronic astrogliosis can become neurotoxic. The first week after surgery can still be considered the acute or early chronic phase of astrocyte activation, which is necessary for restoring homeostasis. It would therefore be of interest to investigate later time points after surgery as well in future studies in order to determine the potential effects of the dietary intervention on chronic astrogliosis.
Finally, it should be noted that the diet investigated in this study is a multi-nutrient supplementation. Therefore, it likely targets multiple pathways involved in cognitive decline following surgery. To identify the individual and possible synergistic effects of the nutrients in the investigational diet and which pathways are affected by each of the nutrients would require further research with single components or multiple combinations of nutrients.

In conclusion, we showed a potential effect of a preventive intervention with a diet containing elevated amounts of specific anti-inflammatory nutrients on the recovery from abdominal surgery in rats. The beneficial effects mainly concerned changes in body weight, the metabolism of brain regions involved in motor activity and anxiety, and regulation of astrocyte activation in the cerebellum and the periventricular zone.

Conflicts of interest This study is part of the Brain Menu project and financially supported by STW-Danone Partnership Program (project number: 11650). J.M. Verkuyl and L.M. Broersen are employees of Nutricia Research and therefore declare potential conflicts of interest. All other authors report no financial interest or potential conflicts of interest.

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Figures and tables

Table 1 Composition investigational diet (on the next page)
### Investigational diet components compared to control diet (per kg diet)

<table>
<thead>
<tr>
<th>Component</th>
<th>Supplier</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Carbohydrates</strong></td>
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</tr>
<tr>
<td>dextrinized corn starch and sucrose substituted by:</td>
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</tr>
<tr>
<td>41.5 wt% maltodextrin (DE6)</td>
<td>Roquette (Lestrem, France)</td>
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<tr>
<td>15.0 wt% free galactose</td>
<td>Inalco (Milan, Italy)</td>
</tr>
<tr>
<td>42.5 wt% isomaltulose</td>
<td>Beneo-Palatinit (Mannheim, Germany)</td>
</tr>
<tr>
<td>1 wt% fructose</td>
<td>Brenntag (Dordrecht, The Netherlands)</td>
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<tr>
<td><strong>Fibers</strong></td>
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<tr>
<td>2.8% cellulose substituted by:</td>
<td></td>
</tr>
<tr>
<td>2% rice fiber RemyLiVe200</td>
<td>Beneo Orafti (Oreye, Belgium)</td>
</tr>
<tr>
<td>0.72% GOS</td>
<td>Friesland Campina (Amersfoort, The Netherlands)</td>
</tr>
<tr>
<td>0.08% Beneo Raftiline HP FOS</td>
<td>Beneo (Leuven, Belgium)</td>
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<td><strong>Proteins</strong></td>
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<td>soy protein isolate 770LN substituted by:</td>
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</tr>
<tr>
<td>1:1 soy protein isolate 770LN</td>
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<tr>
<td><strong>α-lac enhanced whey</strong></td>
<td>Arla Food ingredients (Wageningen, The Netherlands)</td>
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<td><strong>Lipids</strong></td>
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<tr>
<td>27.5 g Nissui anchovy oil</td>
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<td>6.5 g Biopure DHA IF tuna oil</td>
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<td>7.6 g soy lecithin Emulpur</td>
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<td>extra vitamins (reaching 200 % value as compared to the control diet): vitamin A, B6, B12, D2, folic acid</td>
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</table>
Table 2 The main effects of the surgery and their modulation by the investigational diet.

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<th>parameter</th>
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<td>SC vs. CC</td>
<td>SI-post/pre vs. SC</td>
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<td>Anxiety</td>
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<td>~</td>
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<tr>
<td>Spatial memory</td>
<td>(↓)</td>
<td>~</td>
</tr>
<tr>
<td>Glucose metabolism in motor cortex</td>
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</tr>
<tr>
<td>Neuroinflammation measured with [11C]PK11195</td>
<td>↑</td>
<td>~</td>
</tr>
<tr>
<td>Astrocyte expression in hippocampus</td>
<td>↑</td>
<td>~</td>
</tr>
<tr>
<td>Astrocyte expression in cerebellum</td>
<td>↑</td>
<td>↓</td>
</tr>
<tr>
<td>Astrocyte expression in periventricular zone</td>
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<td>↓</td>
</tr>
</tbody>
</table>
**Figure 1** Study design. Three experimental groups with a different dietary regimen were subjected to abdominal surgery at experimental day 0. The surgery control group (SC) was fed with the control diet for the whole experiment, the surgery group treated postoperative dietary intervention with the investigational diet (SI-postoperative) were fed with the investigational diet from the day after surgery (day 0) until the end of the experiment (day 7), and the surgery groups receiving preoperative treatment with the investigational diet (SI-pre) were fed with the investigational diet from two weeks before surgery (day -14) until the end of the experiment (day 7). The control group (CC) was not subjected to surgery or anesthesia. Behavioral tests (B) were performed on post-operative day 2 and 3, and PET scans were performed on post-operative day 4 ([18F]FDG) and day 7 ([11C]PK11195).
**Figure 2** Changes in body weight following surgery. Body weight on each postoperative day is displayed as percentage of the body weight on the day of surgery (day 0). The surgery control group (SC) and the surgery group treated postoperatively with the investigational diet (SI-post) were significantly different from the control group (CC) at all time points after surgery (day 1-7). The surgery groups receiving pre- and postoperative preoperative treatment with the investigational diet (SI-pre) had significantly different body weight as compared to the controls at days 1-5, while at days 6 and 7 this difference was not statistically significant anymore. *p<0.05 for SC, SI-post and SI-pre when compared to CC (GEE model).
Figure 3 Behavioral tests. Open field; a scheme of the open field arena indicating the corners, side areas and central part (A), the time spent walking (B) and the time spent in the center of the open field (C). Novel object (NOR) and novel location recognition (NLR): the time (%) spent on the exploration of the left object during the exploration phase (D), the time (%) spent on exploration of the novel object (E), and the time (%) spent on exploration of the relocated object (F). CC=control group, SC=surgery + control diet, SI-post=surgery + postoperative dietary intervention SI-pre= surgery + preoperative dietary intervention p < 0.05, **p<0.01, ***p<0.001
Figure 4 Neuroinflammation on postoperative day 7: Neuroinflammation was assessed by comparing the binding potentials determined by [11C]PK11195 PET in: A cortex, B hippocampus and C cerebellum in groups subjected to the surgery and different dietary treatment, significant differences were observed, as compared to CC, as indicated with * (p < 0.05). CC=control group, SC=surgery + control diet, SI-post=surgery + postoperative dietary intervention SI-pre= surgery + preoperative dietary intervention. D An example of a transaxial, coronal and sagittal [11C]PK111195 PET image of the brain of a rat from SC group and its overlay with an MRI-based template with selected regions for analysis, as indicated by different colours and the numbers: 1=cortex, 2=olfactory bulb, 3=prefrontal cortex, 4=septum, 5=caudate putamen, 6=hippocampus, 7=cerebellum, 8= corpus collosum, 9=amygdala, 10=thalamus, 11=hypothalamus, 12=pons, 13=medulla.
Figure 5 Radioactive metabolites derived from [11C]PK11195 measured in plasma on postoperative day 7. Differences in tracer metabolism in rats subjected to the surgery and different dietary interventions as compared to CC were observed, as indicated by * (p < 0.05). Significant differences as compared to the SC group are indicated by # (p<0.05). CC=control group, SC=surgery + control diet, SI-post=surgery + postoperative dietary intervention SI-pre= surgery + preoperative dietary intervention.
**Figure 6** Astrocyte activation: GFAP staining of astrocyte activation in: A hippocampus, B cerebellum, and C periventricular zone. Astrocyte activation is assessed by area coverage of GFAP staining in the region of interest. The data are displayed as percentage of CC. Statistically significant differences compared to CC are indicated by * (p < 0.05). Significant differences as compared to the SC group are indicated by # (p<0.05). CC=control group, SC=surgery + control diet, SI-post=surgery + postoperative dietary intervention SI-pre= surgery + preoperative dietary intervention.
Figure 7  Brain metabolism on postoperative day 4: Sagittal, coronal and transverse projections of the brain regions with voxels representing a significant increase (A) or decrease (B) in [18F]FDG uptake in the brain of animals subjected to the surgery as compared to the control group. Only significant differences between the groups are displayed. CC=control group (n=8), SC=surgery + control diet (n=8). C an example of a transaxial and sagittal [18F]FDG PET image of the brain of a rat from SC group and its overlay with a region used for voxel-based analysis (representing the whole brain).
Supplementary information

Table 1 Brain metabolism on postoperative day 4. Table shows the clusters with significantly increased (left) or decreased (right) [18F]FDG uptake in the brains of rats subjected to surgery as compared to the control animals or rat that underwent surgery and received dietary intervention as compared to rats that received surgery alone. The voxels are organized in clusters, which may be spread over (parts of) multiple brain regions. The peak of each cluster (coordinates with highest significant difference) is listed next to the cluster number. (on the next page)
## Dietary intervention in POCD

<table>
<thead>
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<th>DECREASE</th>
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<tr>
<td></td>
<td>±0.1701</td>
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References


[14] Hovens IB, van Leeuwen BL, Nyakas C, Heineman E, van der Zee EA, Schoemaker RG. Prior infection exacerbates postoperative cognitive dysfunction in
Dietary intervention in POCD


[28] Doorduin J, de Vries EFJ, Willemsen ATM, de Groot JC, Dierckx RA, Klein HC.


[41] Doorduin J, de Vries EFJ, Dierckx RA, Klein HC. PET imaging of the peripheral benzodiazepine receptor: monitoring disease progression and therapy response in


