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Contrasted central effects of n-3 versus n-6 diets on brain functions in diet-induced obesity in minipigs

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Abstract

Introduction: N3 polyunsaturated fatty acids (n-3 PUFAs) exert anti-inflammatory effects for the hypothalamus, but their extra-hypothalamic outcome lack documentation. We evaluated the central consequences of the substitution of saturated fatty acids with n-3 or n-6 PUFA in obesogenic diets.

Methods: Twenty-one miniature pigs were fed *ad libitum* obesogenic diets enriched in fat provided either as lard, fish oil (source for n-3 PUFAs), or sunflower oil (source for n-6 PUFAs) for ten weeks. The blood-brain barrier (BBB) permeability was quantified by CT perfusion. Central autonomic network was evaluated using heart rate variability, and PET 18FDG was performed to assess brain metabolism.

Results: BBB permeability was higher in lard group, but heart rate variability changed only in fish oil group. Brain connectivity analysis and voxel-based comparisons show regional differences between groups except for the cingulate cortex in fish oil vs. sunflower oil groups.

Discussion: The minute changes in brain metabolism in obese pigs feed with fish oil compared with saturated fatty acids were sufficient to induce detrimental changes in heart rate variability. On the contrary, the BBB's decreased permeability in n-3 and n-6 PUFAs groups was protective against an obesity-driven damaged BBB.

Keywords: polyunsaturated fatty acids; brain blood barrier; brain connectivity; brain metabolism; diet induced obesity; miniature pig.

Introduction

n-3 PUFAs have beneficial effects on learning and memory, enhance synaptic plasticity and neurogenesis (1), and regulate microglia function (2), (3). They also increase glucose uptake in non-human primates (4), (2). However, these effects were obtained during either n-3 PUFAs supplementation or depletion in an otherwise balanced, non-obesogenic diet (5). Conversely, the central effects of n-3 PUFAs have been poorly studied in the context of obesity and western diets, with the notable exception of the hypothalamus (6). A meta-analysis of 9 randomized clinical trials, concluded to no effect on body mass index in obese (7) but DHA supplementation, a provider of n-3 PUFAs, increased prefrontal cortex activity (8), an area with reduced activity in obese patients (9). Obesity-induced brain inflammation has been partially reversed by dietary supplementation with n-3 PUFAs in mice (10), further supporting the possibility of a possible protective effect of n-3 PUFAs towards the deleterious effects of obesity on the brain (2). Otherwise, obesity causes changes to several cell types belonging to the neurovascular unit that ultimately alter the blood-brain barrier (BBB) integrity (11). However, in HF fed rats (12), these alterations were variable depending on the brain structure ranging from increased permeability in the hippocampus to unchanged permeability in the prefrontal cortex and the striatum. Conversely, increased permeability of cortical structures only (13) was reported in rabbits fed a HF diet enriched in cholesterol. Equally conflicting results were reported for the impact of n-3 PUFAs on the BBB permeability. Hong et al. demonstrated that DHA supplementation in rats (5 mg/kg) diminished BBB damage induced by middle cerebral artery occlusion (14). Conversely, Yang et al. using the same model of BBB damage established a detrimental effect of DHA (dose of DHA is not reported) with an increase in cerebral ischemia/reperfusion-induced alterations (15). Finally, in another study, n-3 PUFAs were reported to have no effect or contrasted effects depending on the brain structure: positive in the paraventricular nucleus of the hypothalamus, but negative in the anterior pituitary (6).

The present study aimed to assess the cerebral consequences of the long-term substitution of saturated fatty acids (SFAs) with either n-3 PUFAs from fish oil or n-6 PUFAs from sunflower oil within Western-type diets (16). For that purpose, we used the adult Pitman-Moore miniature pig, an animal model known to rapidly develop obesity and insulin resistance in response to a high-fat/high-sucrose diet (17) and to have a relatively large brain with well-defined cerebral circumvolutions (18). We hypothesized that n-3 PUFAs substitution would

antagonize various facets of brain function as altered by obesity, leading to (i) a region-dependent restoration of the BBB, (ii) an improvement of the reduced prefrontal or limbic glucose metabolism (19), and (iii) a reconditioning of the metabolic network (20). Accordingly, BBB permeability was measured using perfusion CT (21) and analyzed using impulse residue function to extract the quantitative parameters CBF and CBV that represent the gold standard to investigate BBB permeability (22). The brain metabolism was obtained region wise using PET ¹⁸FDG imaging (23) analyzed for regional activations using spatial parameter mapping and metabolic connectivity by network analysis (24). The functional impact of these central changes was investigated with frequency analysis of the heart rate variability, a function of the vagal dorsal complex modulated by higher brain centers (25), and which is a proxy of the autonomic dysfunction in central obesity (26).

Materials and methods

The experiment was conducted in accordance with the current ethical standards of the European Community (Directive 2010/63/EU), Agreement No. A35-622 (now C35-275-32). The Regional Ethics Committee in Animal Experimentation of Brittany (France) has validated and approved the entire procedure described in this paper (R-2011-MO-01).

Animals

A total of 21 female Pitman-Moore miniature pigs, aged 240 days and weighing 23.6 ± 0.60 kg at the beginning of the experiment were used. The exclusive usage of female animals was dictated by possible gender differences in PUFAs metabolism (27). The animals were ovariectomized before the beginning of the experiment as they were likely to go into one or two heat during the length of the study and because the ovarian cycle induces large variations in brain metabolism (28) and changes in DHA brain concentration (27). The surgery was performed in laparoscopic conditions to limit the experimental bias associated with an open abdomen laparotomy (29). The animals were allowed to recover from surgery during two weeks before the onset of the experiment. The animals were housed individually in 2m² cages, enriched with toys to encourage physical exercise. Free visual but limited physical contact between animals was permitted by the design of the cages.

Experimental diets and protocol

The animals were fed *ad libitum* once daily at 9:00 a.m. with one of the three diets depending on their assigned experimental group. Refusals were removed daily and weighted. Water was given *ad libitum*. All the diets supplied had an identical energy density (18.01 MJ/kg of feed) (Table 1). They were supplied in about 1.5 times the recommended energy intake of 1.2 MJ/kg BW^{0.75} (30) for the duration of the experiment. The experimental diets were designed to generate large differences in n-6 and n-3 PUFAs contents (Table 2) while maintaining about 13 % of crude fat, which is the maximum allowed in pig diet in the context of HF diet without reduction in diet palatability and for making possible the feed granulation.

The animals were divided in three groups (n = 7 each): lard, fish oil (supplying n-3 PUFAs), and sunflower oil (supplying n-6 PUFAs). They were weighed weekly. After 10 weeks of the regimen, CT-scan imaging was performed to evaluate fat deposition and fat-free mass. During

the same imaging session, contrast medium was also administered in a peripheral vein to challenge the permeability of the BBB that was quantified using CT-based perfusion maps, and heart rate was recorded to evaluate autonomic balance. One week after, brain PET imaging was performed to assess glucose metabolism after the administration of ^{18}F FDG, a glucose analog radioactive tracer.

HRV measurements

Heart rate variability (HRV) was analyzed from 5-minute-long electrocardiographic recordings obtained using II derivation (17). Briefly, the electrocardiogram (ECG) was recorded using an ECG machine (GE, USA) in a driven right leg configuration and digitized at 400 Hz (NI-USB-6008, National instruments, USA) after low pass filtration (analog Butterworth filter set at 200 Hz) to cancel aliasing. The digitized signal was streamed continuously as a TDMS (Technical Data Management System) file and analyzed afterward with Labview[®] biomedical toolkit (National instruments, USA). After QRS recognition, the RR intervals were detrended and analyzed in the frequency domain with an autoregressive algorithm to extract low (LF - 0.04 to 0.15 Hz) and high (HF - 0.15 to 0.40 Hz) frequency components and their ratio. LF (related to sympathetic activity) and HF (related to parasympathetic and notably vagal activity) components were normalized according to the total power of the autoregressive spectrum calculated between 0 to 0.4 Hz.

CT scanning

CT scanning was performed in isoflurane-anesthetized animals under artificial ventilation (AS3, GE, France). Minimal alveolar concentration (MAC) of isoflurane was set to 1.6 to reduce the possible changes in CBF due to the anesthetic compound and to cancel the inter-subject variations. Ventilation parameters were adapted for a spO₂ of 98% and a spCO₂ of 4.5%.

CT scans for abdominal fat assessment were achieved according to previously validated method (31) using the same CT machine as the one used afterwards for BBB permeability measurement. Scanning was performed using a Hipped Nx/i CT (GE, France) with the animal in ventral recumbency. A pre-heated low-osmolarity nonionic iodine contrast medium (Optiject 300, Guerbet, France) was injected intravenously by a power injector (Angiomat 6000, Liebel-Flarsheim, USA) as a short bolus of 50 ml (4 ml/sec) through a catheter in the

marginal vein of the ear followed by 20 ml of saline solution. First, a non-enhanced axial CT was performed initially to locate the adequate slice for dynamic scan. This slice was centered to the thalamus for later identification of the anterior cerebral artery, which was used for calculation of the arterial input function (32). Second, CT cine mode was performed to dynamically acquire 50 thick slices (1 sec per slice, 6 mm thick, 120 KV, 200 mA). The contrast medium was injected 5 seconds before initiating the dynamic scans.

CT-scan image analysis

Each brain slice was reconstructed into a 512 x 512 matrix using a smooth kernel filter. Voxel dimensions were anisotropic 0.15 x 0.15 x 6 mm³. CBF, CBV and MTT were calculated semi-automatically using an in-house software written under Labview/IMAQ 2015 IDE (National Instruments, USA). Briefly, perfusion maps were created using a Fourier transform-based deconvolution analysis of the arterial input function with the tissue concentration of contrast medium (33). To quantify perfusion maps, it was assumed that contrast agent concentration was proportional to X-ray attenuation value with a proportionality constant of 1g/mL/HU. Similarly, after automatic segmentation of extra-cerebral tissue, baseline scans were subtracted to perfusion scans assuming a brain density of 1.04 g/mL. Partial volume effects in the arterial input function were corrected using rescaling of the arterial input function with peak concentration value within the interior sagittal sinus.

The perfusion maps were co-registered to a 3D CT scan template/brain atlas template (34) resliced to the same area and voxel matrix size than the one used for perfusion maps. This allowed obtaining quantitative perfusion parameters for 7 brain areas: frontal, insular, occipital, parietal and temporal cortices plus part of the striatum and the anterior part of the thalamus. Cerebral blood flow (CBF), cerebral blood volume (CBV) and mean transit time (MTT) images were computed and their values averaged for all the 7 brain areas.

PET scanning

After a 12-h fasting period, the animals were anesthetized using the same conditions than those described for CT-scan measurements. The animals were placed in ventral recumbency on the bed of a whole-body high-resolution PET and a venous catheter was inserted into their left ear in order to inject the radiolabel. The ears and eyes of the animals were sealed with cotton and surgical tape, respectively, to minimize auditory and visual stimulations. The body

temperature was maintained at 38°C by a heating blanket.

The radiolabel (^{18}F FDG, 200 MBq, IBA France) was injected 45 min before PET imaging. PET data were acquired on a CTI/Siemens HR+ Scanner in 3D mode (Siemens ECAT, HR+). A 30-min 3D emission scan was performed using an axial field of view of 15.52 cm. It was corrected for attenuation by a 15-min transmission scan using rotating ^{68}Ge rods. Following scatter, dead time and random corrections, PET transaxial images were obtained by iterative reconstruction using a ramp filter (Kernel FWHM = 6 mm) providing 63 contiguous slices. Spatial resolution after reconstruction was 0.64 mm per pixel in the x and y directions and 2.42 mm per pixel in the z-axis. Pixel depth was encoded using the Standard Uptake Value (SUV) method.

PET images analysis

Images were normalized using normalized mutual information build in PMOD (Pmod Switzerland) with the pig FDG template co-registered with pig brain atlas (34). Due to the anatomical peculiarities of the pig skull, co-registration was performed using data masking. The binary mask was manually drawn for each data set using the masking feature of PMOD. Once co-registered, the images were normalized to the mean SUV of the cerebellum grey matter for each subject (35). We selected the cerebellum grey matter as a reference tissue because its CMRglu , obtained from time-activity curves analysis, was unaffected by obesity in miniature pigs, unlike whole brain CMRglu (17, 20). The images were either analyzed pixel-wise using statistical brain mapping or VOI-wise (volume of interest) engulfing large brain regions to increase statistical power while minimizing type-2 error. The VOI were selected according to those successfully investigated in obese pigs (19) and obese glucose-intolerant humans (36). Collectively, the cumulative volume of these 7 VOI's accounted for 79% of the cerebrum grey matter. VOI-based analysis was performed after partial volume correction based on the method for PET brain studies (37). Differences at the voxel level were analyzed using SPM (SPM 12, Wellcome Trust Centre for Neuroimaging, London, UK) with differences between diet groups being investigated using a One-way (1 x 3) between-subjects ANOVA model. A significance level of $P \leq 0.001$ with FDR (False detection rate) correction was used to exclude random brain activation (cluster size = 50 voxels).

Metabolic connectivity analysis (38) was performed using NetPET software on quantitative

CMRglu images to identify significant network components (NetPET V1, courtesy of L. Moro, M. Veronese and M. Arcolin). Brain networks were visualized with BrainNet Viewer (39) and the circular graph subroutine (courtesy of P Kassebaum). Brain connectivity matrices were thresholded using the corresponding probability matrices for $P \leq 0.001$.

Statistical analysis

Data are presented as means \pm SEM. $P < 0.05$ was considered as significant except for brain image analysis (see below). Data were compared using one and two-way analysis of variance depending on the dataset using Prism 8 (Graphad, USA) unless otherwise notified. Time-dependent analyses (changes in body weight) were corrected for multiple comparisons using the Sidak test and T-values were obtained from Tukey pairwise comparison tests performed in Stata 14 (StataCorp, USA). Body weight was analyzed by Ancova with final weight as the dependent variable and pre-diet weight as the covariable. The same procedure was also used for CT-based abdominal fat and lean mass measurements.

Results

Body composition

The body weight of the pigs steadily increased during the 12 weeks of the dietary intervention from 23.6 ± 0.60 kg to 41.7 ± 0.82 kg. This 43.4 % increase was not significantly different between groups. A slight but significant difference between PUFA's groups and SFA's group was observed at 63 and 70-days after the beginning of feeding the experimental diets ($P = 0.0419$ and 0.0161 , respectively) but it was not observed anymore 77 and 84 days post intervention ($P = 0.3930$ and 0.0508 , respectively). After 12 weeks of dietary intervention, visceral fat mass, and lean mass (representing the cumulative volumes of the *gluteus medius*, *longissimus dorsi*, *iliocostalis*, *psoas* and *serratus ventralis* muscles) evaluated by CT-scans were similar between the 3 groups (Figure 1).

Heart rate variability

After dietary intervention, heart rate was not different between groups (92 ± 5.6 , 88 ± 5.7 and 99 ± 4.0 beat per minute for lard, fish oil and sunflower oil respectively, $P = 0.327$). However, the LF component of the variability spectrum was significantly higher for the fish oil group (Figure 2, $P = 0.048$) while it was not significantly different between the lard and sunflower oil groups ($P = 0.971$). There was no difference for the HF component ($P = 0.083$). Therefore, the LF/HF ratio reflected a sympathetic LF component significantly higher for the fish oil group ($P = 0.043$).

Blood-brain barrier (BBB) permeability

Cerebral blood flow (CBF), *i.e.* the instantaneous capillary flow in the brain parenchyma as a whole, was significantly modified by the diet ($P < 0.001$) but this was not region-specific ($P > 0.999$). The same feature was also observed for CBV for which the modifications were also not region-specific. Mean CBF and CBV measured for all brain areas were about doubled for lard group as compared to fish oil and sunflower oil groups (Figure 3). There were no differences between fish oil and sunflower groups for CBF and CBV ($P = 0.535$ and 0.610 respectively). Mean transit time, which measures the length of time a certain volume of blood spends in the cerebral capillary circulation, was not different between groups ($P > 0.999$) or between brain areas ($P > 0.999$).

Glucose brain metabolism

Ancova analysis of voxel-wise linear comparisons between the three diet groups did not show any difference at $P < 0.001$ FDR corrected. Using a smaller yet physically realistic cluster size (cluster size = 30 instead of 50 voxels) or removing grand-mean correction were equally ineffective to identify activated or de-activated clusters. T-test comparisons were also unable to point out significant brain activation with the notable exception of fish oil group that exhibited a significant activation of the dorsal right cingulate cortex ($p=0.001$) in comparison to the sunflower group (Figure 4).

Volume of interest (VOI) analysis, a more powerful statistical analysis at the expense of spatial localization, was also unable to identify a statistical difference between glucose metabolism irrespective of the area under scrutiny. Nevertheless, the glucose uptake was significantly greater for the fish oil group compared to sunflower oil group for the cerebrum, *i.e.* the encephalon without the cerebellum (Figure 4). This suggests that the metabolic alterations, if any, were more widely distributed in the brain.

Metabolic connectivity analysis showed that all networks were thalamic-centric irrespective of the group. However, significant changes in networks were observed between groups (Figure 5). For the lard group, the network engulfed limbic structures encompassing the putamen and the insula. For the fish oil group, projections of the network were observed in the right cingulate cortex whereas the network remained thalamic-centric for the sunflower oil group.

Discussion

Summary of main findings

This is the first study that aimed at quantifying comprehensively the effects of large amounts of dietary PUFAs fed chronically on brain function in an obesogenic diet context. We demonstrated that a diet rich in n-3 PUFAs was associated with a significant change of the heart rate variability (HRV), indicating a parallel modification of the autonomic tone. The n-3 and n-6 PUFA-containing diets were equally effective to restrict the BBB permeability compared to the SFAs-rich diet. Despite these large changes in BBB permeability, the brain glucose metabolism was almost not regionally modified unlike the metabolic networks that presented extensive variations.

Changes in heart rate variability

While primarily controlled by the dorsal vagal complex, substantial evidence showed that HRV correlates with the central autonomic network, particularly the cingulate cortex, the insula, and the putamen (40, 41). Unsurprisingly, the same structures were found to be part of the network instantiated in fish oil group while LF/HF was also primarily modified in the same group. Indeed, we found that a n-3 PUFAs-rich diet was associated with a resting network extending to the cingulate cortex, a structure that was also found activated when compared with sunflower oil group. Altogether, these data demonstrate that the replacement, within a high-fat diet, of SFAs by a large amount of n-3 PUFAs was able to modify brain metabolism and function while in a resting and unstimulated condition during brain imaging. Such unconsidered feature so far (42) makes crucial the nature of the diet-related HRV change: an increase in LF spectral component. The engagement of LF component, presumed to represent the sympathetic activity, in the n-3 PUFAs obese animals is opposite to the increase in HF observed by Ninio et al (43) in DHA/EPA supplemented obese patients both at rest and during sub-maximal exercise. This is also at odds with our previous observations in humans, when we showed that n-3 PUFAs from fish oil decreased sympatho-adrenal activity in healthy humans submitted to a mental stress on one hand (44), and in haemodialysis patients on the other (45). The n-3 PUFAs-induced increase in LF in our obese minipigs could be detrimental if extrapolable to obese patients with a risk for coronary artery disease (46). However, the usefulness of LF/HF ratio as a predictor for heart disease in the context of obesogenic diet relies on incomplete epidemiological evidence (26).

Alterations of the brain blood barrier

In small animals, brain blood barrier integrity is classically inferred from brain histology after a carotid infusion of Evans blue, a simple solution but with severe limitations, especially for the BBB permeability quantification (47). We used perfusion computed tomography associated with compartmental modeling as a more accurate proxy since it is sensitive enough to detect permeability default in inflammatory diseases without the limitations of dye injection (48). The analysis of the fate of the tracer within the brain enable us to extract three parameters which are equally essential to characterize the permeability of the BBB: CBF represents the instantaneous capillary flow in the brain, CBV describes the blood volume of the cerebral capillaries and venules per cerebral tissue volume and MTT measures the length of time a particular volume of blood spend in the cerebral capillary circulation. The larger CBF in the lard group compared to the low-SFAs groups was expected since it reflected BBB integrity that is challenged, particularly at the cortical level in some animal models of obesity (49). The paralleled CBV increase in the lard group was novel information since we were unaware of such measurement in obese animals or patients. Taken together with the increase in CBF, CBV changes were a quantitative objective translation of brain hyper-perfusion (50). The increased flow was somewhat unexpected since, in obese type-2 diabetes patients, several studies have reported a decrease in CBF (51). However, these reports were based on SPECT and ASL imaging that concentrate more on the transfer coefficients of water than on microcrystalline solutions such as the one used for CT-based CBF measurements (52). Nevertheless, both fish oil and sunflower oil diets were able to reduce significantly CBF and CBV compared to the standard high-fat western diet, confirming the protective role of unsaturated fat on the BBB permeability (53). Unfortunately, it is not possible to correlate these changes with the underlying cellular and metabolic processes responsible for the BBB permeability, i.e., the interplay between the intracellular and trans-membrane tight junctions, the enzymatic-driven metabolism of blood-borne products, and the numerous transport systems (21).

Modifications of brain metabolism and networks

The small changes in brain metabolism induced by the otherwise significant differences in the origin of fat in the experimental diets were not expected. To ascertain the robustness of the otherwise small deviations in glucose metabolism between groups, we tested several statistical strategies. They included VOI-based measurement that were thought less prone to type-II error

and were statistically more powerful at the expense of spatial accuracy than spatial parameter mapping. The converging results from these multiple analytic sources argue for the lack of localized alteration of brain metabolism. On the contrary, we found substantial changes between groups in the metabolic connectivity, which spread towards the insula and the putamen for lard group compared to the low-SFAs groups. Similar changes have been already described in obese animals (20) and patients (54) supporting the role of an altered communication with the reward structures that, in turn, account for the obesity-related alterations in the reward processing of food stimuli (55). Cingulate cortex expansion of the otherwise thalamic-centric network, present in all groups was identified in n-3 group. Altered connectivity was observed also in obese patients between the anterior cingulate cortex and the thalamus using resting state functional magnetic resonance imaging (fMRI) (56). Since fMRI was able to identify the direction of the change, it was possible to confirm that obesity reduced the activity of the cingulate cortex. On the contrary, in healthy older adults, dietary fish oil supplementation increases BOLD signal in the posterior cingulate cortex during higher working memory load, suggesting enhanced neuronal response to working memory challenge (57). A reverse relationship was found in healthy adults with DHA supplementation resulting in a negative correlation between the amount of circulating DHA and the glucose uptake by the anterior cingulate (58). Unfortunately, our analysis of brain network is semi-quantitative compared to what could have been performed using dynamic PET or fMRI (59). We acknowledge that the changes in brain glucose metabolism might be underestimated because we used static PET image recorded 45 minutes after the radiolabeled compound injection instead of a dynamic PET sequence (60). Nevertheless, in grey mouse lemur, a species for which a tridimensional brain atlas is missing hence limiting interpretation, n-3 PUFAs supplementation increased the brain glucose metabolism in a non-regional specific manner (61).

Limitations of the study

The amount of PUFAs contained in the experimental diets is arguably large compared to the dose used in dietary interventions in patients with neurodegenerative diseases or mood disorders (2). Nevertheless, the EFSA Panel on Dietetic Products, Nutrition and Allergies stated that «the available data are not sufficient to establish a tolerable upper intake level for n-3 PUFA (DHA, EPA, and DPA, individually or combined) for any population group» (62). Based on mean daily food intake of 400 g, the amount of EPA supplied to pigs in fish oil group was in the same range that reported in high EU consumers of fatty fish (3,5 versus 2,7 g per

day). Furthermore, the dose of DHA consumed by the fish oil group is also close that found without toxicity concerns (6 g per day) in humans (63).

Implications for future research and clinical practice

In conclusion, the permeability of the BBB in n-3 and n-6 PUFAs groups was about halved compared to the saturated fat group. This protective pattern against an obesity-driven damaged BBB was not region-specific and was probably at the origin of the insubstantial regional changes of brain metabolism. Despite the absence of focal brain activation in n-3 PUFAs group characteristics of the obese brain, the extension of the resting network towards the cingulate cortex could explain an alteration of heart rate variability with an increase in LF spectral component. The large amount of PUFA present in n-3 diet, while protecting against the damage of the BBB, could be detrimental for heart function. The importance of the heart rhythm dysfunction in the context of long-term substitution of saturated fatty acids with n-3 PUFAs in Western-type diets warrants further investigations on heart perfusion and metabolism.

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Guarantor's statement: Charles-Henri Malbert is the guarantor of this work and, as such, had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

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Authors' contribution - C-H.M. planned the experiments, conducted the studies, analyzed the data, and was primarily responsible for writing the manuscript. D.V-L. conducted the studies and contributed to writing the manuscript. P.M. contributed to the analysis of the data. J-P.L planned the experiments, formulated the experimental diet and contributed to writing the manuscript. J.D. contributed to writing the manuscript.

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Table 1: Composition and nutritional value of the experimental diets

| Composition (%) | | | |
|----------------------------|------|----------|---------------|
| | Lard | Fish oil | Sunflower oil |
| Wheat | 45 | 45 | 45 |
| Soybean meal | 17 | 17 | 17 |
| Lard | 12 | - | - |
| Fish oil | - | 12 | - |
| Sunflower oil | - | - | 12 |
| Corn starch | 11 | 11 | 11 |
| Beet pulp | 5.1 | 5.1 | 5.1 |
| Cane molasses | 5 | 5 | 5 |
| Beet molasses | 2 | 2 | 2 |
| Calcium carbonate | 1.7 | 1.7 | 1.7 |
| Oligoelements and vitamins | 0.5 | 0.5 | 0.5 |
| Sodium chloride | 0.3 | 0.3 | 0.3 |
| Dicalcium phosphate | 0.4 | 0.4 | 0.4 |

Nutritional value (calculated; % or MJ/kg)

| | | | |
|----------------------|-------|-------|-------|
| Metabolisable energy | 18.01 | 18.01 | 18.01 |
| Net energy | 11.79 | 11.79 | 11.79 |
| Dry matter | 88.3 | 88.3 | 88.3 |
| Crude protein | 13.37 | 13.37 | 13.37 |
| Crude fat | 13.13 | 13.13 | 13.13 |
| Cellulose | 2.92 | 2.92 | 2.92 |
| Minerals | 5.67 | 5.67 | 5.67 |

Table 2 - Measured fatty acids (FA) composition of the three experimental diets

| FA % of total FA | Lard | Fish oil | Sunflower oil |
|-------------------------|-------------|-----------------|----------------------|
| Crude fat | 12.7 | 11.9 | 12.3 |
| Saturated FA | 35.4 | 28.1 | 11.7 |
| C18:0 | 9.9 | 4.6 | 3.69 |
| C16:0 | 23.9 | 18.5 | 7.35 |
| C18:1 (OA) | 42.0 | 14.5 | 26.1 |
| PUFA | 16.5 | 47.2 | 60.3 |
| C18:2 n-6c (LA) | 14.4 | 8 | 59.7 |
| C18 :3 n-3 (ALA) | 1.29 | 1.06 | 0.56 |
| C18 :4 n-3 | 0.04 | 1.11 | 0 |
| C20:5 n-3 (EPA) | 0 | 7.5 | 0 |
| C22:6 n-3 (DHA) | 0 | 23.8 | 0 |
| Total n-6 | 14.7 | 10.2 | 59.8 |
| Total n-3 | 1.6 | 36.8 | 0.6 |
| n-6/n-3 | 8.9 | 0.3 | 107.4 |

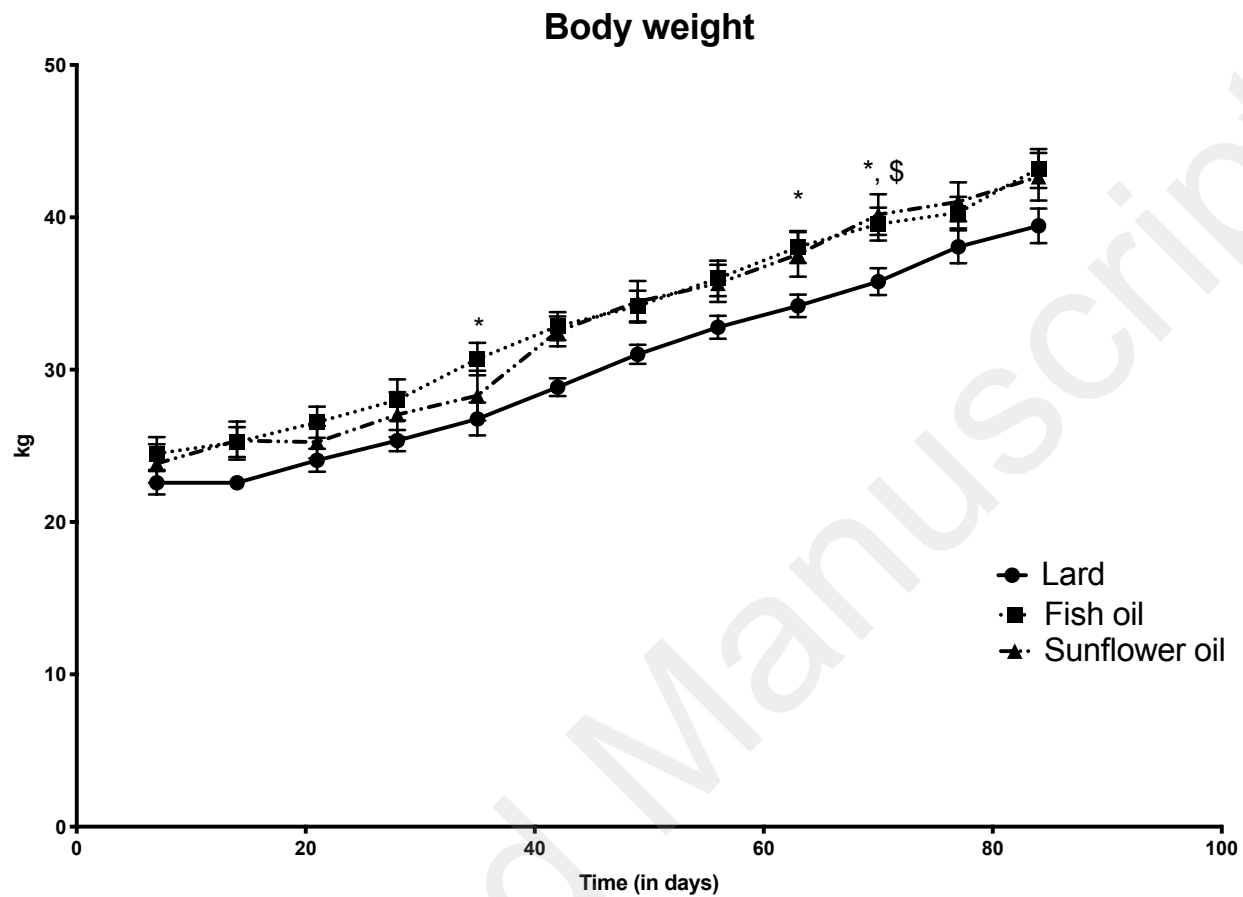
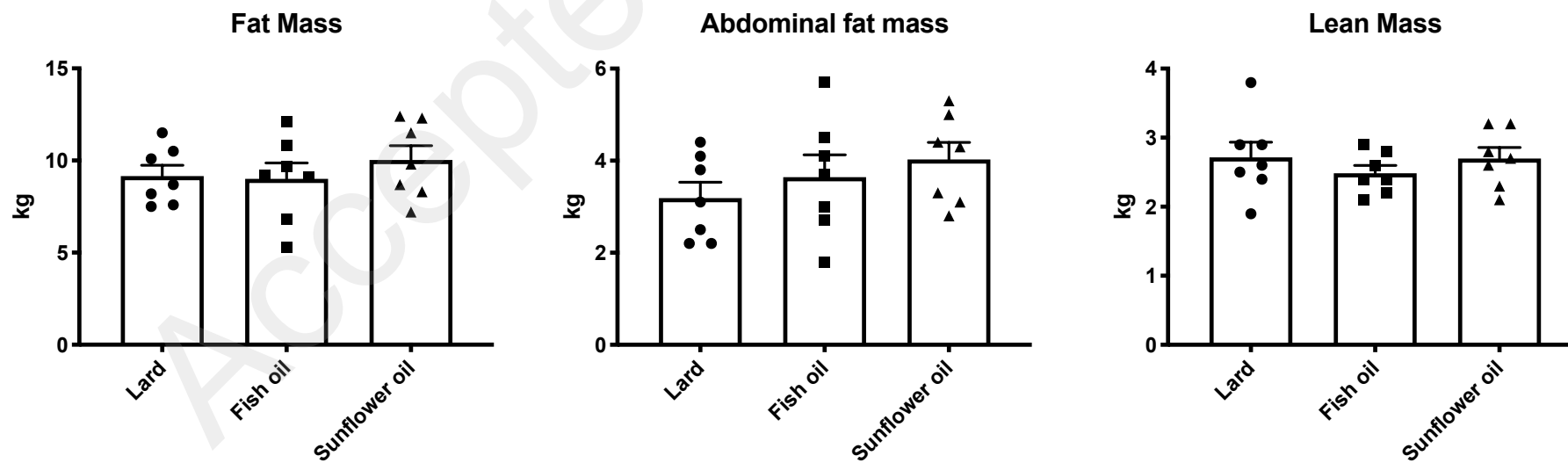
Figure 1: A - Changes in pigs' live body weight in the three experimental groups: Lard, Fish oil and Sunflower oil. * different at $P < 0.05$ between Lard and Fish oil. \$ different at $P < 0.05$ between Lard and Sunflower oil. B - Body composition obtained from image analysis of the CT-scan slices located at T13, L2 and L3 vertebra. Note that the amount of fat (including subcutaneous and abdominal fat), abdominal fat and lean tissue mass were not significantly different between groups.

Figure 2: Spectral analysis of the heart rate variability (HRV) obtained from 5-minute-long recordings. High-frequency component, representative of the parasympathetic (vagal) cardiac modulation, was unchanged irrespective of the diet whereas Fish oil group presented a significant increase in the low frequency sympathetic component and consequently higher LF/HF ratio.

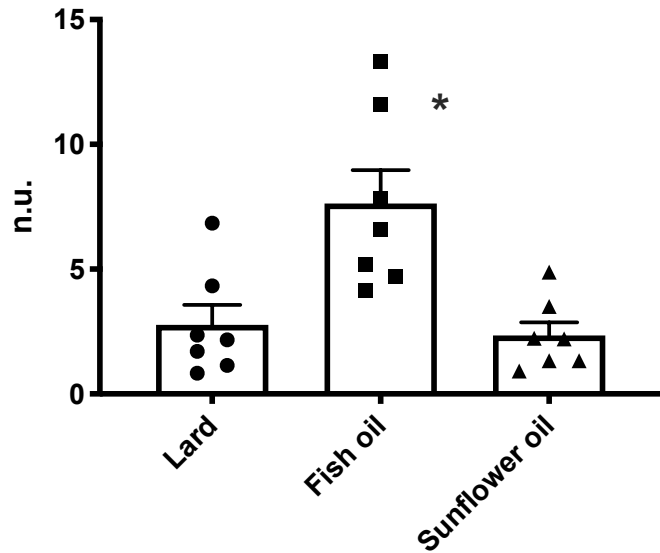
Figure 3: A - Cerebral flow (CBF) coded images obtained from the analysis of the injected dynamic CT image. The insert represents the seven brain structures used for the calculation of rCBF (see text). B - CBF, CBV and MTT calculated as a mean over the 7 regions of interest. * Significantly different ($P < 0.05$) from Lard and Sunflower oil, \neq significantly different ($P < 0.05$) from Lard group.

Figure 4: A - Statistical parameter mapping image of the differences in normalized glucose uptake relative to the cerebellum between fish oil and sunflower oil groups (in yellow) overlaid on T1 coded atlas. Note the activation of the dorsal right cingulate cortex. B - Tridimensional representation of the dorsal cingulate cortex over a porcine brain glass. C - Three-dimensional brain representation showing the 7 volumes of interest (VOI). D - VOI analysis of the regional glucose uptake. Cerebrum values were cumulative uptake divided by the number of voxels. \ddagger Significantly different from Lard group ($P < 0.05$). * Significantly different from Lard and Fish oil group ($P < 0.05$).

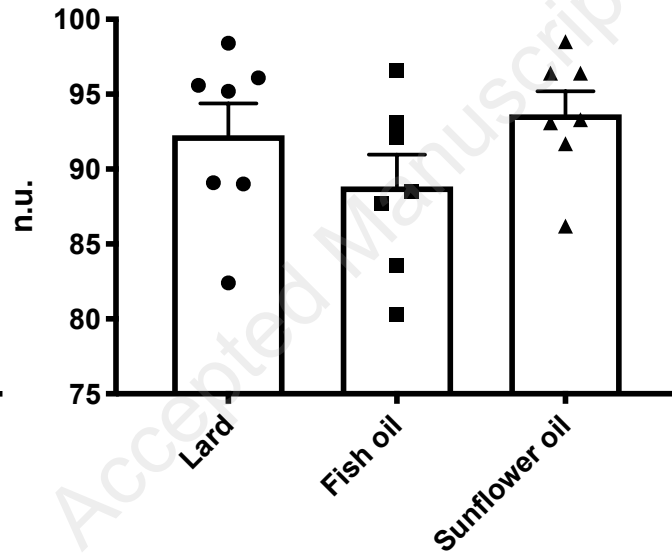
Figure 5: Metabolic connectivity analysis for Lard, Fish oil and Sunflower oil groups. Left - Metabolic connectivity matrices were calculated using NetPET software. Components were presented using BrainNet Viewer with a threshold of $P \leq 0.005$ on the probability map associated to the correlation map. The unconnected nodes were not represented to ease visual comparison. The thickness of the edges symbolized the intensity of the correlation. Right - Metabolic connectivity represented as circular graphs with a threshold of $P \leq 0.001$. The description of the nodes and their abbreviations were given in supplementary information 1.

A**B**

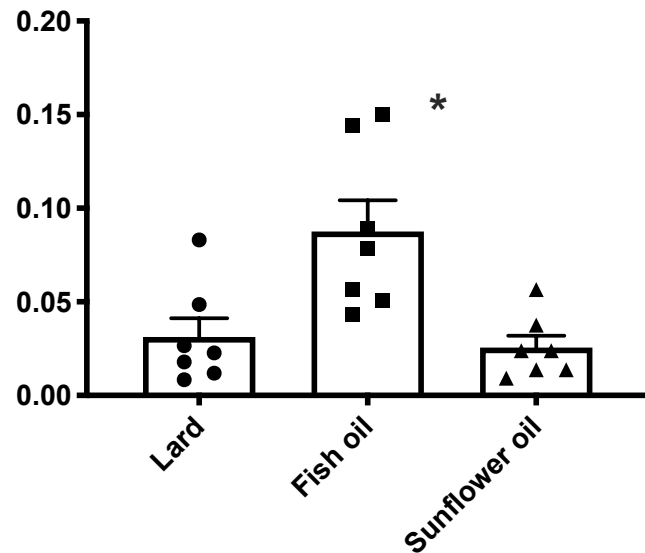
LF Norm

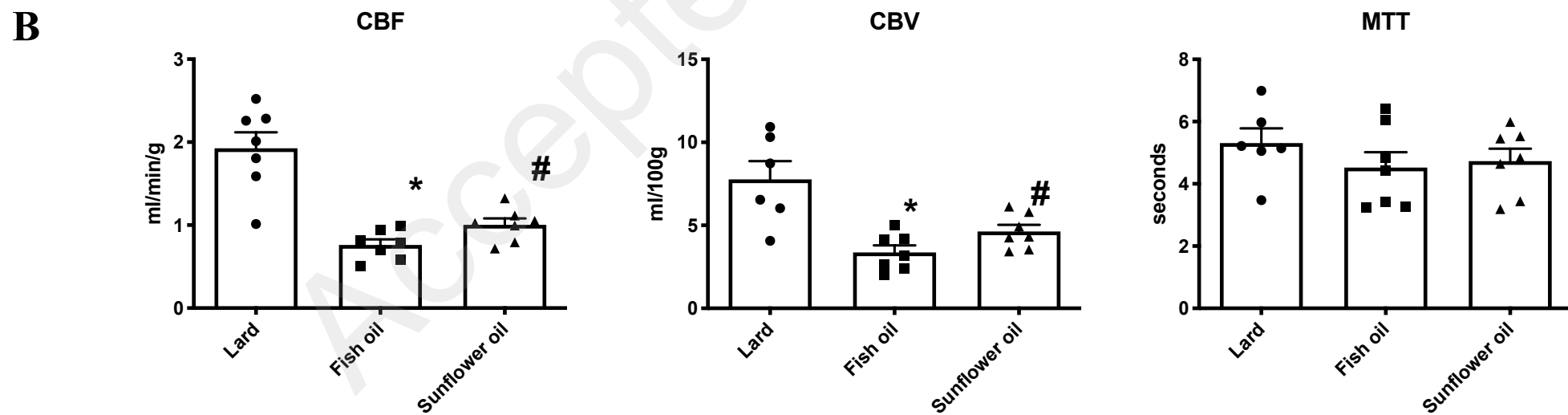
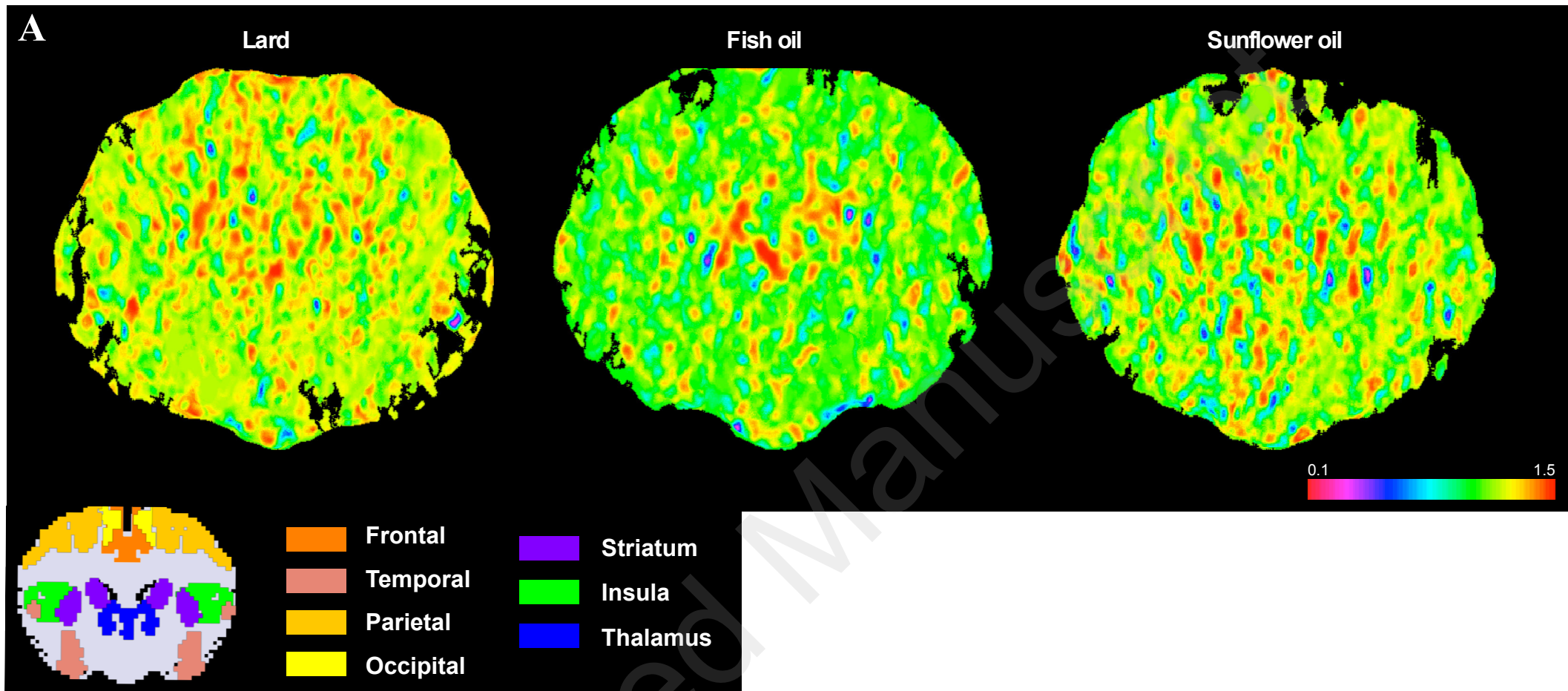


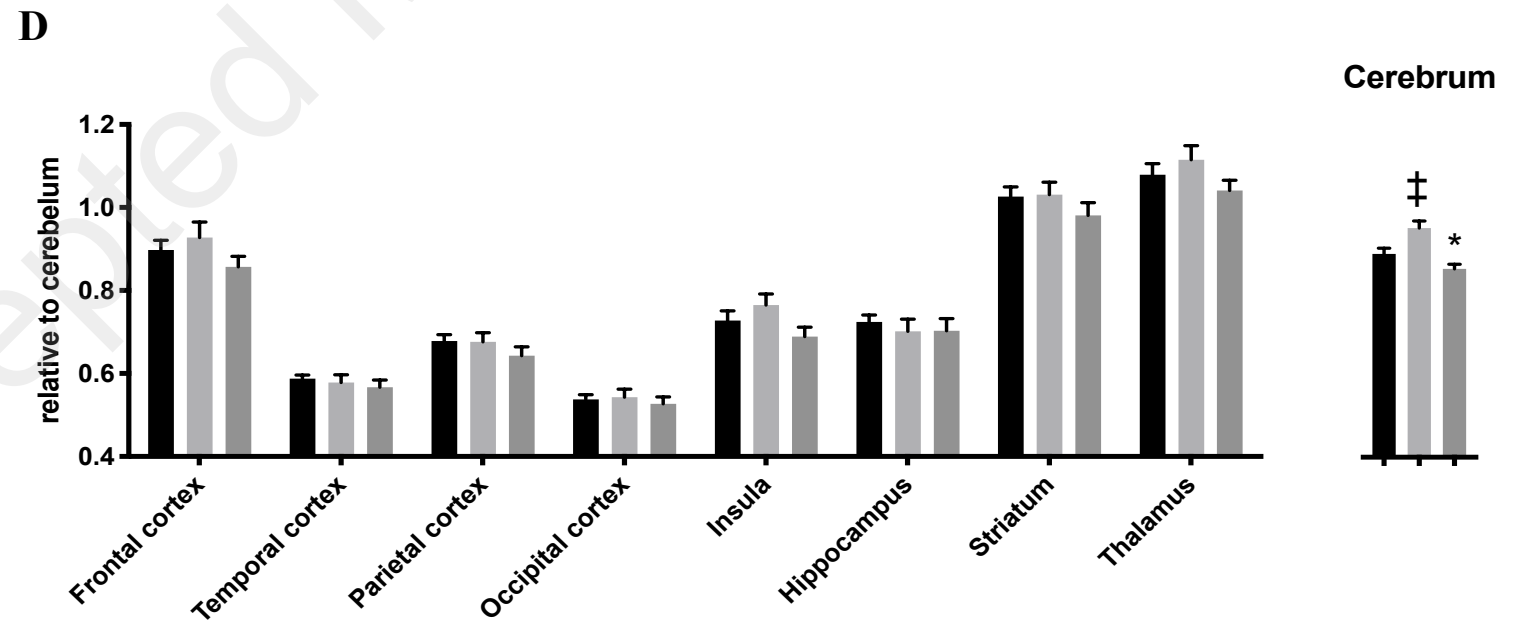
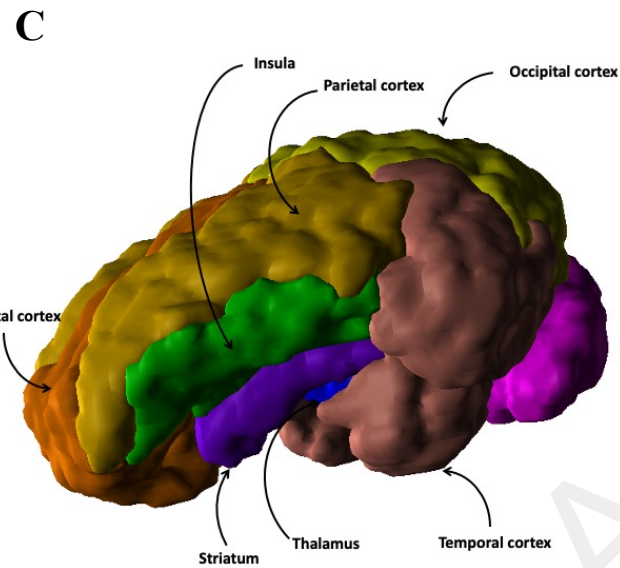
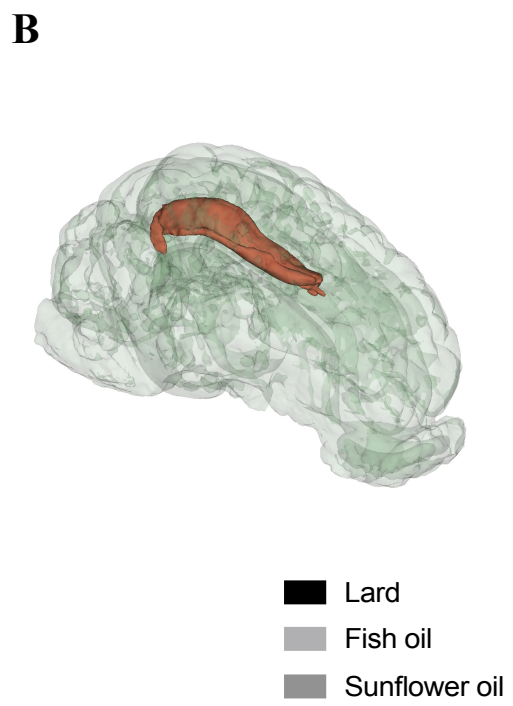
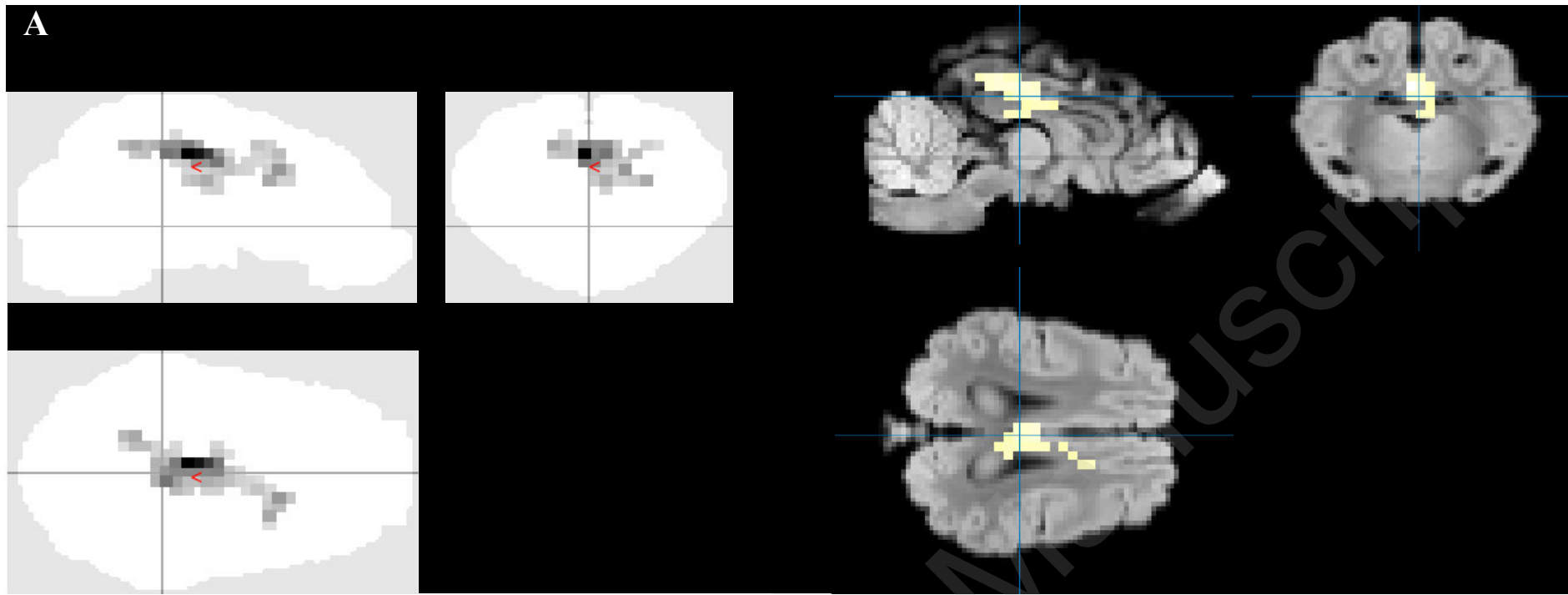
HF Norm



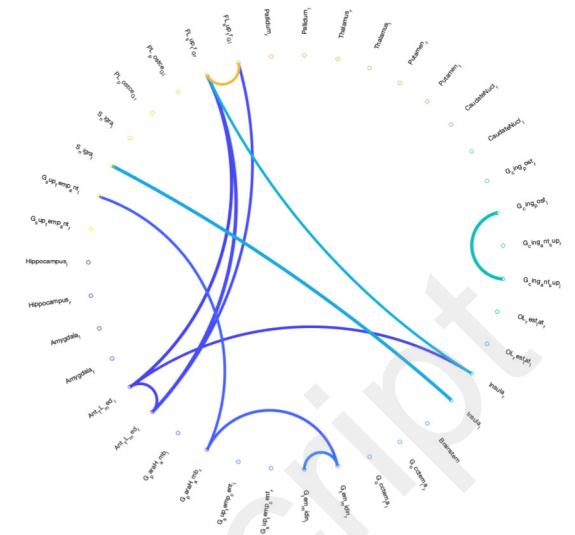
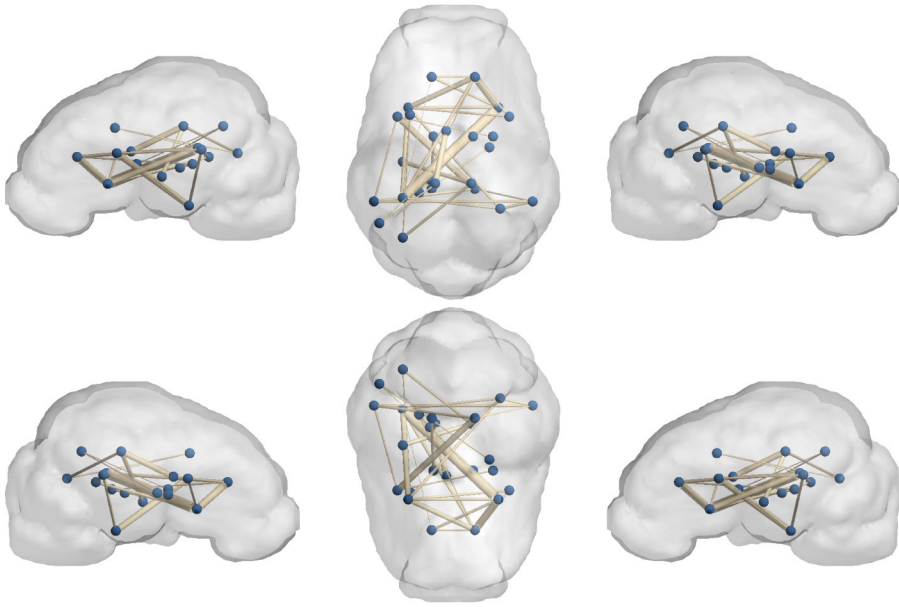
LF/HF



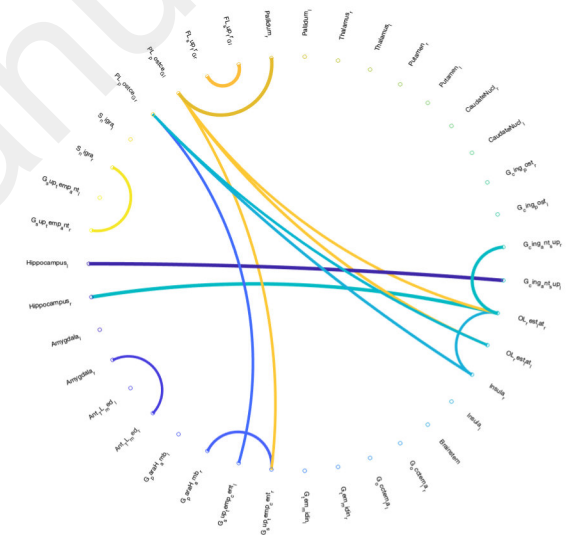
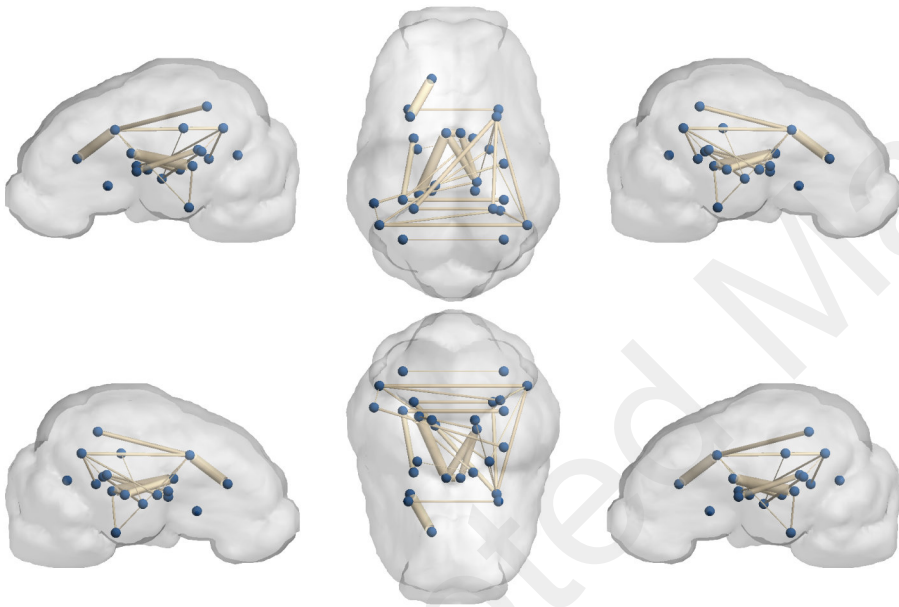




Lard



Fish oil



Sunflower oil

