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Long-chain n-3 PUFAs from fish oil enhance resting state brain glucose utilization and reduce anxiety in an adult nonhuman primate, the grey mouse lemur

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Abstract **Decreased brain content of DHA, the most abundant long-chain n-3 polyunsaturated fatty acid (n-3 LCPUFA) in the brain, is accompanied by severe neurosensorial impairments linked to impaired neurotransmission and impaired brain glucose utilization. In the present study, we hypothesized that increasing n-3 LCPUFA intake at an early age may help to prevent or correct the glucose hypometabolism observed during aging and age-related cognitive decline. The effects of 12 months' supplementation with n-3 LCPUFA on brain glucose utilization assessed by positron emission tomography was tested in young adult mouse lemurs (*Microcebus murinus*). Cognitive function was tested in parallel in the same animals. Lemurs supplemented with n-3 LCPUFA had higher brain glucose uptake and cerebral metabolic rate of glucose compared with controls in all brain regions. The n-3 LCPUFA-supplemented animals also had higher exploratory activity in an open-field task and lower evidence of anxiety in the Barnes maze.jlr Our results demonstrate for the first time in a nonhuman primate that n-3 LCPUFA supplementation increases brain glucose uptake and metabolism and concomitantly reduces anxiety.—** Pifferi, F., O. Dorieux, C-A. Castellano, E. Croteau, M. Masson, M. Guillermier, N. Van Camp, P. Guesnet, J-M. Alessandri, S. Cunnane, M. Dhenain, and F. Aujard. **Long-chain n-3 PUFAs from fish oil enhance resting state brain glucose utilization and reduce anxiety in an adult nonhuman primate, the grey mouse lemur. *J. Lipid Res.* 2015.** 56: **1511–1518.**

Supplementary key words brain glucose hypometabolism • long-chain n-3 polyunsaturated fatty acids • positron emission tomography imaging

Brain cell membranes of vertebrates have high concentrations of long-chain polyunsaturated fatty acids (LCPUFAs) of the n-3 and n-6 series, mainly DHA (22:6 n-3) and arachidonic acid (AA; 20:4 n-6) (1). The accretion of DHA during perinatal development is considered to be essential for the proper functioning of the mammalian central nervous system, especially in primates. The functional role of DHA has been mainly investigated in animal models, mainly rodents, deprived of any dietary source of n-3 PUFAs during perinatal development. Dietary deficiency of n-3 PUFAs leads to decreased brain content of DHA, which is accompanied by severe neurosensorial impairments (learning, memory, and anxiety) that have been linked to changes in neurotransmission processes (2). Neurotransmission is very energy consuming, particularly the restoration of membrane potential by Na-K-ATPase after an action potential, which consumes about 50% of brain ATP (3). Thus, impairment of neurotransmission in animals fed an n-3 PUFA-deficient diet could be due in part to suboptimal brain energy metabolism.

Early work relating n-3 PUFAs and brain energy metabolism came from studies by Bourre's group (4), which demonstrated that activity of brain Na-K-ATPase was 40% lower in nerve terminals of rats made deficient in n-3 PUFAs. This change paralleled significantly lower performance on learning tasks. Later on, Ximenes and colleagues (5) demonstrated that animals fed an n-3 PUFA-deficient diet exhibited 50% lower glucose utilization in cerebral cortex

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Abbreviations: AA, arachidonic acid; AD, Alzheimer disease; CMR_{glu}, cerebral metabolic rate of glucose; FDG, fluorodeoxyglucose; GLM, generalized linear model; GLUT, glucose transporter; LCPUFA, long-chain polyunsaturated fatty acid; PET, positron emission tomography; SUV, standard uptake value; VOI, volume of interest.

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and hippocampus by using autoradiographic 2-deoxyglucose method, and 25–30% lower rate of oxidative phosphorylation by measuring cytochrome oxidase activity (5).

In a recent study, we confirmed that rats on an n-3 PUFA-deficient diet exhibited lower brain uptake of glucose (6). Such a decrease can, at least partly, explain the behavioral changes observed during n-3 PUFA deficiency. Brain glucose hypometabolism can occur in healthy older people in the absence of any measurable cognitive decline (7). This brain glucose hypometabolism seems to be more marked during age-related cognitive decline, such as Alzheimer disease (AD) (8), and there is a positive association between glucose hypometabolism and cognitive decline during mild cognitive impairment or in AD patients (9, 10). Brain glucose uptake is highly dependent on glucose transporter (GLUT) activity and especially GLUT1, which is localized in both endothelial cells of the blood-brain barrier and astrocytes. Interestingly, n-3 PUFA-deficient rats have lower expression of GLUT1 at both the gene and protein levels (11, 12). The effect was specific to this GLUT because no change in neuronal GLUT3 expression was observed. Altogether, these results suggest an important role of n-3 PUFAs in the regulation of brain glucose metabolism, in part due to the regulation of the endothelial and astroglial GLUT1.

Interestingly, in vitro studies on rat brain endothelial cells depleted of DHA show that subsequent addition of DHA to the culture medium increased both glucose transport activity by 35% and GLUT1 density (13, 14). Modulating DHA dietary intake may therefore help prevent or correct the glucose hypometabolism observed during age-related cognitive decline (15). In vivo PUFA supplementation studies confirmed the possible relation between ω3 PUFA and expression of brain energy metabolism genes including cytochrome-C oxidase, NADH dehydrogenase, and ATP synthetase (16). In 2000, Tsukada and colleagues (17) demonstrated that supplementing aged monkeys with DHA for 1 to 4 weeks (a very short-term dietary supplementation) led to increased regional cerebral blood flow, a parameter closely linked to neuronal activation. On the basis of these observations, we hypothesize that a dietary source of n-3 LCPUFAs containing DHA will improve cognitive performance by enhancing brain glucose utilization, which we tested in nonhuman primates.

The gray mouse lemur (*Microcebus murinus*) is a nocturnal prosimian primate originating from Madagascar with a life expectancy of 8–10 years. It presents specific characteristics that make it a good model to evaluate the effects of long-term dietary treatments on behavioral and cognitive parameters in primates. In particular, it is small (80–120 g), it is omnivorous, and its behavioral and cognitive performances can be assessed with specific tasks developed and adapted in our laboratory (18). There is particular interest in determining whether dietary PUFAs affect brain functions in adults, inasmuch as the mean dietary intakes of n-3 LCPUFAs in adults are below the levels of recommendation in developed countries (19). We recently reported the effect of n-3 LCPUFA supplementation for 5 months on behavioral, cognitive, and locomotor performance in adult and aged gray mouse lemurs (20, 21). We showed for the first time in a nonhuman primate species that n-3 PUFA supplementation decreased anxiety and spontaneous locomotor activity and concomitantly improved cognitive performance. The n-3 PUFA-supplemented diet initiated later in life specifically modified the exploratory behavior without improving the spatial memory of these aged lemurs. The very limited effect of long-term ω3 PUFA supplementation in aged animals (20) on behavior and cognitive performances drove us to intervene at an earlier age in the present study. We focused on young adult mouse lemurs given a level of n-3 LCPUFAs corresponding to the recommendation for the French adult population (22). In the present study, we compared the effects of a 12-month supplementation with n-3 LCPUFAs or with monounsaturated fatty acids (isocaloric control diet) on brain glucose metabolism assessed by positron emission tomography (PET) imaging. In parallel, we used a spatial memory task (adapted from the rodent Barnes maze) to assess spatial reference memory and an open-field task to assess exploratory behavior and anxiety, both of which we have already extensively validated in gray mouse lemurs (18, 20, 21).

MATERIALS AND METHODS

Animals and diets

All experiments were performed in accordance with the *Principles of Laboratory Animal Care* (National Institutes of Health publication 86-23, revised 1985) and the European Communities Council Directive (86/609/EEC). The Research was conducted under the authorization number 91–305 from the "Direction Départementale des Services Vétérinaires de l'Essonne" and the Internal Review Board of the UMR 7179. All the experiments were done under personal license (authorization number 91–460, issued June 5, 2009) delivered by the Ministry of Education and Science. All efforts were made to minimize nociception. Reporting of the experiments is following the Animal Research: Reporting of In Vivo Experiments (ARRIVE) guidelines. Twelve young adult male gray mouse lemurs (*M. murinus*, Cheirogaleidae, Primates) were included at the age of 23 ± 4 months. Animals were raised on fresh fruit and a mixture of cereals, milk, and egg prepared daily in the lab. Water and food were given ad libitum. Animals were randomly assigned to each experimental group (n = 6/group) and maintained in individual cages during the supplementation period. The n-3 LCPUFA-supplemented group received the home-made food supplemented with tuna oil (Polaris, Pleuven, France) rich in n-3 LCPUFAs, while the control group received the food isoenergetically supplemented with the same volume of olive oil (<1% n-3 PUFAs under the form of short-chain α-linolenic acid) (**Table 1**). Both groups were supplemented for 12 months. In the n-3 LCPUFA-supplemented group, the intake of EPA (20:5 n-3) and DHA (22:6 n-3) represented ∼0.06% and 0.3% of total energy, respectively, which is equivalent to the highest level of consumption of French coastal populations (23) and corresponds to the recommended daily intake of EPA and DHA for the French population (22). These proportions correspond to a daily intake of about 6 mg EPA and 30 mg DHA per animal. Experiments were performed the last 2 weeks of the final month of supplementation, starting with 1 week of cognitive

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TABLE 1. Fatty acid composition of olive oil (control group) and fish oil (n-3 LCPUFA-supplemented animals) composing the diets

	Olive Oil	Fish Oil
Fatty acids	g/100 g	
14:0	—	3.9
16:0	11.3	18.7
18:0	2.0	4.8
Σ Saturated	13.3	30.0
16:1 n-7	1.3	5.4
18:1 n-9	71.3	14.5
18:1 n-7	—	2.4
20:1 n-9	—	1.7
Σ Monounsaturated	72.6	26.5
18:2 n-6	9.8	1.7
20:4 n-6[a]	—	2.0
22:5 n-6	—	1.5
Σ n-6 Polyunsaturated	9.8	5.8
18:3 n-3	0.8	0.6
18:4 n-3	—	1.2
20:5 n-3[b]	—	8.3
22:5 n-3	—	1.3
22:6 n-3[c]	—	25.8
Σ ω3 Polyunsaturated	0.8	37.7

Minor fatty acids (excluding 18:3 n-3) are not reported because they represented <1% of total fatty acids.
[a]20:4 n-6, AA.
[b]20:5 n-3, EPA.
[c]22:6 n-3, DHA.

testing. Blood sampling, PET, and MRI experiments were performed during the second week. Body weights were measured throughout the study and were not significantly affected by dietary treatments.

Lipid analysis

Blood was collected on heparin and centrifuged, and plasma was stored at −80°C until analysis. Total lipids were extracted from plasma with chloroform-methanol (2:1, v/v) using the method of Folch (24). Total plasma phospholipids were isolated by solid phase liquid chromatography on silica cartridges; sequential elution was made with chloroform and then methanol, which contained the phospholipid fraction. All eluents were dried under nitrogen, and the phospholipid fractions were transmethylated with 10% boron trifluoride (Fluka, Sokolab) at 90°C for 20 min. Fatty acid methyl esters were analyzed by gas chromatography (25); the fatty acid composition is expressed as a weight percentage (g/100 g of total fatty acids).

Circular platform task

The circular platform task apparatus was an adaptation for mouse lemurs of the device described by Barnes (21). It consisted of a white circular platform (diameter, 100 cm) with 12 equally spaced circular holes (each 5 cm in diameter) located 3 cm from the perimeter. The platform could be rotated. The maze platform was placed 60 cm above the floor, and a cardboard nest box (10 cm × 10 cm × 20 cm) could be inserted and removed beneath each hole and served as a refuge (goal box). A black, small plywood box could be slid beneath the nongoal holes to stop the lemurs from jumping through these holes while still permitting head entering. To prevent the mouse lemur from escaping, the platform was entirely surrounded with a white wall 25 cm high and covered with a transparent Plexiglas® ceiling that permitted the mouse lemurs to see the extramaze visual cues. The apparatus was surrounded by a black curtain hung from a square metallic frame (length of the side, 120 cm) located 110 cm above the floor. The center of the frame was a one-way mirror to allow observation. Attached beneath the one-way mirror and along the perimeter of the maze (about 50 cm above the platform) were 24 evenly spaced 2 W lights, illuminating the maze. Between the one-way mirror and the upper edge of the wall, various objects were attached along the inner surface of the curtain to serve as visual cues. The starting box was an open-ended dark cylinder positioned in the center of the platform. Transparent radial Plexiglas partitions (25 cm high × 20 cm long) were placed between the holes to prevent the strategy used by some mouse lemurs to go directly to the periphery of the platform and then walk along the wall and inspect each hole one by one. Consequently, animals had to return to the center of the platform after each hole inspection.

Testing procedure. Animals were given one session of habituation and training (day 1) and one session of testing (day 2). Each session included four trials, each of which began with placement of the animal inside the starting box. After 30 s, the box was lifted to release the animal. For the lemurs, the objective was to reach the goal box positioned beneath one of the 12 holes, the position of which was kept constant relative to the cues for all trials. When the animal entered the goal box, the trial was stopped, and the animal was allowed to remain in the goal box for 3 min. After each trial, the platform was cleaned and randomly rotated on its central axis to avoid the use of intramaze cues, although the position of the goal box was kept constant relative to the cues.

On day 1, trials 1 and 2 consisted of placing the animal in a four-walled chamber containing only the opened goal hole (one-choice test). For trials 3 and 4, the platform comprised six evenly spaced open holes (six-choices test). These two trials permitted the animal to explore the maze, observe the visual cues, and further learn the position of the goal box. On day 2 (testing day), 12 holes were opened during the four trials. Performance was assessed based on the time required for the animal to reach the right exit (expressed in seconds) and the number of errors and visits prior to reaching the goal box. For each group, the rate of success was also defined as the ratio of successful trials to the total number of trials during the testing day, expressed in percent.

Open-field task

This system was an open-field consisting of bright and opaque Plexiglas® walls (100 × 100 × 20 cm) and covered with a transparent Plexiglas® ceiling. Four white lights of 15 W were placed at each corner of the system. The open-field session was recorded by camera, and the data were analyzed after the session, which rendered unnecessary the presence of an observer in the room during the test. The mouse lemurs were placed in the open-field for free exploration for 30 min. At the end of the session, the nest box of the mouse lemur was placed in a corner of the open field (the same corner for all animals) to allow it to return to the nest box with minimal stress. Because of persistent immobility, peripheral tracking and limited exploration are index of stress and anxiety in mouse lemurs when placed in a novel environment. We determined three parameters reflecting the degree of anxiety for each animal: total distance traveled during the test (expressed in centimeters), activity duration during the test (expressed in seconds), and number of crossings of the central zone.

Measurement of brain glucose metabolism

Anesthesia procedure. For MRI, PET, and autoradiography studies, anesthesia was prepared with subcutaneous injection of 0.25 ml atropine (0.025 mg/1 ml) 20 min before induction with isoflurane 5%; anesthesia was maintained with isoflurane 1–2%.

PET data acquisition. Images were recorded on a MicroPET® Focus 220 system (resolution 474 × 474 × 796 μm, 60 min), the time

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framing in seconds was 15 × 2, 6 × 5, 3 × 10, 2 × 15, 3 × 20, 4 × 30, 5 × 120, 3 × 300, 3 × 600. Images were rebuilt with OMSED algorithm, and a 15 min scan transmission with 68 Ga was performed. Brain and liver were within the field of view. Animals were fasted 24 h before the PET but had free access to water. Animals were anesthetized, and respiratory rate and temperature were monitored. Body temperature was monitored rectally and maintained at 36°C using a thermostatically controlled heating pad. A venous catheter (DB Neoflon™ 26G) was inserted into the small saphenous vein after shaving the hind leg. Glycaemia measures were taken after catheter installation and at the end of the PET scan. Fluorodeoxyglucose (FDG; dose 9 µCi/g) was injected at the exact start of the PET scan. Whole blood radioactivity was measured at the end of the scan.

MRI scanning. MR images were recorded for all the animals involved in the study. The main purpose of these images was to provide anatomical landmarks to define volumes of interest (VOIs) in PET images. Brain images were acquired for 32 min on a 7 Tesla spectrometer (Varian) with a surface probe (RapidBiomed, Germany). 2D spin echo images were recorded with an in-plane resolution of 230 µm and slice thickness of 230 µm [repetition time/echo time (TR/TE) = 10,000/17, 4 ms; rapid acquisition with relaxation enhancement (RARE)-factor = 4 = field of view 29.44 × 29.44 mm^3]. The recorded images were then interpolated in the K space ("zerofilling") to provide an in-plane apparent resolution of 115 µm. The acquisition sequence used yielded optimal visual contrast between brain structures and in the cerebrospinal fluid (26) (cerebrospinal fluid appears hyperintense on T2-weighted images). Animals were anesthetized during the exams, and respiratory rate and temperature were monitored.

Image analysis and FDG quantification. MRI and PET images were analyzed with BrainVISA®, Anatomist® (http://brainvisa.info/), and PMOD 3.3 softwares (PMOD Technologies Ltd., Zurich, Switzerland). Brain FDG PET image were analyzed by both semiquantitative [standard uptake values (SUVs)] and quantitative [cerebral metabolic rates of glucose (CMR_{glu})] techniques. For each animal, VOIs were manually segmented using MRI images. In these VOIs, the PET signal (CPET) was calculated (Bq/cc = Bq/ml) on dynamic images and on sum images of the last 30 min and then converted into SUV [SUV = CPET/(injected dose/body mass)]. SUV data were expressed in g/ml. CMR_{glu} (µmol/min/100 g) were calculated using Patlak graphical analysis method (27). The Patlak graphical analysis was performed with the arterial blood time-activity curve (TAC) from the brain. As previously reported by Tantawy and Peterson (28), an image-derived input function was determined from the liver TAC. A lumped constant of 0.344 and an estimated cerebral blood volume of 5% were used (29, 30).

Statistical analysis

All results are reported as mean ± SEM. A Student's *t*-test was used to compare the following parameters between the control and n-3 LCPUFA-supplemented groups: fatty acid content (lipids analysis), percent of successful trials, number of errors, latency in the Barnes maze, and total distance in open field. The effect of treatments on SUV data was compared using a two-way ANOVA (treatment and time effect in each brain region). Because CMR_{glu} data were not normally distributed, data were analyzed using a generalized linear model (GLM) with γ distribution (Shapiro-Wilk normality test: $w = 0.98$; $P = 0.42$). Differences were considered significant with $P < 0.05$. Statistical analyses were performed with GraphPad Prism 5.0 and R 3.0.2 softwares.

RESULTS

Plasma fatty acids

N-3 LCPUFA supplementation increased the n-3 PUFAs and decreased n-6 and monounsaturated fatty acids (**Table 2**). Fish oil-supplemented animals exhibited a 3.4-fold increase in total n-3 PUFAs compared with controls (from 7.9 ± 0.8% of total fatty acids in control to 26.7 ± 0.8% in n-3 LCPUFA-supplemented animals, $P < 0.01$) while total n-6 and monounsaturated fatty acids concurrently decreased ~40% ($P < 0.01$) and 30% ($P < 0.01$), respectively. DHA was 3-fold higher ($P < 0.01$) when AA was close to 2-fold lower ($P < 0.01$) upon n-3 LCPUFA supplementation in comparison with control. The ratio of total n-6:total n-3 PUFAs was equal to 0.7:1 in n-3 PUFA-supplemented animals and to 4.3:1 in the control group. Total saturated fatty acids were not significantly altered by dietary treatment.

Circular platform and open-field tasks

The success rate in the circular platform task is expressed as the number of successful trials to total number of trials during the testing day (%, **Fig. 1A**). Animals of the n-3 LCPUFA group exhibited 55 ± 14% success in this task compared with 21 ± 6% for animals of the control group ($P = 0.041$, $t = 2.334$, df = 10), whereas the number of errors before finding the correct exit was not significantly different (3.7 ± 1.4 vs. 3.9 ± 0.9; $P = 0.908$, $t = 0.1184$, df = 10) (Fig. 1B). Moreover, n-3 LCPUFA-supplemented animals required less time to exit from the maze compared with

TABLE 2. Plasma fatty acids from total phospholipids of control and n-3 LCPUFA-supplemented animals

	Control (n = 6)	Fish Oil (n = 6)
Fatty acids[a]	g/100 g[b]	
12:0	0.6 ± 0.2	2.1 ± 0.2
16:0	25.0 ± 0.5	27.3 ± 0.4 *
18:0	16.8 ± 0.7	14.1 ± 0.2 *
Σ Saturated	43.1 ± 0.0	44.5 ± 0.4
18:1 n-9	9.8 ± 0.1	6.8 ± 0.3 *
18:1 n-7	1.6 ± 0.0	1.5 ± 0.1
Σ Monounsaturated	11.4 ± 0.0	8.3 ± 0.3 *
18:2 n-6	12.9 ± 1.1	8.6 ± 0.6 *
20:4 n-6[c]	15.9 ± 0.2	9.4 ± 0.7 *
22:4 n-6	1.8 ± 0.3	0.2 ± 0.1
22:5 n-6	1.9 ± 0.3	0.3 ± 0.0
Σ n-6 Polyunsaturated	34.8 ± 0.7	19.3 ± 1.2 *
18:3 n-3	0.1 ± 0.0	0.1 ± 0.0
20:3 n-3	0.0 ± 0.0	0.1 ± 0.0
20:4 n-3	0.1 ± 0.1	0.1 ± 0.0
20:5 n-3[d]	0.2 ± 0.1	4.9 ± 0.4 *
22:5 n-3	1.8 ± 0.2	5.1 ± 0.6 *
22:6 n-3[e]	5.7 ± 0.7	16.4 ± 0.5 *
Σ ω3 Polyunsaturated	7.9 ± 0.8	26.7 ± 0.8 *

[a] Minor fatty acids [14:0, 15:0, 17:0, 19:0, 20:0, 22:0, 24:0, 14:1(n-5), 16:1(n-9), 20:1(n-7), 20:1(n-11), 22:1(n-7), 24:1(n-11), 24:1(n-7), 20:3(n-9), and 22:3(n-9)] are not reported because they represented <0.3% of total fatty acids.

[b] Values are means ± SEM, n = 6. Asterisk indicates significant differences between dietary treatments with $P < 0.01$.

[c] 20:4 n-6, AA.

[d] 20:5 n-3, EPA.

[e] 22:6 n-3, DHA.

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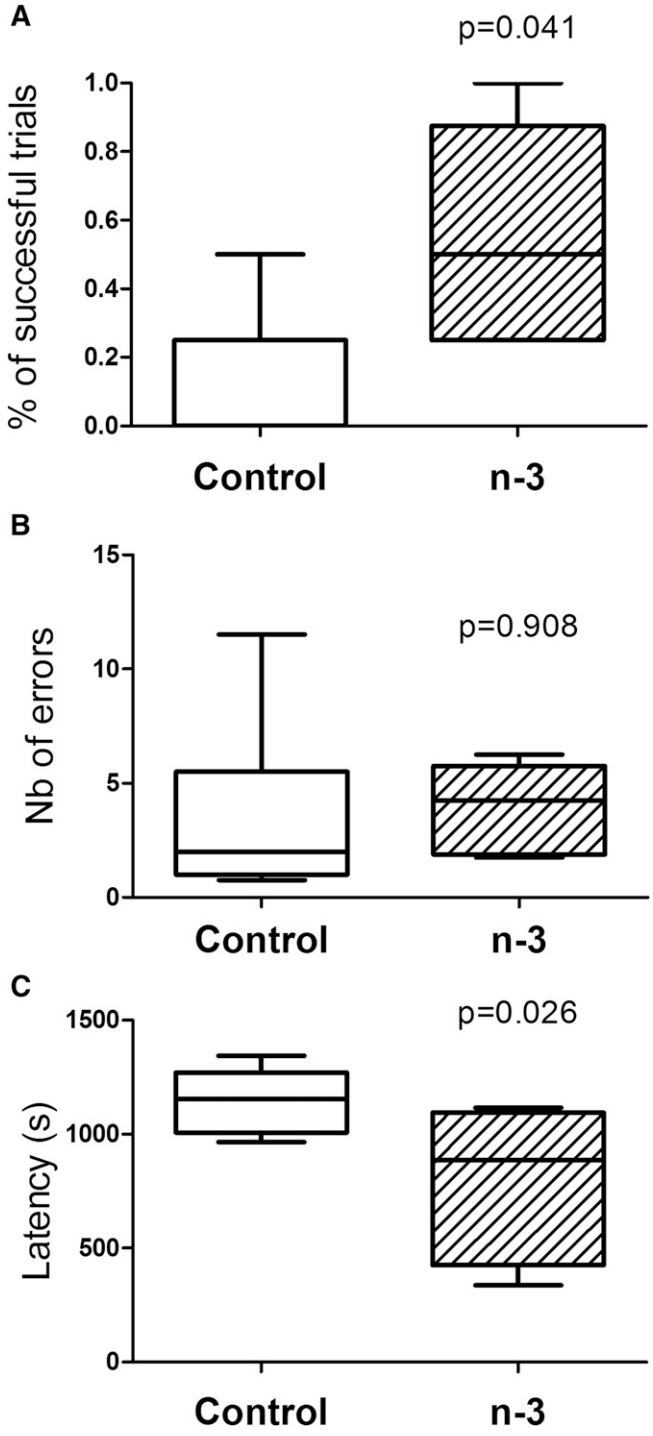

Fig. 1. Performances in a circular maze for control and n-3 LCPUFA-supplemented animals (n-3). A: Rate of success, expressed as the ratio of successful trials on the total number of trials (%). B: Number of errors before reaching the right exit. C: Latency (s) before finding the correct exit. Values are median (minimum, maximum); n = 6 in each dietary group. Differences were considered significant with $P < 0.05$.

controls (785 ± 154 s vs. 1157 ± 52 s; $P = 0.025$, $t = 2.615$, df = 10) (Fig. 1C). The total distance traveled during the open-field task was significantly longer ($P = 0.017$; $t = 2.810$ df = 11) in n-3 LCPUFA -supplemented animals (2,494 ± 549 cm) compared with controls (884 ± 249 cm) (**Fig. 2**).

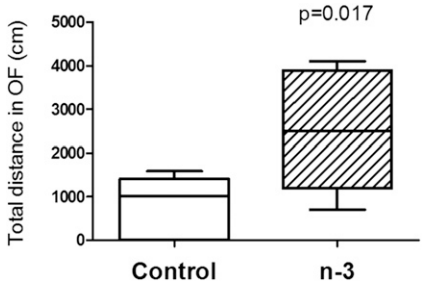

Fig. 2. Total distance in open-field task (cm) for control and n-3 LCPUFA-supplemented animals (n-3). Values are median (minimum, maximum); n = 6 in each dietary group. Differences were considered significant with $P < 0.05$.

Brain glucose uptake and metabolism

The n-3 LCPUFA-supplemented animals exhibited ~30% higher SUVs compared with control in all the selected regions of interest (**Fig. 3**; control vs. n-3: whole brain, $P = 0.015$; hippocampus, $P = 0.004$; cerebellum, $P = 0.026$; caudate nucleus, $P = 0.019$; thalamus, $P = 0.044$; occipital lobe, $P = 0.004$; frontal lobe, $P = 0.041$; temporal lobe, $P = 0.014$). Maximal SUVs (SUV_{max}, in g/ml) were the following (control vs. n-3): whole brain, 2.88 ± 0.21 versus 4.20 ± 0.66; hippocampus, 3.17 ± 0.17 versus 4.53 ± 0.64; thalamus, 3.72 ± 0.34 versus 6.03 ± 1.50; cerebellum, 3.20 ± 0.24 versus 4.77 ± 0.99; caudate nucleus, 3.48 ± 0.24 versus 4.83 versus 0.68; temporal lobe, 2.47 ± 0.14 versus 3.53 ± 0.51; occipital lobe, 2.91 ± 0.13 versus 4.47 ± 0.61; frontal lobe, 2.93 ± 0.27 versus 4.09 ± 0.62). In addition, no significant difference in SUVs was observed between brain regions within the control group or within the n-3 LCPUFA-supplemented group.

CMR_{glu} values of the n-3 LCPUFA-supplemented group were ~30% higher compared with the control group (general effect of treatment, GLM, $P = 0.0003$). CMR_{glu} values (μmol/min/100 g) in the different brain regions were the following (**Fig. 4**) (control vs. n-3): whole brain, 13.9 ± 3.6 versus 20.5 ± 2.1; hippocampus, 11.4 ± 3.9 versus 19.2 ± 1.5; thalamus, 16.8 ± 5.2 versus 25.5 ± 2.0; cerebellum, 19.4 ± 4.9 versus 24.1 ± 2.2; caudate nucleus, 12.8 ± 4.1 versus 20.0 ± 1.9; temporal lobe, 12.1 ± 2.8 versus 17.7 ± 1.9; occipital lobe, 9.6 ± 3.2 versus 16.2 ± 2.0; frontal lobe, 14.0 ± 3.6 versus 20.8 ± 3.0). In addition, no significant differences of CMR_{glu} were observed between brain regions within the control group or within the n-3 LCPUFA-supplemented group.

DISCUSSION

We report here for the first time in a nonhuman primate that 12-month n-3 PUFA supplementation increases brain glucose uptake and concomitantly reduce anxiety in adult mouse lemurs. The n-3 LCPUFA-supplemented animals exhibited higher success and exploratory activity in the Barnes maze and open-field task and had higher brain FDG-uptake and CMR_{glu} compared with control.

Plasma fatty acids from total phospholipids confirmed that animals receiving the n-3 LCPUFA-supplemented diet had significantly higher levels of circulating n-3 LCPUFAs

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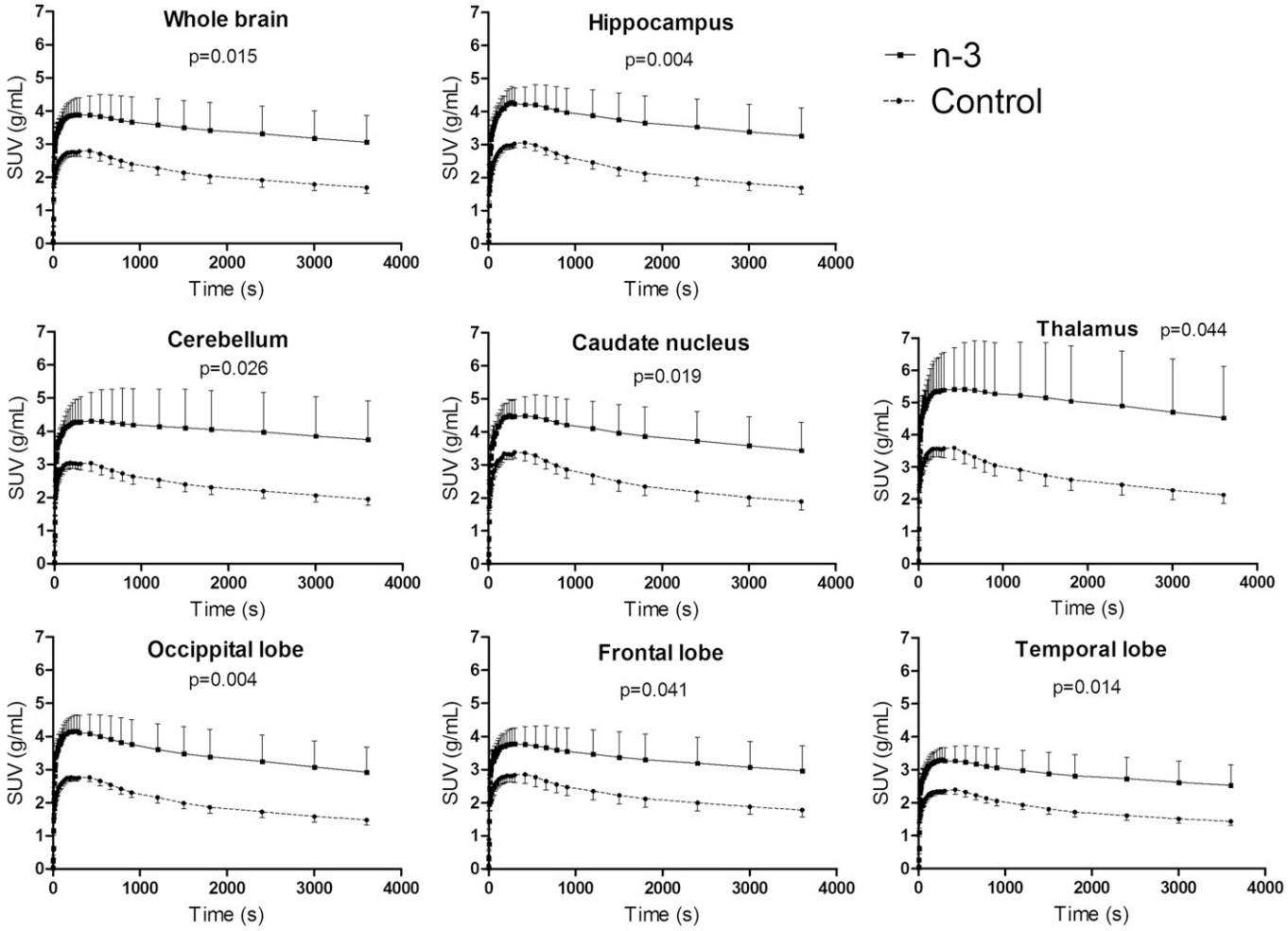

Fig. 3. Standardized uptake values of [^{18}F]FDG in the whole brain and seven regions of interest for control and n-3 LCPUFA-supplemented animals (n-3). Values are means ± SEM; n = 6 in each dietary group. Differences were considered significant with $P < 0.05$.

(including EPA, 22:5 n-3 and DHA) compared with controls (Table 2). Brain fatty acid composition was not accessible without euthanizing the animals, but plasma fatty acids are a reliable marker of organ n-3 PUFA composition under these conditions (31). The increased level of n-3 PUFAs in n-3 LCPUFA-supplemented animals occurred at the expense of both n-6 PUFAs and monounsaturated fatty acids. These changes contributed to improve the balance between n-3 and n-6 PUFAs in the plasma phospholipids of n-3 LCPUFA-supplemented animals with a ratio of n-6:n-3 of 0.7:1 compared with 4.3:1 in the control group. The dietary ratio of n-6:n-3 PUFAs should be close to 1:1 according to recommendations for human health (32).

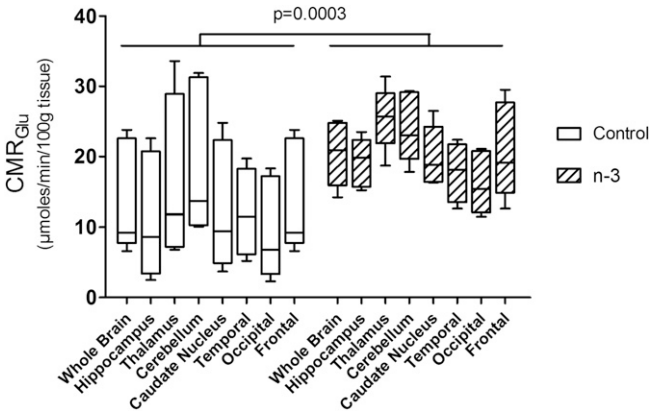

Fig. 4. CMR_{glu} in the whole brain and seven regions of interest for control and n-3 LCPUFA-supplemented animals (n-3). Values are median (minimum, maximum); n = 6 in each dietary group. A significant difference was observed between dietary treatments, independently of the different brain regions we considered.

Our finding that n-3 LCPUFA-supplemented mouse lemurs exhibited both lower anxiety in the open-field task and better performances in the Barnes maze confirms our previous observation in comparable conditions (21) and also suggests that the two outcomes may be directly linked. Indeed, the tendency to higher exploration in the Barnes maze of the n-3 PUFA-supplemented animals (higher number of visits and lower time spent to find the correct exit) may be due to lower anxiety, resulting in a higher percentage of success in comparison with the control group. Thus, the difference of performance between the two groups seems to depend more on their anxiety level than on their intrinsic spatial memory capacity. Similar findings have been made in rodents, which exhibited an increased level of anxiety upon chronic n-3 PUFA dietary deficiency (33, 34) and decreased anxiety upon DHA supplementation (33). A recent study additionally

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reported that fish oil supplementation can attenuate anxiety-like behaviors in a rat model of depression (35). Therefore, it is possible that lower anxiety underlies better cognitive performances of mouse lemurs raised on the tuna oil-enriched diet, an interpretation that is supported by a role of DHA in anxiety disorders in humans (36).

The main novelty of the present study is the observation that brain glucose uptake is higher during long-term dietary supplementation of n-3 LCPUFAs, independently of the brain regions we considered. This result confirms our previous in vivo observations in rodents (13, 14) in which higher expression of the two isoforms of GLUT1 was directly linked to higher brain n-3 PUFA content. These data also confirm the observations made by other groups during very short-term supplementation studies in humans and monkeys. In humans, Jackson and colleagues (37) showed that the impact of a 12-week supplementation with DHA-rich fish oil resulted in a significant increase in the concentrations of oxygenated hemoglobin and total levels of hemoglobin, indicative of increased cerebral blood flow during cognitive tasks. In young and aged monkeys, Tsukada and colleagues (17) demonstrated that supplementing monkeys 1 to 4 weeks with a DHA-rich diet resulted in a significant increase in regional cerebral blood flow response to stimulation. Taken together, these studies confirm the implication of dietary DHA in the coupling mechanism between neuronal activation and regional cerebral blood flow response. The present data confirm that longer-term n n-3 LCPUFA supplementation increases brain glucose uptake and metabolism.

It is possible that n-3 LCPUFAs act on brain glucose uptake and metabolism via effects on blood-brain barrier glucose transport (acting on GLUT1 expression and activity), neuronal glucose transport, and brain energy metabolism. Indeed, we already demonstrated in rats that GLUT1, the GLUT responsible for glucose entry into the brain (i.e., uptake) at both the endothelial cells and astrocytes levels, is affected by dietary n-3 PUFA intake while GLUT3, the neuronal transporter of glucose, is not (12–14). PUFAs are also known to modify the expression of several genes implied in energy metabolism in the brain such as ATP synthase, cytochrome b, cytochrome c oxydase, or NADH dehydrogenase (16). Lower brain DHA is associated with lower activity of oxidative enzymes such as cytochrome oxidase (5).

A pilot study in humans without n-3 PUFA supplementation recently demonstrated a brain regional correlation between plasma n-3 PUFA and regional CMR_{glu} (38), supporting that the link between PUFA and glucose metabolism could also exist in humans. This relation has been recently confirmed in a study examining the relationship between nutrients and brain biomarkers of AD in cognitively normal individuals. Participants received [^{18}F]FDG brain PET examination and completed semiquantitative food frequency questionnaires. In this study, higher consumption of saturated fats was associated with lower brain glucose metabolism supporting a possible relation between fatty acids consumption and brain glucose metabolism (39). Testing this hypothesis, a preliminary intervention study in humans (40) assessed the effect of a 3-week n-3 PUFA supplementation on cerebral glucose metabolism. No difference between healthy control and supplemented elderly people was observed, but a limitation of this pilot study was the short duration of the dietary intervention (3 weeks). The present study, performed during a much longer period (1 year, in a species whose median life span is close to 6 years) confirms the role of n-3 PUFAs in the regulation of brain glucose metabolism. Modulating DHA dietary intake may therefore help to prevent the glucose hypometabolism observed during age-related cognitive decline, but only on the basis of a long-term supplementation, and probably started at younger age, before the onset of glucose hypometabolism. In this context, we already demonstrated that the effects of long-term n-3 LCPUFA supplementation on cognition and behavior (including anxiety) is age dependent (20, 21). Indeed, our previous findings in lemurs indicate that n-3 PUFA supplementation is associated with lower anxiety and higher cognitive performance when started at young age (21) but does not counteract the age-related declines in old lemurs when started at old age (20) (>6 years old). Structural and functional modifications of the brain during aging could explain differential effects of fatty acids on behavior in function of age. Aged animals may differ from young animals due to age-related changes in n-3 PUFA metabolism as suggested by animal studies reporting a significant decrease in the level and turnover of PUFAs during aging (41).

CONCLUSION

The present results demonstrate for the first time that n-3 LCPUFA supplementation can lead to improved glucose entry and utilization into the primate brain. Our data suggest that increasing n-3 LCPUFA dietary intakes, equivalent to recommendations for humans, may improve brain glucose utilization in adults. Thus, n-3 LCPUFA supplementation, started before the onset of aging, could potentially help prevent the glucose hypometabolism observed during aging. We suggest that timing and duration of the supplementation is the key point influencing the positive and significant effect of n-3 LCPUFA dietary supplementation on brain function, particularly brain glucose utilization: the supplementation must be established on a long-term basis, and, very importantly, it must be started early enough to prevent the negative effects of aging, as suggested by our previous behavioral studies (20, 21).

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