Protective Effects of Uridine Plus Docosahexaenoic Acid in a Rat Model of Parkinson’s Disease

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Abstract

Parkinson's disease (PD) causes degeneration of midbrain dopaminergic neurons and diminishes striatal dopaminergic transmission. We previously showed in normal animals that administering the phosphatide precursors uridine (as uridine-5'-monophosphate [UMP]) and docosahexaenoic acid (DHA) can increase the quantity of synaptic membrane per brain cell, and that uridine can enhance dopamine release. We have now tested their effects on rotational behavior and brain composition in a rat model of PD. Rats started receiving a control or UMP-supplemented (0.5%) diet, and/or, by gavage, DHA (300 mg/kg) on the first day of the study; they were injected with 6-hydroxydopamine (6-OHDA) into the right striatum on day 4; tested for d-amphetamine-induced rotational behavior on day 25; and sacrificed on day 29. Giving UMP, DHA, or both reduced d-amphetamine-induced ipsilateral rotations by 48%, 47%, or 57% three weeks following 6-OHDA injections. The combination also increased dopamine levels, tyrosine hydroxylase (TOH) activity, TOH protein levels, and phosphatide levels in lesioned striata, and dopamine and phosphatide levels in intact striata. Synapsin-1 levels, reduced in lesioned striata of control rats, were restored following all three treatments. These data indicate that administering the phosphatide precursors uridine and DHA can ameliorate behavioral and biochemical defects in a rat model of PD.
Parkinson's disease (PD) is characterized by the progressive degeneration of dopamine-containing neurons in the midbrain, particularly in the pars compacta of the substantia nigra\(^1,2\), and by a reduction in dopamine (DA) levels and release in the basal ganglia. Moreover, the striatal DA depletion is associated with a decrease in dendritic spine density on striatal medium spiny neurons\(^3\), observed both in postmortem studies\(^4,5\) and in animal models of PD\(^3,6\). This impairment in dopaminergic neurotransmission leads to characteristic motor symptoms including akinesia, rigidity, and resting tremor\(^7\).

Current treatment of PD can be surgical, ie., pallidotomy, deep brain stimulation, or foetal graft implantation\(^8\), or, more commonly, medical, using drugs to increase synaptic DA (e.g. Levodopa [L-dopa]), or various anticholinergic or antiglutamatergic agents\(^9\). Various allegedly neuroprotective agents have been proposed as treatments, for example riluzole\(^10\), coenzyme Q10\(^11\), glial-derived neurotrophic factor (GDNF)\(^12\) and vitamin E\(^13\). However, compelling supporting data are lacking for clinical use of any of these compounds\(^9\). No treatment is available that has been shown to increase the numbers or sizes of dopaminergic nigrostriatal terminals in Parkinsonian brains.

We previously observed that chronic oral administration of two circulating phosphatide precursors, uridine (as uridine-5'-monophosphate [UMP]) and docosahexaenoic acid (DHA), to gerbils increases the amounts of brain phosphatides and specific pre- and postsynaptic proteins per brain cell\(^14,15\), and that chronic administration of uridine to rats enhances potassium-evoked striatal dopamine release\(^16\). Chronic administration of UMP and DHA also increases the number of hippocampal dendritic spines in gerbil brains\(^17\). We have now tested the effects of UMP and/or DHA on chemical and behavioral aspects of impaired DA neurotransmission in a rat model of PD.
MATERIALS AND METHODS

Animals

Male Sprague Dawley rats (200-250 g BW; Charles River, Wilmington MA) were housed at room temperature, under 12-h light/12-h dark conditions with ad libitum access to food and water. Experiments were conducted according to the National Institutes of Health Guide for the Care and Use of Laboratory Animals (NIH Publications No. 80-23) revised 1996, and formal approval to conduct them was obtained from MIT’s Committee on Animal Care. All efforts were made to minimize the number of animals used and their suffering.

Surgery

Rats anesthetized intraperitoneally with ketamine and xylasine (80 and 10 mg/kg BW, respectively) received unilateral injections of 6-hydroxydopamine (6-OHDA; Sigma, St. Louis, MO), each containing 8 μg dissolved in 2 μl of 0.3% L-ascorbic acid/0.9% saline, into their right striata. Two small burr holes were drilled using a bone drill (Ideal micro-drill; CellPoint Scientific, Gaithersburg, MD) on the right side of the skull; injections were made into each using a 10-μl microsyringe (Hamilton Company, Reno, NV) fitted with a 26-gauge steel cannula. Coordinates for the injection sites were: (first injection site) anterior-posterior (AP) = +0.5 mm relative to bregma, medial-lateral (ML) = -2.4 mm from the mid-line, dorsal-ventral (DV) = -5.0 mm from dura; (second injection site) AP =-0.5 mm relative to bregma, ML = -4.2 from the mid-line and
DV = -5.0 mm from dura, with the tooth bar set at 0 mm. The 6-OHDA was injected at a rate of 1 μl/min using a microinfusion pump (CMA/100; Bioanalytical Systems, W. Lafayette, IN).

Treatments

In one set of experiments, control rats were given access to standard 0.1% choline-containing rodent diet (Teklad Global 16% protein rodent diet, Harlan-Teklad, Madison, WI); a second group received this diet supplemented with uridine monophosphate (UMP; 0.5%); a third group of rats received the control (unsupplemented) diet plus, each day, 300 mg/kg of docosahexaenoic acid (by gavage in 1 ml/kg of 5% Arabic gum solution dissolved in deionized water); and a fourth group received the 0.5% UMP supplemented diet plus, by gavage, 300 mg/kg of DHA. All treatments were given for 4 weeks starting 3 days prior to 6-OHDA injections. Animals not receiving DHA were gavaged daily for 4 weeks with its vehicle, 5% Arabic gum solution.

In the other set of experiments, the same protocol was followed except that a lower dose (100 mg/kg) of DHA, in combination with UMP-supplemented diet was also tested.

Assessment of Rotational Behavior

Each animal was systematically handled on a regular basis throughout the study. Drug-induced (d-amphetamine; 5 mg/kg, i.p.) rotational behavior was tested 3 weeks following 6-OHDA treatment. After receiving the d-amphetamine, animals were placed
into metal cylinders (35 cm diameter, 37 cm height) and their movements recorded between 15 and 45 minutes after injection, using a camcorder placed 1.5 meters above the cylinders. Ipsilateral (towards the lesioned side) rotations by each rat were counted by a blind observer. Rats were allowed a drug washout period of three days before sacrifice.

**Biochemical Assays**

Treatment with phosphatide precursors was terminated on the 28th day (24 days after lesioning). The following day rats were sacrificed and their brains were removed; striata were dissected, transferred into pre-weighed eppendorf tubes, and frozen immediately on dry ice. Samples were subsequently assayed for DA, tyrosine hydroxylase (TOH) activity, TOH and Synapsin-1 protein levels, and phospholipid composition.

DA was assayed using an ESA Coulochem II 5100A detector (E1= -175 mV; E2= 325 mV; Eguard= 350 mV) with an ESA Microdialysis Cell (model 5014B, ESA, North Chelmsford, MA). The flow rate of the mobile phase (MD-TM, ESA) was 0.4 mL/min. The column (ESA MD 150, 3 x 150 mm, 3 μm) was kept at 40°C. Samples were injected and analyzed by Alltech AllChromsystem (Alltech, Deerfield, IL) as described previously [16].

TOH activity was measured according to a previously-described protocol19. The rate of 14CO2 formation from 1-[carboxy-14C]tyrosine (Perkin-Elmer, Waltham, MA) was measured, and TOH activity was expressed as nmol DOPA formed per hour per mg protein tissue.
The levels of TOH and Synapsin-1 proteins were determined by Western blot as described previously. The following primary antibodies were used: mouse anti-Synapsin-1 (Calbiochem, EMD Chemicals, San Diego, CA), and rabbit anti-tyrosine hydroxylase (Abcam, Cambridge, MA).

Striatal phospholipids were extracted and measured as described previously.

Statistics

Data were analyzed using one-way analysis of variance (ANOVA) followed by post hoc Tukey test. Student’s t-test was applied as appropriate. Values of P less than 0.05 were considered to be significant. Data are presented as the mean ± S.E.M.

RESULTS

As expected, intrastriatal injection of 6-OHDA, a neurotoxin that destroys dopaminergic nerve terminals, caused 64% and 65% decreases in striatal DA levels and TOH activity, respectively. There was a 35% loss in TOH protein and a 15% loss in Synapsin-1, while phospholipid levels did not change in the lesioned striatum compared with the intact striatum.

Rotational Behavior

Intraperitoneal injection of d-amphetamine (5 mg/kg) 3 weeks following 6-OHDA injections into right striata induced ipsilateral rotations in all rats. Compared with control rats (receiving the unsupplemented diet and DHA’s vehicle by gavage), oral
administration for 24 days of the phosphatide precursors UMP, DHA, or UMP plus DHA significantly reduced the number of rotations by 48%, 47%, or 57%, respectively (all P<0.05) (Table 1). In a separate experiment, the effect of DHA on rotational behavior was confirmed with the 300 mg/kg dose, but not with a 100 mg/kg dose (data not shown).

**Striatal DA Levels**

DA levels in the lesioned (right) striata were 36% of those in the intact (left) striata of control rats (0.25 ± 0.02 vs 0.70 ± 0.02 nmol/mg protein) (Table 2). Chronic administration of the UMP-supplemented diet alone increased DA levels in the lesioned but not the unlesioned striata (by 41%; P<0.01) (Table 2). Combining UMP and DHA increased DA levels in both the lesioned (by 37%; P<0.05) and the intact (18%; P<0.01) striata (Table 2). Supplementation of DHA alone tended to increase DA levels in lesioned striata, but not significantly (Table 2).

**TOH Activity and TOH Protein Levels**

TOH activity in the lesioned (right) striata was 35% of that in intact striata (1.40 ± 0.06 vs 3.98 ± 0.26 nmol DOPA formed/h/mg protein) (Table 3A). UMP administration, alone or with DHA, increased TOH activity in lesioned striata by 53% or 52%, respectively (Table 3A), but had no effect on TOH activity in intact striata.

DHA supplementation alone, and when given with UMP, increased TOH protein levels in the lesioned striata, by 21% and 22%, respectively (Table 3B); UMP failed to produce this significant increase. TOH protein levels in lesioned striata were reduced by about 35% compared with those in intact striata of control rats (data not shown).
Synapsin-1 Levels

Levels of Synapsin-1, a specific presynaptic protein located on vesicular membranes, were reduced in the lesioned striata by 15% (P<0.001), and restored by all treatments (Figure 1).

Phospholipid Levels

As expected from the neurochemical specificity of its toxicity, injection of 6-OHDA into right striata did not change phospholipid levels significantly (i.e., total phospholipids in lesioned striata and intact striata of control rats were 376 ± 11 and 377 ± 16 nmol/mg protein, respectively) (Table 4). Administration of both UMP and DHA increased total phospholipid levels by 15% (P<0.05) and 21% (P<0.001) in lesioned and the intact striata, respectively (Table 4). Levels of individual phospholipids such as phosphatidylcholine (PC), phosphatidylethanolamine (PE), phosphatidylserine (PS) and sphingomyelin (SM) were also significantly increased in lesioned and intact striata, by 18-60% and 19-45% following UMP plus DHA treatment, respectively (Table 4). Phosphatidylinositol (PI) levels in intact striata were increased by 2-fold and 2.7-fold following DHA supplementation alone and in combination with UMP, respectively, while PI increased by 50% in lesioned striata with either treatment (Table 4). DHA supplementation also alone increased striatal PE and PS, levels by 19% and 27% in the intact striata, respectively (Table 4).
DISCUSSION

These data show that chronic oral administration of the phosphatide precursors uridine (as UMP) and/or DHA significantly reduces the number of d-amphetamine-induced ipsilateral rotations in rats with unilateral striatal lesions caused by 6-OHDA (Table 1). UMP alone or in combination with DHA significantly increased DA levels (Table 2) and TOH activity (Table 3A) in lesioned striata, while UMP plus DHA increased DA levels significantly in intact striata (Table 2). DHA alone enhanced TOH protein levels (Table 3B) and brain PI levels (Table 4) significantly, while its combination with UMP likewise enhanced levels of TOH protein (Table 3B) and all phosphatide classes investigated (i.e., PC, PE, PS, SM, and PI) in lesioned striata as well as in intact striata (Table 4). Moreover, levels of Synapsin-1, a presynaptic vesicular protein, which were reduced in lesioned striata of control rats, were restored following UMP, DHA or UMP plus DHA treatment (Figure 1).

In the present study, the injection of 6-OHDA (8 µg) into two different regions of the right striatum caused about a 65% decrease in TOH activity in the lesioned striata, compared with that in the intact (left) striata of control rats (1.40 ± 0.06 vs 3.98 ± 0.26 nmol DOPA formed/h/mg protein) (Table 3A). Similarly, DA levels in the lesioned striata decreased by about 64% compared with those in the intact (left) striata of control rats (0.25 ± 0.02 vs 0.70 ± 0.02 nmol/mg protein) (Table 2). These observations are in good accord with previous studies which reported a 50-80% reduction in TOH-immunoreactive cells in rat striatum following two injections of 6-OHDA (each 10 µg)18. It has long been known that peripheral administration of d-amphetamine to rats with
unilateral striatal lesions induced by 6-OHDA causes ipsilateral circling by acting
presynaptically, presumably by releasing DA from nigrostriatal terminals which are more
abundant on the intact side. Consistently, rats in our study exhibited ipsilateral rotations
following intraperitoneal injection of d-amphetamine 3 weeks after their right striata were
lesioned by 6-OHDA. Chronic oral supplementation with the phosphatide precursors
uridine and/or DHA ameliorated this rotational behavior: Giving UMP alone, DHA
alone, or a combination of UMP and DHA reduced d-amphetamine-induced ipsilateral
rotations by 48%, 47%, or 57%, respectively (Table 1). These reductions were
accompanied by recoveries in biochemical parameters, discussed below.

Consistent with our previous studies, chronic oral supplementation with uridine
enhanced by 41% the reduced DA levels in lesioned striata following 6-OHDA injections
(Table 2). Moreover, a combination of uridine and DHA also increased striatal DA levels
(by 37%) on the lesioned side (Table 2). TOH activity which was reduced by 65% in the
lesioned striata likewise was partially restored by 53% and 52%, following treatment
with UMP alone or with UMP plus DHA, respectively (Table 3A). TOH protein levels,
which were reduced by 35% in the lesioned striata were enhanced significantly by 21%
and 22% following DHA, or UMP plus DHA administrations (Table 3B). Decreases (by
15%) in levels of the presynaptic vesicular protein Synapsin-1 in lesioned striata were
totally restored by all three treatments (Figure 1). Levels of striatal phosphatides did not
differ in lesioned vs intact striata (Table 4). UMP plus DHA treatment increased the
amounts of total phosphatides, as well as of individual phosphatide classes, in both
lesioned and intact striata (Table 4). DHA alone also significantly increased PI levels in
the lesioned, and PE, PS and PI levels in the intact striata (Table 4). That the amounts of
phosphatides did not differ significantly, and those of Synapsin-I were only 15% lower in lesioned vs intact striata was probably due to the fact that dopaminergic terminals constitute only a small fraction of all neuronal structures in the striatum. These data confirm our previous results which showed enhanced amounts of synaptic membranes\textsuperscript{14,15} and numbers of hippocampal dendritic spines\textsuperscript{17} following chronic oral administration of the phosphatide precursors uridine and DHA.

Synthesis of brain PC, the most abundant membrane phosphatide, via the predominant Kennedy pathway\textsuperscript{23} utilizes various circulating compounds, two of which are a pyrimidine (e.g., uridine) and a PUFA (e.g., DHA). Uridine, on entering the brain cells by the high-affinity CNT2 transporter located at the blood-brain barrier\textsuperscript{24}, is phosphorylated to UTP by uridine/cytidine kinase (UCK) and then converted to CTP, the compound that usually rate-limits PC synthesis, by CTP-synthase. DHA, on entering the brain\textsuperscript{25,26}, can be activated to docosahexaenoyl-CoA and acylated to the sn-2 position of DAG\textsuperscript{27} to form DAG species rich in DHA\textsuperscript{28}. Each step in the incorporation of uridine or DHA into brain phosphatides is catalyzed by a relatively low-affinity enzyme; this characteristic allows the administration of each precursor to affect the rate of phosphatide synthesis\textsuperscript{14,29}.

In conclusion, these data show that, chronic oral administration of the phosphatide precursors uridine and DHA, probably by enhancing the amount of synaptic membranes, can ameliorate the loss of dopaminergic terminals in 6-OHDA-lesioned striata. Administering uridine plus DHA to patients could possibly enhance the efficacy of current treatments for PD, by partially restoring dopaminergic nigrostriatal transmission.
Acknowledgements

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REFERENCES


15. Cansev M, Wurtman RJ. Chronic administration of docosahexaenoic acid or eicosapentaenoic acid, but not arachidonic acid, alone or in combination with uridine, increases brain phosphatide and synaptic protein levels in gerbils. Neuroscience In Press.

17. Sakamoto T, Wurtman RJ. Increased dendritic spine density in gerbil hippocampus following oral UMP and DHA supplementation. 10th International Conference on Alzheimer’s Disease and Related Disorders, Madrid, Spain, 2006.


Figure Legend

Figure 1. Synapsin-1 Levels

Experiments were carried out as described in Table 1 legend. On the 29\textsuperscript{th} day of treatment rats were sacrificed; brains were removed, and striata were dissected and assayed for Synapsin-1 levels using Western Blotting. ***P<0.001 compared with left (intact) striatum using Student's t test.
Rats were given, daily for 28 days, either a control or a UMP-containing (0.5%) diet (both also contained 0.1% choline), and received, by gavage, DHA (300 mg/kg; in a vehicle of 5% Arabic gum solution) or just its vehicle. Three days after the treatment had started (day 4), 8 μg of 6-OHDA dissolved in 2 μl of 0.3% L-ascorbic acid/0.9% saline was injected into two different sites within their right striata. Three weeks following the 6-OHDA injections (day 25), ipsilateral (towards the lesioned side) rotations by each rat were recorded for 30 minutes, between 15 and 45 min, after the i.p. injection of d-amphetamine (5 mg/kg). *P<0.05; and **P<0.025 compared with Control diet + Vehicle group using One Way ANOVA followed by post hoc Tukey test.

### Table 1. Rotational Behavior

<table>
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<tr>
<th>Treatment</th>
<th>Ipsilateral rotations/30 min</th>
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<tr>
<td>Control diet + Vehicle</td>
<td>151 ± 21</td>
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<tr>
<td>UMP diet + Vehicle</td>
<td>79 ± 22*</td>
</tr>
<tr>
<td>Control diet + DHA</td>
<td>81 ± 12*</td>
</tr>
<tr>
<td>UMP diet + DHA</td>
<td>65 ± 18**</td>
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Table 2. Dopamine (DA) Levels

<table>
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<tr>
<th>Treatments</th>
<th>Dopamine Levels (nmol/mg protein)</th>
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<tr>
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<td>Left (Intact) Striatum</td>
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<tr>
<td>Control diet + Vehicle</td>
<td>0.704 ± 0.024</td>
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<tr>
<td>UMP diet + Vehicle</td>
<td>0.749 ± 0.022</td>
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<tr>
<td>Control diet + DHA</td>
<td>0.745 ± 0.021</td>
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<tr>
<td>UMP diet + DHA</td>
<td>0.830 ± 0.022**</td>
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</table>

Experiments were carried out as described in Table 1 legend. Striata were assayed for DA levels. *P<0.05; and **P<0.01 compared with Control diet + Vehicle group using One Way ANOVA followed by post hoc Tukey test.
Table 3. Tyrosine Hydroxylase (TOH) Activity and TOH Protein Levels

**Table 3A**  
TOH Activity (nmol DOPA formed/h/mg protein)

<table>
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<th>Left (Intact) Striatum</th>
<th>Right (Lesioned) Striatum</th>
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</thead>
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<tr>
<td>Control diet + Vehicle</td>
<td>3.983 ± 0.26</td>
<td>1.405 ± 0.06</td>
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<tr>
<td>UMP diet + Vehicle</td>
<td>3.591 ± 0.20</td>
<td>2.144 ± 0.19*</td>
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<tr>
<td>Control diet + DHA</td>
<td>4.014 ± 0.12</td>
<td>1.906 ± 0.17</td>
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<tr>
<td>UMP diet + DHA</td>
<td>4.189 ± 0.24</td>
<td>2.131 ± 0.17*</td>
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</table>

**Table 3B**  
TOH Protein Levels (Percent of Control)

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<th>Right (Lesioned) Striatum</th>
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<tr>
<td>Control diet + Vehicle</td>
<td>100 ± 3</td>
<td>100 ± 3</td>
</tr>
<tr>
<td>UMP diet + Vehicle</td>
<td>95 ± 3</td>
<td>114 ± 4</td>
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<tr>
<td>Control diet + DHA</td>
<td>98 ± 5</td>
<td>121 ± 2**</td>
</tr>
<tr>
<td>UMP diet + DHA</td>
<td>100 ± 5</td>
<td>122 ± 6**</td>
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Experiments were carried out as described in Table 1 legend. Striata were assayed for TOH activity (Table 3A) using radioenzymatic method and TOH protein levels (Table 3B) using Western Blotting. *P<0.05; and **P<0.01 compared with Control diet + Vehicle group using One Way ANOVA followed by post hoc Tukey test.
Table 4. Phospholipid Levels

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<th>PE</th>
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<td>128 ± 5</td>
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<td>UMP diet + Vehicle</td>
<td>394 ± 15</td>
<td>126 ± 4</td>
<td>139 ± 5</td>
<td>25 ± 1</td>
<td>16 ± 1</td>
<td>11 ± 1</td>
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<tr>
<td>Control diet + DHA</td>
<td>402 ± 9</td>
<td>142 ± 3</td>
<td>149 ± 4*</td>
<td>28 ± 1**</td>
<td>16 ± 1</td>
<td>20 ± 1**</td>
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<tr>
<td>UMP diet + DHA</td>
<td>455 ± 9***</td>
<td>152 ± 4**</td>
<td>158 ± 7**</td>
<td>32 ± 1***</td>
<td>21 ± 2*</td>
<td>27 ± 3***</td>
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<table>
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<tr>
<th>Treatments</th>
<th>Total PL</th>
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<tr>
<td>Control diet + Vehicle</td>
<td>376 ± 11</td>
<td>130 ± 4</td>
<td>127 ± 6</td>
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<td>424 ± 14</td>
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<td>Control diet + DHA</td>
<td>419 ± 13</td>
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<td>144 ± 7</td>
<td>25 ± 1</td>
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<td>UMP diet + DHA</td>
<td>433 ± 14*</td>
<td>153 ± 4**</td>
<td>150 ± 3*</td>
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Experiments were carried out as described in Table 1 legend. *P<0.05; **P<0.01; and ***P<0.001 compared with Control Diet + Vehicle group using One Way ANOVA followed by post hoc Tukey test. Total PL, Total Phospholipids; PC, Phosphatidylcholine; PE, Phosphatidylethanolamine; PS, Phosphatidylserine; PI, Phosphatidylinositol; SM, Sphingomyelin.
Control Diet + Vehicle

Control Diet + DHA

UMP Diet + Vehicle

UMP Diet + DHA

270×355mm (300 x 300 DPI)
RICHARD J. WURTMAN

ITINERARY FOR
TUESDAY SEPTEMBER 4 – SUNDAY SEPTEMBER 9, 2007

BOSTON—AMSTERDAM—NICE—LONDON—BOSTON

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</tbody>
</table>

Note: Dr. Tremblay’s mobile number: 06 07 55 94 00