Diurnal rhythm of plasma EPA and DHA in healthy adults

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ABSTRACT

Knowledge of the diurnal variation in circulating omega-3 polyunsaturated fatty acids (n-3 PUFAs) may be an important consideration for the development of dosing protocols designed to optimise tissue delivery of these fatty acids. The objective of the current study was to examine the variation in plasma concentrations of eicosapentaenoic acid (EPA; 20:5n-3) and docosahexaenoic acid (DHA; 22:6n-3) over a 24-hour period in healthy adults under eating and sleeping conditions generally approximate to a free-living environment. Twenty-one healthy participants aged 25 to 44 years took part in a single laboratory visit encompassing an overnight stay. EPA and DHA were measured in plasma samples collected every two hours from 22:00 until 22:00 the following day, with all meals being provided at conventional times. Cosinor analysis was used to estimate the diurnal variation in each fatty acid from pooled data across all participants. A significant diurnal variation in the pooled plasma concentrations of both fatty acids was detected. However, evidence of distinct rhythmicity was strongest for DHA. The timing of the peak concentration of DHA was 17:43 with a corresponding nadir at 05:43. In comparison, the observed acrophase for EPA was delayed by three hours, occurring at 20:41, with a nadir at 08:41. This is the first time that the diurnal variation in these important bioactive fatty acids has been described in a sample of healthy adults following a normal pattern of eating and sleeping. In the absence of any dietary intake of EPA and DHA, circulating levels of these fatty acids fall during the overnight period and reach their lowest point in the morning. Consumption of n-3 PUFAs at night time, which counteracts this pattern, may have functional significance.

Keywords: Docosahexaenoic acid; eicosapentaenoic acid; diurnal rhythm; healthy population

Abbreviations: EPA, eicosapentaenoic acid; DHA, docosahexaenoic acid; n-3 PUFA, omega-3 polyunsaturated fatty acids

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1. Introduction

Following several decades of investigation into the effects of the bioactive omega-3 polyunsaturated fatty acids (n-3 PUFAs) eicosapentaenoic acid (EPA; 20:5n-3) and docosahexaenoic acid (DHA; 22:6n-3) on cognition, firm conclusions regarding their specific effects are still to be drawn. Evidence from cross-sectional studies is generally consistent: across the lifespan, increased consumption of n-3 PUFAs is associated with better cognitive function [1-5]. On the other hand, critical and systematic reviews of clinical trials are more tentative, often citing methodological variations underpinning the inconsistency in the evidence [6-8]. Recommendations regarding dose, duration of intervention, outcome measures and populations from which study samples are drawn are now being proposed, which may lead to stronger evidence in time [6, 7, 9, 10].

Due consideration is also being given to the formulation of the n-3 PUFA intervention itself, so that maximal delivery of DHA and/or EPA to tissues is ensured [11-13]. With regards optimal digestion and absorption, a further factor that may influence the efficacy of n-3 PUFAs is the time at which the supplement is consumed. Evidence from rodent studies suggests circadian variation in lipid metabolism that may be an important consideration. For example, under normal light-dark conditions where animals have *ab libitum* access to food, the peak time for lipid digestion and absorption and for fatty acid oxidation occurs at the very

beginning of the active/awake phase [14], suggesting consumption of fats is more desirable in the morning.

Specific investigation of the diurnal variation of blood levels of n-3 PUFAs in humans is limited to two studies, the results from which do not agree. Cornelissen et al. [15] estimated peak whole blood (fingerprick) concentrations of total n-3 PUFAs (EPA, DHA, alpha linolenic acid, docosapentaenoic acid) collected from one male and one female to occur shortly after 08:00, with an estimated nadir occurring at 20:00. On the other hand, the highest concentrations of plasma EPA and DHA were reported by Dallmann et al. [16] at around noon in their sample of ten males, after which they rapidly declined. Whilst both studies recruited a small number of participants, key methodological differences between these studies may have influenced the reported findings. For example, both participants in the study by Cornelissen et al. had a number of health issues, and the authors reported that one of the participants followed an odd schedule on the day of data collection that the authors suspected may have affected the results. On the other hand, despite the fact that all of the participants recruited to the study reported by Dallmann et al. were healthy, the blood samples were collected under a strict regimen of hourly feeding around the clock and participants were not able to sleep under a constant routine protocol. The current study therefore aimed to investigate the diurnal rhythm of plasma concentrations of EPA and DHA in humans under normal eating and sleeping conditions. The results described below comprise a secondary outcome from a larger study that also evaluated the bioavailability of acute doses of n-3 oils, which together informed the design of a randomised controlled trial investigating the effects of n-3 oils on cognitive function.

2. Materials and Methods

2.1. Participants

All study procedures were reviewed and approved by Yorkshire & The Humber - Bradford Leeds National Health Service Research Ethics Committee (REC 16/YH/0224) and were conducted according to the principles of the Declaration of Helsinki. All participants gave their written informed consent before inclusion in the study. The trial is registered at ClinicalTrials.gov (NCT02661698).

A convenience sample of 21 adults aged 25 to 44 years, recruited from within the staff and student population of Northumbria University, completed the study (n = 10 males, 11 females; mean age 31.86 years, mean BMI 25.30 kg/m^2 and self-reporting an average intake of 0.5 portions of oily fish per week). Participants declared themselves to be in good health. Participant demographics are displayed in **Table 1**. Exclusion criteria were: consumption of more than one oily fish meal per week; BMI < 18 or > 35 kg/m^2; high blood pressure (defined as systolic > 159 mmHg or diastolic > 99 mmHg); smoking; food allergies or insensitivities; pregnancy; breast feeding; currently taking any dietary supplements; sleep disturbances and/or taking sleep aid medication; chronic health conditions; gastrointestinal problems; active infections and any health condition that would prevent the fulfilment of the study requirements. The study was conducted from March to October 2016 at the Brain, Performance and Nutrition Research Centre (BPNRC), Northumbria University, UK.

2.2. Study design

Participants attended four identical study visits. During the first of these visits, all participants received a placebo treatment (olive oil capsules) in a single blind design. This approach fulfilled a dual purpose; the first was to meet the objective of the current paper, namely to investigate the diurnal variation in plasma EPA and DHA. The second was to serve as a 'practice' visit for a randomised controlled trial assessing the enrichment of plasma fatty acids following bedtime consumption of n-3 oils—which comprised the remaining three visits—as it was anticipated some participants might not feel comfortable with the extended, overnight laboratory protocol. Only data collected at the first visit are presented here. Sample size was calculated for the intervention study; the effect size from a previous in-house bioavailability study (unpublished data) showed that the effect size for 24- hour Area Under the Curve (AUC) increase in plasma DHA and EPA concentrations following DHA-enriched oil was f = 0.49. Assuming similar, large effect size, an a priori calculation suggested that in order to achieve 80% power and 95% statistical confidence, the sample size required was 24, inclusive of a 20% drop out rate.

The placebo treatment comprised three soft gelatine capsules providing a total of 3 g refined olive oil. The blinding and labelling of the treatment was carried out on site by a third party who had no further involvement in the study.

2.3. Procedure

Participants arrived at the Brain, Performance and Nutrition Research Centre (BPNRC) at Northumbria University at 19:30 following a minimum two-hour fast. They were immediately fitted with a cannula and a sample was drawn to check the participant was comfortable with the procedure and to check for patency; this sample was discarded. At 20:00, participants

were given an evening meal consisting of pasta, tomato sauce, grated cheese and olive oil (624 Kcal, 26 g fat). They then rested until 22:00 when the treatment (i.e. olive oil) was administered with 200 mL of water. Participants were then settled into their room in the Northumbria Centre for Sleep Research (adjacent to BPNRC) and lights out was at 22:30. Lights on was at 06:00 the following morning and a breakfast of cereal and toast was served at 07:00 (504 Kcal, 13 g fat). Participants spent the remainder of the visit in a comfortable waiting room in BPNRC where they read, watched TV, listened to music etc. Lunch, comprising a cheese sandwich, an apple and a sweet biscuit (479 Kcal, 17 g fat), was provided at 12:00, and an afternoon snack was provided at 16:00 comprising a yoghurt, a banana and a packet of savoury cracker snacks (402 Kcal, 15 g fat). The identical evening meal to the previous day was given at 20:00 and participants went home by public transport or by taxi at 22:15. Hot drinks (e.g. tea, coffee) were offered along with the study meals and participants drank water *ad libitum* throughout the visit.

In total, from each participant, thirteen blood samples were drawn for analysis via cannula, with the first being drawn immediately prior to treatment administration at 22:00. Samples were then collected every two hours on the hour for the remainder of the study visit. The last sample was drawn at 22:00, twenty-four hours post treatment. Samples were always collected prior to the study meals. To keep the vein patent throughout the entire study visit, after each draw the vein was flushed with 0.5 mL saline solution (PosiFlush, BD, UK).

2.4. Fatty acid analysis

Blood was collected in EDTA vacutainers that were refrigerated at 4°C for a maximum of four hours before being processed. Samples were then centrifuged for 20 minutes at 3000 x g at

135 4°C. Resulting plasma was pipetted off and frozen at -80°C prior to analysis, which took place
136 at the University of Southampton.

137 Lipid was extracted from plasma using 5 mL of chloroform/methanol (2:1; v/v) containing 0.2
138 M butylated hydroxytoluene as antioxidant. 1 M sodium chloride (1 mL) was added and the
139 sample vortexed and then centrifuged. The lower solvent phase was aspirated and
140 evaporated to dryness under nitrogen at 40°C. The lipid extract was redissolved in 0.5 mL
141 toluene and fatty acids were released from esterified lipids and simultaneously derivatized to
142 methyl esters by incubation with 1 mL 2% H_2SO_4 in methanol for a minimum of 2 h at 50°C to
143 form fatty acid methyl esters. The samples were then neutralized and fatty acid methyl esters
144 transferred into hexane for analysis by gas chromatography. Fatty acid methyl esters were
145 separated on a BPX-70 fused silica capillary column (30 m x 0.2 mm x 0.25 µm, manufactured
146 by SGE) in a HP6890 gas chromatograph fitted with a flame ionisation detector. Gas
147 chromatography run conditions were as described elsewhere [17]. Dipentadecanoyl
148 phosphatidylcholine added into the initial plasma sample was used as an internal standard
149 for quantification purposes and a Supelco® 37 Component FAME Mix was used as a calibration
150 reference standard (Sigma-Aldrich). Plasma concentrations of EPA and DHA are expressed as
151 µg/mL.

152

153 **2.5. Statistical analysis**

154 In order to investigate the diurnal variation in plasma EPA and DHA, data collected over the
155 24-hour period were evaluated using cosinor analysis. In this procedure, a cosine curve is
156 fitted to the data based on the least squares method from which the MESOR (Midline
157 Estimating Statistic Of Rhythm; a mean value), amplitude (the difference between the MESOR

158 and peak plasma concentrations) and acrophase (time of the peak plasma fatty acid
159 concentration) parameters are estimated [18, 19]. In order to establish the presence of a
160 common rhythm between participants, data points (µg/mL) from each participant were firstly
161 expressed as a percentage of their respective mean value and then pooled for the cosinor
162 analysis [15]. Four data points were missing due to technical issues during blood sampling.
163 These missing data were imputed by averaging the value of the samples drawn immediately
164 prior to and after the missing sample. Linear regression was used to determine the goodness
165 of fit of the estimated cosine curve where the R^2 value, referred to here as Percentage Rhythm
166 (PR), relates to the percentage of the variability accounted by the curve and the *p* value of the
167 F test is the likelihood of the data fitting a straight line as opposed to a cosine curve [20].

168 A post-hoc exploratory analysis was carried out to assess the difference between males and
169 females at each of the sampling time points. This was conducted using the MIXED procedure
170 in SPSS and included the terms sample and sex. Subject was included as a random effect.

171 Significance was set at $p < 0.05$. Calculations of the cosine curve parameters were made in
172 Microsoft Excel 2016 and the regression analyses were carried out in SPSS version 24 (IBM,
173 USA).

174

175 **3. Results**

176 Of the 25 participants who received treatment, four participants withdrew from the study; in
177 all cases this occurred during the study visit due to feeling uncomfortable with the overnight
178 blood sampling. Mean (±SD) plasma fatty acid concentrations for EPA and DHA collected at
179 the first pre-treatment sample (22:00) were 14.27 ± 9.08 µg/mL and 32.41 ± 14.07 µg/mL,

180 respectively. When expressed as relative percentages of total fatty acids (EPA, 0.87 ± 0.79;
181 DHA, 1.94 ± 0.95), these values are largely in line with what has been observed in other
182 samples from the UK population [21].

183 Results from the cosinor analysis of the pooled data are presented in **Table 2**. This table
184 includes the cosinor analysis of all twenty-one fatty acids that were quantified in the plasma
185 samples, although only EPA and DHA were of interest in the current study. A cosine curve,
186 indicating diurnal variation, was fitted to the data with statistical significance for both DHA
187 and EPA. In clock time, the acrophase for DHA and EPA was estimated to occur at 17:43 and
188 20:41, respectively, with the nadir of each occurring exactly 12 hours previously (i.e. at 05:43
189 and 08:41 respectively). A similar pattern was observed for all analysed fatty acids. Individual
190 data points, plotted as a function of time, are presented together with the 24-h cosine curve
191 for DHA and EPA in **Figure 1**. Plasma concentrations of DHA and EPA across the 24-hour
192 period for each individual are presented in **Supplementary Material Figure 1.**

193 In the exploratory analysis, the DHA and EPA data were analysed separately for males and
194 females. For both fatty acids, the rhythm was phase advanced in females although this was
195 more evident for DHA. Specifically, the acrophase was estimated to occur at 16:16 in females
196 and 18:57 in males for DHA. For EPA, the estimated acrophase occurred at 20:03 and 20:57
197 for females and males, respectively. Raw Individual data points, plotted as a function of time,
198 are presented together with the 24-h cosine curve for DHA and EPA in males and females
199 separately in **Supplementary Material Figure 2**.

200 The results of the linear mixed model analysis showed no main effect of sex or interaction
201 with time with regards the raw plasma concentrations (both $p < 0.05$). Data are displayed in
202 **Supplementary Material Table 1**.

203

4. Discussion

The current study aimed to investigate the diurnal variation of plasma concentrations of the n-3 PUFAs EPA and DHA under eating and sleeping conditions generally approximate to what might be considered a 'normal' pattern for adults living in the UK. The cosinor analysis revealed a significant diurnal rhythm for both DHA and EPA. Whilst there was evidence of a similar general pattern for both these fatty acids and indeed all the analysed fatty acids—where the lowest concentrations were detected in the morning and the highest in the evening—the phase was advanced by three hours for DHA, compared to EPA.

Apart from the obvious sleep-wake cycle, a number of physiological functions including body temperature, blood pressure and metabolism are known to oscillate daily in mammals [22]; therefore, our observation of the presence of diurnal variation in plasma EPA and DHA is somewhat expected. Biological rhythms are controlled via clock genes organized in a transcriptional feedback loop (e.g. *Clock, Bmal1, Per* and *Cry*), with the 'master clock' located within the superchiasmatic nucleus of the hypothalamus [23]. Clock-controlled genes have also been found in most peripheral tissues, with a large number of physiological functions controlled by endogenous circadian clocks that can oscillate independently from sleep and feeding cues [16]. To date, circadian rhythms have been identified at all stages of lipid metabolism including absorption, mitochondrial transport and partitioning [24]. Diurnal variation is therefore evident in tissues such as plasma, and it would appear that different lipid species display distinct rhythms in humans. For example, in one study wherein circadian variation was measured over a 28-hour period, the acrophase of the majority of di- and triglycerides occurred in the morning whereas peaks in phosphatidylcholine (PC) species were

observed in the evening [25]. Other trials have shown diurnal variation in plasma free fatty acids, with peak concentrations reported in the afternoon and evening [26, 27].

Previous contradictory reports of the timing of the peak concentration of n-3 PUFAs specifically indicate that this occurred in the morning [15] or at lunchtime [16], whereas we observed these to occur in the evening. A key reason underpinning this observed difference could be the more naturalistic pattern of eating and sleeping we aimed to achieve with our protocol. Indeed, Singh et al. [28] reported peak total plasma lipids at around 18:00 in 60 healthy young adults (21-40 years) who were synchronized to follow a diurnal pattern of eating and sleep/activity similar to the current study. Given that the standard meals provided to participants throughout the course of the study visit were free from n-3 PUFAs, the observed diurnal rhythms for DHA and EPA most likely reflect daily variations in the transfer of these fatty acids between different lipid pools e.g. plasma phospholipids, triglycerides, and cholesteryl esters, cellular phospholipids and adipose and other tissues. In plasma, higher levels of EPA and DHA are esterified to phosphatidylcholine (PC) compared to other fractions after repeated dosing [19]. Given that PC species have been shown to peak in the evening [25, 29], this suggests that the diurnal variation we observed was probably for EPA and DHA esterified to phospholipids, and possibly PC in particular. It is a limitation of the current study that the concentration of EPA and DHA was measured in total plasma lipids rather than within specific lipid fractions, which would have enabled us to address this issue directly.

In contrast to previous studies, we did not find any evidence of a difference in absolute concentrations of DHA and EPA across the 24-hour period between males and females. Previously, analysis of plasma metabolites revealed that concentrations of unsaturated fatty acids were higher in females and also more stable across BMI compared to males [30]. In

addition, total plasma lipids were reported to be higher in females than males regardless of age between 06:00 and 18:00 [28]. In the current study, the sex differences in plasma concentrations of DHA and EPA were analysed post hoc, and should therefore be interpreted with caution, especially taking into consideration the small number of participants included in this sub-group analysis. However, it is interesting that we did observe a difference of almost three hours in the acrophase of DHA, with females peaking earlier than males. This pattern was also observed for EPA, but to a much lesser extent (~1-hour). Sex differences in the timing of melatonin secretion, body temperature, sleep onset and the expression of clock genes in the brain have been observed, with earlier timing consistently demonstrated in females [31]. While hormonal differences such as the circadian rhythm of testosterone and the monthly fluctuations in estradiol and progesterone have been cited as common reasons underpinning any observed differences between males and females with regards lipid metabolism, it has also been argued that the action of insulin, also known to vary by sex, could be key [32]. Therefore, future studies designed specifically to investigate sex differences in diurnal variation of lipid metabolism should seek to control for monthly hormonal fluctuations, menopausal status and insulin- and glucoregulation.

It has been demonstrated that lipid metabolism in humans is under endogenous circadian clock control [16]. However, although we observed a significant diurnal variation in both EPA and DHA at the group level, our data also revealed a great degree of inter-individual variability as evidenced by the low (<30%) percentage rhythm detected for both fatty acids. This, coupled with the variability in the previously reported rhythmicity of circulating levels of n-3 PUFAs [15, 16], would suggest some degree of entrainment with rest/activity and feeding cues. We aimed to mitigate any circadian misalignment due to altered sleep patterns with the exclusion of night shift workers from the study, and all data were collected from April to

October during British Summer Time. However, a limitation of the current study is a lack of information regarding participants' average sleep and eating habits, which could have been used to determine how closely the enforced laboratory schedule actually mimicked their normal day. A seven-day entrainment protocol—wherein participants follow the laboratory schedule at home during the week prior to the study visit— could have also been implemented, which may have helped to reduce inter-individual variability to some degree. However, our study is not the first to report such variability. Indeed, based on evidence showing poor agreement in both the presence and phase of rhythmicity between participants across all lipid species [25], it has been suggested that there may be distinct circadian metabolic phenotypes, which will require further investigation [24]. It must also be noted that although the amplitude of the phase for plasma concentrations of EPA was significant, the percentage rhythm value observed for the variation of EPA was much lower than DHA, and especially so when considering females separately. This finding echoes what has been observed previously [15], and suggests that plasma EPA does not follow as predictable a daily variation as DHA does. One reason for this may be differences in fate and function between the two fatty acids with EPA—as an eicosanoid precursor—being released from adipocytes and potentially other tissues in response to spontaneous endogenous changes such as inflammation, for example [33, 34].

The observed overnight decrease in DHA, and to a lesser extent EPA, is interesting from a functional perspective. If regarded as a lipid transport pool [19], the nocturnal decline in plasma concentrations of these n-3 PUFAs suggests tissue uptake of these fatty acids during sleep. The aim of the current study was to investigate the diurnal variation in circulating plasma EPA and DHA, in order to inform optimal timing of intake of these n-3 PUFAs in the context of maximising their tissue delivery. The timing of delivery of DHA in particular may be

297 important for brain function, since the brain is especially enriched in this n-3 PUFA [35] that
298 has key structural and functional roles. For example, Umhau et al. [36] suggest that alterations
299 in the specific composition of newly formed neurons or synaptic membranes may underpin
300 changes in neuropsychiatric function following supplementation protocols of just a few weeks
301 that are unlikely to impact the overall concentration of brain DHA. Adult hippocampal
302 neurogenesis is known to occur during sleep [37], and may play an important role in
303 supporting learning and cognitive flexibility [38, 39] and memory consolidation [40].
304 Therefore, we suggest that intake of n-3 PUFAs later in the evening—which would ensure an
305 overnight increase in plasma concentrations of EPA and DHA at a time when background
306 levels are at their daily lowest—presents as a novel strategy to optimise the efficacy of n-3
307 PUFA supplementation with regards cognitive function. Circadian studies in mice suggest that
308 lipid absorption is lowest at the end of the active/awake phase [41, 42], which would need to
309 be taken into account if this dosing protocol was adopted. Of course, testing this hypothesis
310 by observing effects following morning and evening or indeed afternoon dosing protocols
311 would be required. What the data presented herein suggest more concretely, is that the time
312 at which DHA and EPA plasma samples (and other fatty acids more broadly) are collected is
313 an important consideration. Therefore it is recommended that within the same study,
314 samples should be collected at the same time; any conclusions drawn from comparisons to
315 data presented by other studies should be mindful of differences in sampling time points as
316 well.

317 In conclusion, the current study revealed the presence of diurnal variation in plasma
318 concentrations of EPA and DHA in a sample of healthy young adults with low habitual oily fish
319 consumption. We observed that peak concentrations occur towards the end of the day and
320 fall overnight, with the nadir of each fatty shown to occur in the morning. We suggest that an

evening dosing protocol may have implications for efficacy studies with regards cognitive function, which we aim to address in the future. Lastly, given their daily variation, caution should be exercised when comparing absolute concentrations of plasma fatty acid concentrations across studies.

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Author Contributions

All authors designed research. HA, JK and JF conducted research; PAJ analysed data; PAJ, CH, SOH and PCC wrote the paper; PAJ had primary responsibility for final content. All authors and read and approved the final version of the manuscript.

Conflicts of Interest

C.H and S.O.H are employees of BASF AS. P.C.C. is an advisor to and has received funding from BASF AS.

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Table 1. Participant demographics. N = 21 including 10 males and 11 females

	Mean	SD	Min	Max
Age (years)	31.85	6.44	25.00	44.00
Systolic BP (mmHg)	120.57	12.56	92.50	139.50
Diastolic BP (mmHg)	80.10	9.12	65.00	98.00
BMI (kg/m^2)	25.30	3.76	18.68	33.02
Oily fish (portions/week)	0.50	0.40	0.00	1.00
Ethnicity (N)				
White	12			
Asian	6			
Black	1			
Hispanic	1			
Mixed	1			

Table 2. Results from the cosinor analysis of pooled data with a period of 24 hours

Fatty acid	N	*p*	PR	A	Φ	Acrophase (hrs:min)
DHA	21	< 0.001	23.3	8.99	-94.18	17:43
EPA	21	< 0.001	13.5	7.76	-49.64	20:41
14:00	21	< 0.001	51.2	40.00	-85.93	18:16
16:00	21	< 0.001	36.7	12.70	-77.40	18:50
16:1n-7	21	< 0.001	8.2	7.47	-95.42	17:38
18:00	21	< 0.001	45	14.33	-69.40	19:22
18:1n-9	21	< 0.001	31.8	15.40	-50.84	20:36
18:1n-7	21	< 0.001	16.5	8.56	-65.94	19:36
18:2n-6	21	< 0.001	24	8.75	-57.81	20:08
18:3n-6	21	< 0.001	7.7	6.57	-109.21	16:43
18:3n-3	21	< 0.001	36.7	23.33	-55.47	20:18
20:00	21	< 0.001	36.2	22.80	-86.22	18:15
20:1n-9	21	< 0.001	19.2	14.69	-40.38	21:18
20:2n-6	21	< 0.001	8.2	6.67	-63.25	19:47
20:3n-6	21	< 0.001	24.4	10.80	-87.79	18:08
20:4n-6	21	< 0.001	24.9	8.92	-74.57	19:01
20:4n-3	21	0.077	1.9	5.29	-76.21	18:55
22:00	20	< 0.001	9.8	18.97	-64.66	19:41
22:5n-3	21	< 0.001	11.7	10.31	-104.40	17:02
24:00	21	0.001	5.5	6.20	-80.49	18:38

24:1n-9	21	0.002	4.5	7.01	-93.72	17:45

p, p-value derived from the regression test; PR, percentage rhythm or proportion of variance in the data accounted for by the cosine wave (R^2 x 100); A, 24-hour amplitude; Φ, acrophase or the time at which the peak amplitude occurs, measured in degrees; EPA, eicosapentaenoic acid; DHA, docosahexaenoic acid.

Figure 1. Diurnal variation of pooled data (N = 21) in plasma concentrations of DHA (a) and EPA (b) expressed as a percentage of their respective mean 24-hour value. A significant rhythm is detected for both DHA and EPA. The equation of the cosine curve fitted by least squares to the data is presented on the graph. PR, percentage rhythm or proportion of variance in the data accounted for by the cosine wave (R^2 x 100).