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Plasma and red blood cell total phospholipid fatty acid status of nonpregnant female Vervet monkeys (*Cercopithecus aethiops*) on a high carbohydrate maintenance diet

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Abstract: Nonhuman primates are of interest as models of human physiology to study the effect of multiple pregnancies on birth weight. Reference plasma and red blood cell (RBC) total phospholipids fatty acids were established in nonpregnant breeding female Vervet monkeys. Twenty-three clinically healthy nonpregnant Vervet monkeys (*Cercopithecus aethiops*), contained in a controlled closed environment and consuming a high carbohydrate diet (68 E%) that contained 20 E% fat and 12 E% protein were sampled for blood during a cross-sectional study. A low intake of $\omega 3$ fatty acids was reflected by a high $\omega 6/\omega 3$ ratio (66:1) of the diet. Inverse relations were seen between plasma and RBC total phospholipid fatty acids, 18:2 $\omega 6$, 20:3 $\omega 6$, and 20:4 $\omega 6$, which suggested selective incorporation in membranes. Low levels of 20:5 $\omega 3$ and 22:6 $\omega 3$ of plasma and RBC total phospholipids render Vervet monkeys as ideal subjects to study the effect of $\omega 3$ fatty acid supplementation on pregnancy outcomes.

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Introduction

Nonhuman primates are considered appropriate models for human physiology because of their close phylogenetic relationships to humans [10,24]. They are used to study the regression of atherosclerosis [2,3], the immune system [29], and visual function [11]. A history of low birth weight in a previous pregnancy is a risk factor for reduced birthweight in man [16] and in Vervet monkeys (unpublished data from our primate unit). Vervets are indigenous to southern Africa and are readily available for research which make them ideal subjects to study nutritional problems relevant to man [22]. Colony bred nonpregnant Vervets on a high carbohydrate (HC) maintenance diet provide ideal subjects to study the effect of multiple pregnancies on birth weight because a controlled environment [22] excludes confounding factors such as smoking, alcohol consumption, and diet [27]. Knowledge of the

fatty acid status of nonpregnant female Vervet monkeys on their usual HC maintenance diet is essential to study the effect of fatty acid supplementation during pregnancy, as the growing fetus is likely to exhaust the mother's reserves of essential fatty acids [21], especially during multiple pregnancies in breeding female Vervet monkeys.

Plasma [4] and erythrocyte [15] phospholipids are excellent markers of dietary long-chain $\omega 3$ fatty acids. The purpose of this study was to establish the plasma and RBC phospholipid fatty acid status of healthy nonpregnant Vervet monkey breeding females maintained on a HC diet.

Material and methods

Animals and environment

The 23 female primates, aged 6–17 years, included in this cross-sectional study were contained in a con-

trolled closed environment, housed in single cages with squeeze back mechanisms for ease of anaesthesia. Their average weight was 3.56 kg (± 0.39). Birth rate was three infants (± 2.3) per Vervet monkey (range: 0–9). The diet consisted of maize meal and kernels, white kidney beans, whole egg powder, sugar, sunflower oil and seeds, and vitamin and mineral mixes. The food was prepared in the kitchen of the primate unit in the form of a patty. Apples (70 g/day) were added to the diet daily and water was provided ad libitum via an automatic watering system. The composition [17] of the HC maintenance diet per Vervet monkey per day is provided in Table 1. Female Vervets are sexually mature at 3 years of age and remain reproductive until death [5]. Breeding schedules were described before [22]. The study protocol was approved by the Medical Research Council's Ethics Committee for Research on Animals.

Sampling techniques

All samples were taken from clinically healthy, normally cycling nonpregnant animals within a 2 month period during the months May and June. Following an overnight fast (18 hours), the animals were sedated with ketamine HCl at 10 mg/kg intra muscular. The blood samples were taken from the femoral artery or vein without venous stasis within 10 minutes following the ketamine administration. One

sample was obtained from each animal. The blood was collected in EDTA (K₃) tubes (Vacutainer), protected from light, and kept on ice (4°C). The plasma was separated from RBC within 30 minutes by centrifugation for 15 minutes at 3,000 rpm. Haemolyzed and lipemic samples were omitted.

Laboratory techniques

An external standard (41.6 µg L- α -Phosphatidylcholine, diheptadecanoyl; Sigma Chemical Co., St. Louis, MO) was added to 200 µl plasma and co-extracted [13] with chloroform/methanol (2:1, v/v) containing butylated hydroxytoluene (BHT; 454×10^{-6} mol/l) as anti-oxidant. Lipid classes were then separated by thin layer chromatography (TLC) [2]. RBC were washed three times with 150 mmol/l NaCl solution. Packed RBC (600 µl) were haemolyzed by adding an equal volume of distilled water. The haemolyzed cells were then added to 6 ml of methanol containing BHT (136×10^{-4} mol/l) and mixed for 30 seconds. Twelve milliliters of chloroform was added and mixed for a further 30 seconds. The extraction mixture was then filtered through Whatman No. 1 filter paper to separate the RBC membranes. Five milliliters of a 150 mmol/l NaCl solution was added to the filtrate and mixed for 10 seconds. The mixture was centrifuged at 1,500 rpm for 10 minutes. The organic solvent was removed by aspiration and dried under a stream of nitrogen. The lipid residue was re-dissolved in chloroform/methanol/150 mmol/l NaCl (86:14:1; v/v/v) and applied to TLC plates [2]. After separation of neutral lipids, the total phospholipid spots residing at the origin were scraped off into glass stoppered tubes and transmethylated [25]. Analysis of the fatty acid methyl esters (FAME) was performed on a model 3700 Varian gas-liquid chromatograph using fused silica megabore DB-225 columns (J&W Scientific, Cat. No. 125-2232) [23,25]. Gas flow rates were hydrogen (carrier gas), 5–8 ml/min; medical air, 250 ml/min; and hydrogen, 25 ml/min. Temperature programming was linear at 3°C/minute, initial temperature 165°C, final temperature 220°C, injector temperature 240°C, and detector temperature 250°C. The FAME were identified by comparison of the retention times to those of a standard FAME mixture (Nu-Chek-Prep, Inc., MN).

Table 1. Composition of the high carbohydrate maintenance diet per Vervet monkey per day

Nutrient	g/day	Energy % per total calories	Fatty acids % w/w ¹
Carbohydrates	97.5	67.6	
Fibre	11.9		
Protein	17.6	12.2	
Plant protein	15.7		
Animal protein	1.9		
Total fat	12.9	20.1	
SFA	1.94	3.0	16.2
MUFA	3.27	5.1	27.3
PUFA	6.75	10.5	56.4
P/S ratio	3.48		
$\omega 6/\omega 3$ ratio	66.1		
Fatty acids			
16:0	1.27	2.0	10.6
16:1 $\omega 7$	0.06	0.1	0.5
18:0	0.58	0.9	4.8
18:1 $\omega 9$	3.19	5.0	26.7
18:2 $\omega 6$	6.60	10.3	55.2
18:3 $\omega 3$ (mainly)	0.09	0.1	0.8
20:0	0.03	trace	0.3
20:4 $\omega 6$	0.01	trace	0.1
22:0	0.04	0.1	0.3
22:6 $\omega 3$	0.01	trace	0.1
24:0	0.01	trace	0.1

¹Based on an average energy intake of 2,412 kJ/day or 577 kcal/day. Cholesterol 64 mg/day [17].

Results

Diet

The animals received 20% of their total caloric intake from fat, 68% from carbohydrate, and 12% from protein. The predominant fatty acids were

palmitic (16:0; 11% of total fatty acids), stearic (18:0; 5%), oleic (18:1 ω 9; 27%), and linoleic (18:2 ω 6; 55%). Most of the calories from fat (15.6 energy %) in the animal's diet was derived from mono-unsaturated fatty acids (MUFA) and poly-unsaturated fatty acids (PUFA). The intake of ω 3 fatty acids was low as reflected by the high ω 6/ ω 3 ratio (66:1) of the diet. Saturated fatty acids (SFA) provided 3% of energy from total calories. The diet had a high polyunsaturated to saturated (P/S) fatty acid ratio of 3.48.

Fatty acids

Plasma and RBC total phospholipid fatty acid profiles are shown in Table 2. There are clear differences between the fatty acid profiles of plasma and RBC phospholipids. These differences are apparent in the saturated and unsaturated fatty acid components. The proportion of total saturated fatty acids (Σ SFA) and total polyunsaturated fatty acids (Σ

PUFA) was similar in the plasma and RBC phospholipids. The total monounsaturated fatty acid (Σ MUFA) content was, however, lower in the plasma than in the RBC phospholipids. Similar inverse relationships can be seen between 18:2 ω 6, eicosatrienoic acid (20:3 ω 6) and arachidonic acid (20:4 ω 6). The ratio of 18:2 ω 6 to 20:3 ω 6 is lower in plasma than in the RBC phospholipids (5.5:1 vs. 11:1). In contrast, the ratio of 18:2 ω 6 to 20:4 ω 6 is higher in plasma than in red blood cells (2.6:1 vs. 1.0:1). This suggests a selective incorporation of long-chain fatty acids into membranes [8]. The discrepancy of the ω 6/ ω 3 fatty acid ratio between the diet and in red blood cells suggest an active desaturase system for the accretion of ω 3 fatty acids in the plasma and RBC phospholipids of these monkeys. The higher content of ω 3 fatty acids relative to the ω 6 fatty acids in the plasma and red blood cells than in the diet may be of particular physiological relevance in neurological tissue [12] and during pregnancy when the demands of the fetus for ω 3 fatty acids is increased [21].

Table 2. Fatty acid composition (%wt/wt) of plasma and red blood cell total phospholipids of non-pregnant breeding female Vervet monkeys (*Cercopithecus aethiops*) on a high carbohydrate diet (n=23) (mean, SD)

Fatty acid	Plasma composition (% wt/wt)	Red blood cell composition (% wt/wt)
Σ SFA	44.30 (2.35)	41.60 (2.66)
14:0	0.20 (0.06)	0.18 (0.04)
16:0	22.16 (1.51)	22.37 (1.58)
18:0	21.05 (2.25)	15.90 (1.06)
20:0	0.18 (0.05)	0.40 (0.13)
22:0	0.38 (0.10)	1.11 (0.24)
24:0	0.34 (0.09)	1.65 (0.33)
Σ MUFA	8.65 (1.00)	13.75 (1.27)
16:1 ω 7	0.19 (0.18)	0.10 (0.05)
18:1 ω 9	7.58 (0.86)	9.94 (0.88)
20:1 ω 9	0.22 (0.09)	0.37 (0.08)
22:1 ω 9	0.05 (0.08)	0.29 (0.15)
24:1 ω 9	0.61 (0.14)	3.05 (0.49)
Σ ω 9	8.46 (0.90)	13.65 (1.25)
Σ PUFA	47.05 (2.68)	44.65 (3.30)
18:2 ω 6	24.98 (2.74)	17.24 (1.64)
18:3 ω 6	0.05 (0.05)	0.02 (0.02)
20:2 ω 6	1.16 (0.37)	0.78 (0.15)
20:3 ω 6	4.58 (2.39)	1.57 (0.77)
20:4 ω 6	9.74 (1.41)	16.80 (2.28)
22:2 ω 6	0.08 (0.02)	0.23 (0.03)
22:4 ω 6	1.24 (0.19)	2.28 (0.46)
Σ ω 6	39.49 (2.85)	40.09 (3.09)
18:3 ω 3	0.18 (0.08)	0.13 (0.04)
20:3 ω 3	0.07 (0.03)	0.07 (0.04)
20:5 ω 3	0.13 (0.07)	0.15 (0.07)
22:3 ω 3	0.96 (0.20)	1.01 (0.20)
22:5 ω 3	0.47 (0.12)	0.84 (0.29)
22:6 ω 3	3.41 (0.82)	3.50 (0.78)
Σ ω 3	5.23 (1.03)	5.70 (0.96)
ω 6/ ω 3 ratio	7.84 (1.68)	7.17 (1.01)
P/S ratio	1.07 (0.11)	1.08 (0.13)
Σ FA (mg/l)	1652.46 (395.95)	1482.47 (142.22)

Discussion

The establishment of suitable animal models for the study of human disease has proven to be invaluable for the study of human cardiovascular disease [3]. As in man [26], the low dietary intake of α -linolenic acid (18:3 ω 3) (0.1%) of nonpregnant female Vervet monkeys is reflected in the RBC phospholipids. In contrast, howler monkeys in their natural habitat have relatively higher dietary intakes of 18:3 ω 3 [7]. Caged nonpregnant female Vervet monkeys in this study had low levels of 18:3 ω 3 in their plasma and RBC phospholipids similar to what is found in human plasma [19,27]. The total ω 3 fatty acids in plasma and RBC phospholipids was nevertheless higher than that of female Rhesus monkeys (respectively, 2.7% and 4.9%) despite a lower dietary intake of ω 3 fatty acids [11]. On the other hand, the female Vervet monkeys had lower RBC phospholipid 18:2 ω 6 and concomitant higher 20:4 ω 6 levels despite a higher dietary intake of linoleic acid than male marmoset monkeys [15]. The Vervet monkeys must therefore have adequate metabolic pathways to desaturate and elongate 18:3 ω 3 to eicosapentaenoic acid (20:5 ω 3) and docosahexaenoic acid (22:6 ω 3) and 18:2 ω 6 to 20:4 ω 6 [28].

An adequate supply of essential fatty acids (EFA) and their longer chain derivatives are necessary for optimal growth and development of the fetus. However, it is known that the maternal EFA supply to the fetus is limited [14]. The lower levels of 20:5 ω 3 and 22:6 ω 3 of plasma total phospholipids than in rhesus monkeys [20] render nonpregnant female

Vervet monkeys therefore as ideal subjects to study the effect of ω 3 fatty acid supplementation on growth of the fetus during pregnancy, since long-chain ω 3 fatty acids are known to increase significantly with dietary supplementation [6,9]. Because the biochemical EFA status of infants is not optimal after a normal pregnancy [1], infants born to female Vervet monkeys on a maintenance diet may be particularly susceptible to altered visual function and/or neurological function if the mother's diet does not contain adequate amounts of long-chain EFA during pregnancy [18].

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