Effects of Alcohol Intake During Pregnancy on Docosahexaenoic Acid and Arachidonic Acid in Umbilical Cord Vessels of Black Women

Skadi Beblo, MD*; Ken D. Stark, PhD*; Mahadev Murthy, PhD*; James Janisse, PhD‡; Helaine Rockett, MS, RD;§; Janice E. Whitty, MD‡; Michelle Buda-Abela, PhD‡; Susan S. Martier, PhD‡; Robert J. Sokol, MD‡; John H. Hannigan, PhD‡‖; and Norman Salem, Jr, PhD*

ABSTRACT. *Objective.* **Alcohol influences the intake and metabolism of several nutrients including long-chain polyunsaturated fatty acids (LC-PUFAs). The LC-PUFAs docosahexaenoic acid (DHA) and arachidonic acid (AA) are particularly crucial for intrauterine growth and brain development. We hypothesized that alcohol consumption adversely affects LC-PUFA levels in pregnant women and their newborn infants.**

Methods. **Pregnant black women ($N = 208$) presenting at a core city antenatal clinic were screened and recruited. Shortly before delivery, maternal plasma was collected. After delivery, umbilical arteries and veins were dissected from the cords, total lipids were extracted from the vessel tissues and maternal plasma, and fatty acid levels were assayed by gas chromatography. For statistical analysis, subjects were categorized according to absolute alcohol intake per day (AAD) and absolute alcohol intake per drinking day (AADD) around the time of conception, with smoking and other potential confounders included in the analyses.**

Results. **Significant differences in fatty acid composition of total lipid extracts were detected in umbilical cord vessels among the AADD groups: abstainers (AADD = 0), moderate drinkers (AADD < 130 g), and heavy drinkers (AADD ≥ 130 g). DHA and AA content in the arterial umbilical vessel wall was ~14% and ~10% higher in the moderate ($n = 127$) and heavy ($n = 32$) alcohol groups, respectively, than in abstainers ($n = 49$). A small, nonsignificant increase (~3%) was seen in the umbilical vein for AA but not for DHA. Alcohol intake was positively correlated to both DHA and AA concentrations in the arterial vessel wall but to neither in the venous wall nor maternal plasma. Maternal plasma DHA was positively correlated with both umbilical arteries and vein DHA, but there were no significant correlations for AA between maternal plasma and either umbilical vessel.**

Conclusions. **Our findings indicate that alcohol intake during pregnancy is associated with altered DHA and AA status in fetal tissues. Although differences may be due to either metabolism and/or distribution, it is most likely a result of a direct influence of alcohol on fetal metabolism.** *Pediatrics* **2005;115:e194–e203. URL: www.pediatrics.org/cgi/doi/10.1542/peds.2004-0202;** ***docosahexaenoic acid, arachidonic acid, nutrition, essential fatty acids, pregnancy, alcohol, fetal alcohol syndrome.***

ABBREVIATIONS. ARND, alcohol-related neurodevelopmental disorder; FAS, fetal alcohol syndrome; LC-PUFA, long-chain polyunsaturated fatty acid; DHA, docosahexaenoic acid; AA, arachidonic acid; SES, socioeconomic status; AAD, absolute alcohol intake per day; AADD, absolute alcohol intake per drinking day; HSD, honestly significantly different; ANCOVA, analysis of covariance; LNA, α-linolenic acid.

Maternal alcohol consumption during pregnancy may lead to alcohol-related neurodevelopmental disorders (ARNDs) in the offspring, of which fetal alcohol syndrome (FAS) constitutes the worst clinical picture.[1–4] The clinical manifestation of both ARNDs and FAS consists of cognitive and/or behavioral impairments of various degrees. FAS is also associated with characteristic morphologic craniofacial abnormalities and prenatal and postnatal growth retardation.[5]

Recent studies have shown that the impact of alcohol on the fetus varies throughout pregnancy and also depend on the pattern of the mother's drinking. For example, binge drinking seems to have a greater effect on the fetus compared with regular drinking of smaller amounts.[6–8] The impact of alcohol on the developing brain seems to be most pronounced during the last trimester of pregnancy, the period coincident with the brain growth spurt.[9] Different mechanisms for the adverse effects of intrauterine alcohol exposure have been identified.[10–12] However, a direct relationship between alcohol intake and any specific clinical outcome associated with ARND or FAS remains unclear.

Alcohol is a potent modulator of fatty acid metabolism and is known to influence the fatty acid profiles of different organs.[13] In animal studies, chronic alcohol exposure has been shown to decrease long-chain polyunsaturated fatty acid (LC-PUFA) concentrations, especially docosahexaenoic acid (DHA) and arachidonic acid (AA) in liver and brain tissues.[14–16] Decreased DHA and AA contents have been observed in the blood of alcoholics (for review, see Neuringer et al[17]). LC-PUFAs are essential components of all cell membranes and modulate membrane functions. In particular, animal studies have estab-

From the *Laboratory of Membrane Biochemistry and Biophysics, NIAAA, National Institutes of Health, Rockville, Maryland; ‡Department of Obstetrics and Gynecology, School of Medicine, and ‖Department of Psychology, Wayne State University, Detroit, Michigan; and §Department of Medicine, Brigham and Women's Hospital, Harvard Medical School, Boston, Massachusetts.

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Reprint requests to (N.S.) National Institute on Alcohol Abuse and Alcoholism, Laboratory of Membrane Biochemistry and Biophysics, 5625 Fishers Lane, Room 3N-07, Rockville, MD 20852. E-mail:nsalem@niaaa.nih.gov

lished that LC-PUFA availability modulates visual and behavioral development,[17–19] and clinical trials have shown that supplementation of healthy newborns and infants with preformed DHA has beneficial effects on visual and psychomotor development.[20–22]

The effects of alcohol on fatty acid metabolism may mediate alcohol's influence on fetal development through 2 mechanisms. First, alcohol intake leads to fatty acid catabolism and therefore can result in decreased LC-PUFA concentrations in the mother, depending on dosage and length of alcohol exposure.[23] Fetal PUFA metabolism depends largely on the maternal LC-PUFA supply, because fetal enzymatic elongation and desaturation alone seem insufficient to meet the LC-PUFA needs for optimal brain development.[15,24–27] Second, recent studies show that alcohol influences placental metabolism and transport function. Alcohol exposure increases thromboxane production in the placenta, and the resulting vasoconstriction may limit oxygenation and nutrient transport to the fetus.[28] Moreover, the placenta transports individual LC-PUFAs selectively,[29] and alcohol exposure can markedly disturb this transport system,[30,31] leading to functional reductions in bioavailability of particular fatty acids.

The umbilical vessel walls are a most important material for analysis because the umbilical cord is constructed over time from LC-PUFAs available in fetal circulation, which means that the umbilical vessel walls reflect longer-term fetal fatty acid status than the umbilical cord plasma levels of LC-PUFAs. This study was designed primarily to investigate the influence of maternal drinking on the fetal fatty acid composition with a secondary aim to relate drinking patterns, particularly binge drinking, to the overall effects of alcohol.

Currently, there is no reliable, objective parameter to determine the actual ingestion of alcohol. Therefore, clinical studies on alcoholism depend on information obtained from the subjects. Although these self-reported measures of alcohol intake are inherently unreliable, validated structured interviews are currently the best available method to determine alcohol consumption and drinking patterns. In particular, pregnant women especially tend to underreport, and this effect is likely to increase during the course of pregnancy. Previous studies have shown that women who drink the most around the time they discover they are pregnant are more likely to continue drinking throughout pregnancy, and to drink in binge patterns, although both the self-reported and actual levels of consumption are usually reduced.[32–36] Therefore, to best estimate the degree of alcohol consumption over the course of the pregnancy, we chose to ask the women at their first visit to the antenatal clinic about their periconceptional drinking habits.

METHODS

The study protocol and procedures had prior approval from the Wayne State University (Detroit, MI) Human Investigations Committee. Written informed consent for the acquisition of maternal dietary information and alcohol and drug-use histories and for the analyses of maternal blood and the umbilical cord specimens after delivery was obtained at recruitment into the study.

Participants

Samples from 244 black mother-infant pairs were collected. Final analyses were limited to 208 pairs, because 27 umbilical cord specimens were unavailable, 7 pairs were excluded due to multiparous deliveries (ie, twins or triplets), and 2 pairs had missing infant data.

At the first visit to the antenatal clinic at Wayne State University, medical and social histories and detailed information on alcohol, drug, and cigarette use in the periconceptional period were obtained. To determine the individual alcohol consumption, maternal drinking histories were included in the larger context of a medical and nutritional history by skilled screeners using structured interviews. These interviews have been designed and validated to limit denial and elicit sensitive drug and alcohol-use information from pregnant women.[33,37] The structured interview included the full-scale Michigan Alcoholism Screening Test[38] and T-ACE (Tolerance, Angry or Annoyance, Cut down or quit, eye opener) scales[39] to identify risk drinkers.[32] Socioeconomic status (SES) was measured by using a modified Hollingshead 2-factor index (occupation and education) of social position.[40] Dietary information at the time of delivery was determined by the Harvard Women's Nutrition Questionnaire,[41] which has been validated for low-income pregnant women.[42] The food-frequency questionnaire was modified by the addition of questions to improve the quantification of selected dietary fats. Individual nutrient intakes were adjusted for total energy intake to reduce measurement error associated with food-frequency questionnaires[43] using the nutrient residual model.[44]

The subjects were grouped according to alcohol consumption at the time of conception in 2 ways for statistical analyses. First, the estimated absolute alcohol intake per day (AAD) around the day of conception was used to determine 3 drinking groups: abstainers (AAD = 0 g/day), moderate drinkers ($<$ 60 g/day), and heavy drinkers ($\geq$ 60 g/day). Second, the mothers were assigned to 3 groups according to the absolute amount of alcohol consumed per drinking day (AADD), which reflects the amount consumed per occasion and not an overall average intake: abstainers (AADD = 0), moderate drinkers (AADD $<$ 130 g), and heavy drinkers (AADD $>$ 130 g). This takes into account a possible influence of the pattern of drinking independently from the total amount that the person drinks. A woman with a relatively low average intake (AAD) but who drinks the whole amount on a single occasion could be categorized "heavy" instead of "moderate" based on AADD.

Fatty Acid Extraction and Analyses

Maternal blood was obtained shortly before delivery by venipuncture of the antecubital vein, and plasma was immediately separated from erythrocytes. At delivery, ~3 cm of umbilical cord was collected proximal to the placenta after clamping. The cord samples were immediately irrigated and rinsed with buffered saline to remove any remaining blood. All samples were frozen at −70°C until additional analysis was performed.

Umbilical cord samples were allowed to thaw before blood vessels were removed. Briefly, cord segments were incised lengthwise, exposing the vein that was peeled from the Wharton jelly bed. Next, the 2 arteries were cut from the surrounding Wharton jelly. Care was taken to ensure that vessels were free of any Wharton jelly. They were then rinsed with phosphate-buffered saline and weighed before homogenization. Total lipid extraction from cord-vessel homogenates and maternal plasma was performed according to the method of Folch et al[45] after the addition of 20 μg of an internal standard (22:3n-3, NuCheck Prep, Elysian, MN). Total lipid extracts were methylated with boron trifluoride in methanol (14% wt/vol) according to the method of Morrison and Smith[46] as modified by Salem et al.[16] Fatty acid methyl esters were then analyzed by gas chromatography as described previously.[16] Fatty acids were expressed as weight percent, which agreed with area percent to within 5% as verified with a 28-component, quantitative standard (Mixture 462, NuCheck Prep). Unidentified peaks were included in the calculation of total fatty acids, and thus the sum of the identified fatty acids presented in the tables was $<$ 100%. The total fatty acid concentrations were

calculated by summing the peak areas of all fatty acids in comparison to that of the internal standard.

Statistical Analyses

SPSS for Windows (version 11.0, SPSS Inc, Chicago, IL) was used for all statistical operations. Significance was accepted for $P < .05$. For all statistical procedures, fatty acids were analyzed as the percentages derived as described in the previous section. Fatty acid percentages in umbilical arteries and veins were compared by paired t test (2-tailed). A smoothing spline fit and visual inspection of the relationships between fatty acid composition and AAD first and then AADD suggested a step function. Therefore, AAD and AADD were both categorized into 3 groups (defined previously) for the purposes of analysis. Data are given as means ± SEM unless otherwise mentioned. Demographic and clinical characteristics were analyzed by 1-way analysis of variance. For fatty acid data comparisons between the AAD and AADD groupings, a multivariate general linear modeling procedure was used. Tukey's honestly significantly different (HSD) procedure was used for all posthoc comparisons. Umbilical tissue DHA and AA percentages were also analyzed by analysis of covariance (ANCOVA) with maternal plasma DHA and AA included as covariates (see "Results"). Pearson's correlation (2-tailed) was used to investigate the relationships between variables. Various demographic and clinical characteristics of the mothers were also included in the statistical analyses, such as SES, parity, age, and education, as well as other substance use (especially smoking). Multivariable linear regression analyses were performed for various independent variables with known potential to influence umbilical tissue DHA and AA. A parsimonious stepwise model that controlled for multicollinearity was selected because numerous variables are correlated with each other.

RESULTS

Clinical Features and Demographics

Maternal demographic and clinical characteristics are summarized in Tables 1 and 2. The maternal age ranged from 15 to 38 years. Only 20% of the participants held a high school diploma, but 85% had continued school beyond the 10th grade. Of all subjects, 47% were smokers, with women that consume alcohol smoking significantly more than abstainers. The number of live births (mean range: 1.4-1.7 among the groups) was not different, with 70% of the mothers being multiparous. The heavy drinkers as determined by both AAD and AADD had significantly higher Hollingshead scores, indicating higher SES as compared with the abstainers. Total energy intake was significantly higher in the group of heavy drinkers as determined by AADD but not significantly so by AAD. Prepregnacy BMI was significantly higher in the heavy AAD group but not the heavy AADD group. However, removal of a single super-super obese participant (defined as BMI ≥ 60) resulted in decreased means (heavy AAD: 31.1 ± 2.0 kg/m^2; heavy AADD: 28.8 ± 1.3 kg/m^2) and no significant differences. No significant differences in maternal dietary intake of DHA or AA were detected both before and after adjustment for energy intake.

Correlations between demographic characteristics and AAD and AADD are presented in Table 3. The number of previous live births was not correlated with drinking. Smoking and prepregnacy BMI were positively correlated with both AAD and AADD. However, the removal of the single super-super obese participant from the analysis resulted in a loss of significance for the correlation between drinking and BMI ($r = 0.12$, $P = .09$ for AAD; $r = 0.14$, $P = .06$ for AADD). Higher SES (lower Hollingshead class) and more education were inversely associated with drinking, although the relationship between AADD and education did not reach significance. A weak positive correlation between age and AADD was detected also.

Fatty Acid Composition of Umbilical Artery and Vein Tissues

The fatty acyl compositions of umbilical artery and vein tissues are shown in Table 4. Umbilical arteries contained considerably higher absolute concentrations of total fatty acids than the vein based on tissue wet weight, with no relation to alcohol consumption. Among individual fatty acids, most were significantly different between arteries and vein. Both

TABLE 1. Demographics and Clinical Characteristics of the 3 Different Drinking Groups According to AADD

	Abstainers ($N = 49$)	Moderate ($N = 127$)	Heavy ($N = 32$)	P Value
Age, y	23.2 ± 0.7	25.4 ± 0.5*	25.9 ± 0.9	.030
SES, Hollingshead class	3.6 ± 0.2	3.9 ± 0.1	4.2 ± 0.2*	.029
Grade, school years	12.0 ± 0.2	11.7 ± 0.1	11.6 ± 0.2	NS
Previous live births	1.5 ± 0.2	1.6 ± 0.1	1.7 ± 0.3	NS
Prepregnancy BMI, kg/m^2	27.6 ± 1.2	27.1 ± 0.6	30.4 ± 2.0	NS
Smoking, cigarettes/d	2.4 ± 1.0	6.0 ± 0.7*	7.4 ± 1.9*	.013
AAD, g/d	0	26.7 ± 1.7	79.7 ± 11.8†	<.001
AADD, g/drinking day	0	64.8 ± 3.0	173.3 ± 8.0†	<.001
Total energy, kJ	8728 ± 544	9042 ± 397	11 113 ± 962*†	.176
Dietary DHA, mg/d	45 ± 7	61 ± 6	68 ± 13	NS
Adjusted dietary DHA, mg/d‡	48 ± 6	62 ± 6	58 ± 12	NS
Dietary AA, mg/d	97 ± 11	105 ± 7	131 ± 23	NS
Adjusted dietary AA, mg/d‡	106 ± 7	107 ± 6	107 ± 19	NS
Maternal DHA, wt%	1.63 ± 0.06	1.77 ± 0.04	1.61 ± 0.06	NS
Maternal AA, wt%	7.1 ± 0.3	7.2 ± 0.1	6.9 ± 0.3	NS
Infant parameters				
Gestational age, wk	38.3 ± 0.4	38.7 ± 0.2	39.0 ± 0.4	NS
Birth weight, kg	3.1 ± 0.1	3.1 ± 0.1	3.1 ± 0.1	NS

All values are given as mean ± SEM. Compared by 1-way ANOVA except for AAD and AADD, for which moderate and heavy drinkers were compared by independent t tests. Significance accepted for $P = .05$. NS indicates not significant.
* Significantly different from abstainers.
† Significantly different from moderate.
‡ Adjusted for energy using the nutrient residual model.

TABLE 2. Demographics and Clinical Characteristics of the 3 Different Drinking Groups According to AAD

	Abstainers (N = 49)	Moderate (N = 140)	Heavy (N = 19)	P Value
Age, y	23.2 ± 0.7	25.7 ± 0.5*	24.3 ± .2	.020
SES, Hollingshead class	3.6 ± 0.2	3.9 ± 0.1	4.5 ± 0.2*	.007
Grade, school y	12.0 ± 0.2	11.8 ± 0.1	11.3 ± 0.4	NS
Previous live births	1.5 ± 0.2	1.7 ± 0.1	1.4 ± 0.3	NS
Pre-pregnancy BMI, kg/m^2	27.6 ± 1.2	27.0 ± 0.5	33.6 ± 3.2*†	.003
Smoking, cigarettes/d	2.4 ± 1.0	5.7 ± 0.7*	10.0 ± 2.9*	.002
AAD, g/d	0	25.9 ± 1.3	121.8 ± 14.3†	<.001
AADD, g/drinking day	0	74.8 ± 3.9	172.4 ± 12.1†	<.001
Total energy, kJ	8728 ± 544	9255 ± 385	10 958 ± 1322	NS
Dietary DHA, mg/d	45 ± 7	63 ± 6	54 ± 14	NS
Adjusted dietary DHA, mg/d‡	48 ± 6	63 ± 6	44 ± 12	NS
Dietary AA, mg/d	97 ± 11	108 ± 7	128 ± 34	NS
Adjusted dietary AA, mg/d‡	106 ± 7	107 ± 6	107 ± 30	NS
Maternal DHA, weight %	1.63 ± 0.06	1.74 ± 0.04	1.75 ± 0.08	NS
Maternal AA, weight %	7.1 ± 0.3	7.1 ± 0.1	7.5 ± 0.4	NS
Infant parameters				
Gestational age, wk	38.3 ± 0.4	38.7 ± 0.2	38.8 ± 0.5	NS
Birth weight, kg	3.1 ± 0.1	3.1 ± 0.1	3.0 ± 0.1	NS

All values are given as mean ± SEM. Compared by 1-way ANOVA except for AAD and AADD, for which moderate and heavy drinkers were compared by independent t tests. Significance accepted for P = .05. NS indicates not significant.
* Significantly different from abstainers.
† Significantly different from moderate.
‡ Adjusted for energy using the nutrient residual model.

TABLE 3. Correlations of Selected Demographics With AAD and AADD

	AAD		AADD	
	r	P Value	r	P Value
Age, y	0.04	NS	0.13	.042
SES, Hollingshead class	0.23	.001	0.13	.045
Grade, school years	−0.16	.015	−0.09	NS
Previous live births	−0.01	NS	0.02	NS
Prepregnancy BMI, kg/m^2	0.16	.014	0.18	.008
Total energy, kJ	0.11	NS	0.19	.0294
Smoking, cigarettes/d	0.28	.0001	0.25	.0003
Infant parameters				
Gestational age, wk	−0.01	NS	0.04	NS
Birth weight, kg	−0.07	NS	−0.04	NS

Pearson's correlation (2-tailed), P values are given for P = .05. NS indicates not significant.

α-linolenic acid (LNA; 18:3n-3) and Mead acid (20:3n-9) were not consistently detected, and if so only in trace amounts in both umbilical cord vessels. It is interesting that the amount of the total n-3 PUFA, especially DHA (22:6n-3) was significantly higher in the artery. Because the total fatty acid content is ~32% higher in the artery than in the vein, this means that absolute amount of DHA is substantially higher in the artery as compared with the vein.

Relationship Between Average AAD and Umbilical Cord Vessel Fatty Acids

AAD was associated with significant differences in fatty acid compositions, particularly in umbilical arteries (Table 5). Posthoc comparisons of individual means identified differences between the abstainers and both the moderate- and heavy-drinking groups ($P < .05$). There were no significant differences between the moderate and heavy drinkers. In umbilical arteries, DHA and AA percentages were higher in the drinkers than in the abstainers, and the percentages of other n-6 PUFAs differed between the drinking groups. Namely, 20:2n-6 and 22:2n-6 decreased, whereas 22:4n-6 increased with alcohol consumption. The percentage of total n-6 PUFA in arteries was higher in drinkers. Among the n-3 PUFAs, only DHA was significantly different: 14% higher in drinkers. Because DHA constitutes the vast majority of n-3 PUFAs in this tissue, the total percentage of n-3 PUFA in arteries was also higher in drinkers. In contrast to the findings in PUFAs, the total amount of monounsaturated fatty acids was decreased with alcohol intake. No differences were found for saturated fatty acids. In the umbilical vein, only differences in the percentages of AA (20:4n-6) reached statistical significance for the moderate drinking group versus abstainers (14.7 ± 0.1% vs 14.1 ± 0.2%, respectively; P = .048, data not shown). Umbilical vein and artery DHA and AA were also examined by ANCOVA with maternal DHA and AA status as estimated by plasma percentages entered as covariates. The significant effect of AAD persisted for arterial DHA and AA and for venous AA.

Relationship Between AADD and Umbilical Cord Vessel Fatty Acids

Some biological effects of alcohol have been shown to depend more on the per-occasion intake than on

TABLE 4. Fatty Acid Percentages in Umbilical Arteries and Vein

Fatty Acids*	Arteries (N = 208)	Vein (N = 208)	P Value (2-Tailed)
Total SFAs	40.2 ± 0.13	42.3 ± 0.13	<.01
14:0	1.47 ± 0.03	1.32 ± 0.02	<.01
16:0	18.6 ± 0.09	21.0 ± 0.09	<.01
16:0 DMA	1.7 ± 0.06	1.3 ± 0.05	<.01
18:0 DMA	1.14 ± 0.04	1.10 ± 0.05	NS
18:0	16.9 ± 0.06	17.4 ± 0.07	<.01
20:0	0.38 ± 0.003	0.35 ± 0.003	<.01
22:0	1.05 ± 0.009	0.84 ± 0.008	<.01
24:0	1.87 ± 0.02	1.50 ± 0.02	<.01
Total MUFAs	20.2 ± 0.18	16.8 ± 0.13	<.01
16:1n-7	2.14 ± 0.03	1.50 ± 0.04	<.01
18:1 DMA	0.39 ± 0.02	0.41 ± 0.02	NS
18:1n-9	11.0 ± 0.12	9.0 ± 0.08	<.01
18:1n-7	2.7 ± 0.02	2.6 ± 0.02	<.01
20:1n-9	0.50 ± 0.01	0.39 ± 0.01	<.01
24:1n-9	3.8 ± 0.05	3.3 ± 0.04	<.01
Total n-6 PUFAs	23.7 ± 0.13	26.9 ± 0.13	<.01
18:2n-6	1.25 ± 0.02	1.91 ± 0.03	<.01
20:2n-6	2.9 ± 0.07	0.55 ± 0.02	<.01
20:3n-6	1.07 ± 0.02	1.55 ± 0.02	<.01
20:4n-6	10.9 ± 0.13	14.5 ± 0.11	<.01
22:2n-6	1.59 ± 0.03	0.62 ± 0.02	<.01
22:4n-6	2.9 ± 0.05	4.9 ± 0.06	<.01
22:5n-6	3.2 ± 0.04	3.0 ± 0.05	<.01
Total n-3 PUFAs	4.3 ± 0.06	4.0 ± 0.06	<.01
20:5n-3	0.26 ± 0.01	0.15 ± 0.01	<.01
22:5n-3	0.22 ± 0.03	0.28 ± 0.03	NS
22:6n-3	3.9 ± 0.05	3.7 ± 0.05	<.01
Total fatty acids, μg/g wet weight	2851 ± 42	1952 ± 52	<.01

* Data are expressed as the mean weight percentages of total fatty acids ± SEM except total fatty acids, which are given as μg/g wet weight ± SEM. 18:3n-6, 18:3n3, 20:3n-3, and 22:1n-9 were not consistently detected, ie, a level < 0.01% of total fatty acids. Compared by paired t test between vein and arteries with significance accepted for P = .05. SFAs indicates saturated fatty acids; DMA, dimethyl acetal; NS, not significant; MUFAs, monounsaturated fatty acids.

the average intake,[47] reflecting a binge pattern of drinking. Therefore, the data were analyzed with the participants grouped according to AADD. Again differences were seen largely in umbilical arteries with significantly higher DHA and AA percentages in moderate and heavy drinkers in comparison to abstainers (Table 6). In addition, the arterial percentages of some other highly unsaturated fatty acids of the n-6 PUFA group differed between the drinkers and abstainers. In particular, 20:2n-6 and 22:2n-6 were decreased in umbilical arteries of women consuming alcohol, whereas 22:4n-6 tended to be increased. The arterial percentages of total n-6 PUFA tended to be higher in drinkers. In the n-3 PUFAs, only DHA was significantly higher for moderate and heavy drinkers compared with the abstainers, which parallels the results for the AAD groups. The arterial percentage of total n-3 PUFA was also higher in drinkers. Total monounsaturated fatty acids were significantly decreased in both moderate and heavy drinkers, whereas no differences among the groups could be found for saturated fatty acids. In the umbilical vein, AA content in moderate drinkers was significantly higher than in abstainers (abstainers: 14.1 ± 0.20; moderate: 14.7 ± 0.14; heavy: 14.4 ± 0.25). There were no other significant differences (data not shown). ANCOVA for an AADD effect on umbilical tissue DHA and AA while controlling for maternal status of these respective fatty acids yielded similar results as those demonstrated by AAD. The significant effect of AADD persisted for arterial DHA and AA and for venous AA. Overall, grouping according to AADD and AAD revealed similar results in both tissues, although the effects for the AADD categories were more pronounced.

Correlations of Umbilical Vessel and Maternal Plasma DHA and AA Status to Alcohol Intake and Smoking

Bivariate relationships between DHA and AA in umbilical vessels and maternal plasma with alcohol intake, smoking, and other demographic characteristics were investigated using Pearson's correlations (Table 7). AAD and AADD correlated positively with the percentages of AA and DHA in the umbilical arteries but not in the umbilical vein. Also, maternal alcohol intake was not correlated with DHA and AA content in maternal plasma. However, a positive correlation was found between maternal plasma and fetal umbilical vessel DHA and AA content. Maternal smoking was positively correlated with umbilical vessel DHA and AA content but not with maternal DHA or AA content. Gestational age was positively correlated with DHA and AA in the umbilical artery and vein. Birth weight exhibited a similar relationship, although somewhat weaker, with the correlation between birth weight and umbilical vein AA failing to reach significance. A negative correlation between maternal plasma AA and infant birth weight was detected also. In the umbilical vessels, DHA in the artery was strongly correlated with ve-

TABLE 5. Selected Fatty Acid Percentages in Umbilical Arteries as Categorized by AAD (g/d)

Fatty Acids*	Abstainers: AAD = 0 (N = 49)	Moderate: AAD >0, ≤60 (N = 140)	Heavy: AAD >60 (N = 19)	P Value
Total SFAs	40.0 ± 0.27	40.2 ± 0.16	40.3 ± 0.49	NS
14:0	1.5 ± 0.05	1.5 ± 0.03	1.5 ± 0.08	NS
16:0	18.6 ± 0.17	18.6 ± 0.11	18.7 ± 0.34	NS
16:0 DMA	1.6 ± 0.11	1.7 ± 0.07	1.6 ± 0.18	NS
18:0 DMA	1.1 ± 0.09	1.2 ± 0.06	1.1 ± 0.14	NS
18:0	16.7 ± 0.14	16.9 ± 0.07	16.7 ± 0.16	NS
20:0	0.37 ± 0.01	0.38 ± 0.01	0.38 ± 0.01	NS
22:0	1.0 ± 0.02	1.0 ± 0.01	1.1 ± 0.03	NS
24:0	1.8 ± 0.04	1.9 ± 0.02	1.9 ± 0.07	NS
Total MUFAs	21.1 ± 0.37	20.0 ± 0.21	19.4 ± 0.69†	.005
16:1n-7	2.2 ± 0.05	2.1 ± 0.04	2.1 ± 0.10	NS
18:1 DMA	0.36 ± 0.03	0.40 ± 0.02	0.37 ± 0.05	NS
18:1n-9	11.4 ± 0.24	10.9 ± 0.14	10.6 ± 0.44†	.047
18:1n-7	2.8 ± 0.05	2.7 ± 0.03	2.6 ± 0.07	NS
20:1n-9	0.55 ± 0.02	0.49 ± 0.01†	0.43 ± 0.03†	.004
24:1n-9	4.0 ± 0.12	3.8 ± 0.06	3.6 ± 0.20†	.037
Total n-6 PUFAs	23.0 ± 0.27	24.0 ± 0.15†	24.0 ± 0.48†	.001
18:2n-6	1.2 ± 0.04	1.2 ± 0.02	1.4 ± 0.08†	NS
20:2n-6	3.2 ± 0.13	2.8 ± 0.08†	2.6 ± 0.23	.028
20:3n-6	1.0 ± 0.03	1.1 ± 0.02	1.1 ± 0.06†	NS
20:4n-6	10.1 ± 0.26	11.0 ± 0.15†	11.3 ± 0.49†	.003
22:2n-6	1.8 ± 0.06	1.6 ± 0.04†	1.4 ± 0.11†	.004
22:4n-6	2.7 ± 0.09	3.0 ± 0.06†	2.9 ± 0.19	.016
22:5n-6	3.1 ± 0.07	3.2 ± 0.04	3.2 ± 0.12	NS
Total n-3 PUFAs	4.0 ± 0.14	4.4 ± 0.08†	4.5 ± 0.17†	.045
20:5n-3	0.22 ± 0.01	0.22 ± 0.01	0.21 ± 0.01	NS
22:5n-3	0.16 ± 0.01	0.20 ± 0.02	0.19 ± 0.03	NS
22:6n-3	3.7 ± 0.13	4.0 ± 0.06†	4.2 ± 0.16†	.023
Total fatty acids, μg/g wet weight	2896 ± 85	2855 ± 52	2707 ± 127	NS

* Data are expressed as the mean weight percentages of total fatty acids ± SEM except total fatty acids, which are given as μg/g wet wt ± SEM. 18:3n-6, 18:3n3, 20:3n-3, and 22:1n-9 were not consistently detected, ie, a level <0.01% of total fatty acids. Compared by MANOVA with significance accepted for P = .05. SFAs indicates saturated fatty acids; NS, not significant; DMA, dimethyl acetal; MUFAs, monounsaturated fatty acids.

† Significantly different from abstainers after posthoc analyses by Tukey's HSD procedure.

nous DHA (r = 0.84; P < .001) but also with AA in the arteries (r = 0.68; P <.001) and veins (r = 0.55; P <.001). Arterial AA was also strongly correlated with venous AA (r = 0.67; P < .001) and DHA (r = 0.39; P < .001). Venous DHA was also correlated with venous AA (r = 0.49; P <.001).

Determinants of Umbilical Cord Tissue DHA and AA by Multivariable Analyses

The associations between umbilical cord tissue DHA and AA and various lifestyle factors were examined in a stepwise multiple linear regression manner to achieve parsimonious models. Variables considered in the model included: AAD, AADD, maternal caloric intake, previous live births, mother's grade, mother's age, prepregnancy BMI, SES, smoking, gestational age at delivery, birth weight and maternal plasma, and dietary AA and DHA. Dietary AA and DHA were entered as both unadjusted and energy-adjusted values separately. When independent variables were correlated with each other, the variable with the weaker association was removed to prevent multicollinearity. The results are presented in Table 8. Gestational age and smoking were positively and significantly associated with DHA and AA in both the arteries and vein. Maternal plasma DHA was also positively associated with arterial DHA and venous AA. Neither AAD nor AADD persisted in the parsimonious model as they were correlated strongly with smoking, and smoking had a stronger association. AADD had the strongest effect when substituted for smoking in the model, and the standardized β values are presented in Table 8 as well. AADD had a significant association with arterial DHA and AA but not in venous tissue.

DISCUSSION

As far as we are aware, the present study is the largest study to investigate the effects of maternal alcohol consumption on levels of PUFAs in plasma and umbilical cord vessels. This cohort is from a relatively homogeneous population of black women who are generally of low SES. This is of special interest because children born of black women have been reported to be at greater risk for fetal alcohol effects, including FAS and ARNDs, than other groups.[48–50]

Umbilical cord vessels from mothers who consumed alcohol during their pregnancy showed higher amounts of DHA and AA than cord vessels from abstainers. This increase is significantly associated with average alcohol amounts consumed per day around the time of conception. We are aware that self-reported alcohol consumption around conception cannot accurately reflect the amount of alcohol intake at the time of sampling, at delivery. However, pregnant women tend to underreport their alcohol consumption,[34,35] and this effect is likely to increase during the course of pregnancy.[32,36] Previous studies have shown that women who drink the

TABLE 6. Fatty Acid Composition of Umbilical Artery as Categorized by the Absolute AADD (g/drinking day)

Fatty Acids*	Abstainers: AADD = 0 (N = 49)	Moderate: AADD >0, ≤130 (N = 127)	Heavy: AADD >130 (N = 2)	P Value
Total SFAs	40.0 ± 0.27	40.2 ± 0.18	40.6 ± 0.32	NS
14:0	1.5 ± 0.05	1.5 ± 0.04	1.5 ± 0.06	NS
16:0	18.6 ± 0.17	18.5 ± 0.11	19.0 ± 0.24	NS
16:0 DMA	1.6 ± 0.11	1.7 ± 0.07	1.7 ± 0.12	NS
18:0 DMA	1.1 ± 0.09	1.2 ± 0.06	1.1 ± 0.10	NS
18:0	16.7 ± 0.14	17.0 ± 0.08	16.8 ± 0.12	NS
20:0	0.37 ± 0.01	0.38 ± 0.01	0.38 ± 0.01	NS
22:0	1.0 ± 0.02	1.0 ± 0.01	1.1 ± 0.02	NS
24:0	1.8 ± 0.04	1.9 ± 0.02	1.9 ± 0.05	NS
Total MUFAs	21.1 ± 0.37	20.1 ± 0.22†	19.5 ± 0.46†	.005
16:1n-7	2.2 ± 0.05	2.1 ± 0.04	2.0 ± 0.07†	.047
18:1 DMA	0.36 ± 0.03	0.40 ± 0.02	0.37 ± 0.04	NS
18:1n-9	11.4 ± 0.24	10.9 ± 0.15	10.6 ± 0.29†	.044
18:1n-7	2.8 ± 0.05	2.7 ± 0.03	2.7 ± 0.06	NS
20:1n-9	0.55 ± 0.02	0.49 ± 0.01†	0.45 ± 0.02†	.007
24:1n-9	4.0 ± 0.12	3.8 ± 0.06	3.6 ± 0.13†	.039
Total n-6 PUFAs	23.0 ± 0.27	24.0 ± 0.16†	24.0 ± 0.33	.007
18:2n-6	1.2 ± 0.04	1.2 ± 0.02	1.4 ± 0.06†‡	.013
20:2n-6	3.2 ± 0.13	2.8 ± 0.08	2.6 ± 0.18†	.013
20:3n-6	1.0 ± 0.03	1.1 ± 0.02	1.1 ± 0.04†	NS
20:4n-6	10.1 ± 0.26	11.0 ± 0.15†	11.3 ± 0.33†	.003
22:2n-6	1.8 ± 0.06	1.6 ± 0.04†	1.4 ± 0.08†	.002
22:4n-6	2.7 ± 0.09	3.0 ± 0.06†	3.0 ± 0.15	.023
22:5n-6	3.1 ± 0.07	3.2 ± 0.05	3.2 ± 0.10	NS
Total n-3 PUFAs	4.0 ± 0.14	4.4 ± 0.07†	4.6 ± 0.18†	.016
20:5n-3	0.22 ± 0.01	0.22 ± 0.01	0.22 ± 0.01	NS
22:5n-3	0.15 ± 0.01	0.20 ± 0.03	0.20 ± 0.01	NS
22:6n-3	3.6 ± 0.13	4.0 ± 0.07†	4.2 ± 0.12†	.022
Total fatty acids (μg/g wet weight)	2896 ± 85	2846 ± 56	2803 ± 91	NS

* Data are expressed as the mean weight percentages of total fatty acids ± SEM except total fatty acids, which are given as μg/g wet wt ± SEM. 18:3n-6, 18:3n3, 20:3n-3, and 22:1n-9 were not consistently detected, ie, a level <0.01% of total fatty acids. Compared by MANOVA with significance accepted for P = .05. SFAs indicates saturated fatty acids; NS, not significant; DMA, dimethyl acetal; MUFAs, monounsaturated fatty acids.
† Significantly different from abstainers after posthoc analyses by Tukey's HSD procedure.

TABLE 7. Correlations of Infant Umbilical Vessels and Maternal Plasma Fatty Acids to Alcohol Intake Variables and Smoking

	Arteries		Veins		Maternal Plasma	
	DHA	AA	DHA	AA	DHA	AA
AAD	NS	NS	NS	NS	NS	NS
AADD	0.19*	0.19*	NS	NS	NS	NS
Age	NS	NS	NS.	0.17†	NS	0.14†
SES	NS	NS.	NS	NS	NS	NS
Grade	NS	NS	NS	NS.	0.19*	NS
Previous live births	NS	NS	NS	NS	NS	NS
Prepregnancy BMI	NS	NS	NS	NS	.29‡	0.34‡
Total energy, kJ	NS	NS	NS	−0.59†	NS	NS
Smoking	0.16†	0.20*	0.14†	0.16†	NS	NS
Maternal Plasma						
DHA	0.25‡	NS	0.27‡	NS	—	0.67‡
AA	0.16*	NS	0.15†	NS	0.67‡	—
Infant parameters						
Gestational age, wk	0.38‡	0.41‡	0.35‡	0.31‡	NS	NS
Birth weight, kg	0.16†	0.16†	0.23†	NS	NS	−0.17†

NS indicates not significant; Pearson's correlation (2-tailed), P values are given for P = .05.
* $P < .01$.
† $P < .05$.
‡ $P < .001$.

most around the time they discover they are pregnant, as assessed by AAD and AADD, are more likely to continue to drink throughout pregnancy, and to drink in binge patterns, although both the self-reported and the actual levels of consumption are usually reduced.[32–36] Therefore, asking the women about their drinking habits around the time of conception seems to better reflect the actual amount of alcohol consumption over the course of the pregnancy than repeated questionnaires.

It is interesting that an index of binge drinking, as assessed by the amount of alcohol (in grams) con-

TABLE 8. Associations With Umbilical Cord DHA and AA as Determined by Stepwise Multiple Linear Regression

Umbilical Fatty Acid	Parsimonious Model*	Model R^2	Standardized β	Standardized β for AADD†	*P* Value
Arterial DHA		0.25			<.001
	Maternal plasma DHA		.26		<.001
	Gestational age		.39		<.001
	Smoking		.25		<.001
				.16	.018
AA		0.20			<.001
	Gestational age		.42		<.001
	Smoking		.22		.002
				.15	.019
Venous DHA		0.25			<.001
	Maternal plasma DHA		.30		<.001
	Gestational age		.35		<.001
	Smoking		.23		.001
				.09	.19
AA		0.09			<.001
	Gestational age		.26		<.001
	Smoking		.18		.013
				.02	.80

* Parsimonious model was generated by using a stepwise multiple linear regression model while controlling for collinearity between independent variables. The independent variables entered included AAD, AADD, maternal caloric intake, previous live births, mother's grade, mother's age, prepregnancy BMI, SES, smoking, gestational age at delivery, birth weight, maternal plasma DHA and AA, and maternal dietary AA and DHA (entered as unadjusted and energy adjusted separately).
† Values were generated by replacing smoking with AADD in the model. AADD had stronger associations than AAD.

sumed per drinking day (ie, AADD), had a more profound influence in predicting DHA and AA levels than did the average daily alcohol consumption (ie, AAD). This observation deserves additional attention. The severity of most prenatal alcohol-related disorders is dose-dependent, increasing with absolute alcohol consumption.[37,51,52] However, some effects of alcohol depend more on the pattern of alcohol consumption, that is, the amount of alcohol consumed on a single occasion, than on long-term daily averages.[53,54] Binge drinking (>5 drinks per occasion) has been determined to be a strong risk factor for FAS and ARND.[55] The altered fetal fatty acid status in the groups according to AADD, one index of binge drinking, is consistent with and supports the hypothesis that changes in fatty acid metabolism may be involved in the pathophysiology associated with FAS.

The influence of alcohol consumption during pregnancy on fetal fatty acid status has been investigated in human and in animal models, yielding contradictory results. It has been shown in adult animals and their offspring that high amounts of alcohol (up to several g/kg body weight) consumed on a chronic basis lead to decreased DHA and AA concentrations in different tissues, notably the central nervous system.[15] This effect seems to be due to increased lipid peroxidation.[56–58] At the same time, alcohol stimulates the hepatic elongation-desaturation enzyme system and thereby increases the autologous synthesis of DHA and AA from their precursors, LNA and linoleic acid, respectively.[59] We found no differences in either the LNA or linoleic acid levels. There is evidence that the elongation-desaturation system for fatty acid metabolism functions to a limited extent even in very low birth weight infants and in this early stage of life, although the fetus and newborn may not be able to fully meet their needs by endogenous production of DHA and AA alone.[25,26] Alcohol may inhibit the uptake of DHA and AA by the fetus from blood, thus limiting their deposition into different vital organs.[60] The effects of alcohol on both the mother and the fetus must be considered as possible explanations for the present findings.

Additionally, a direct effect of ethanol on maternal fatty acid metabolism could indirectly alter the fatty acid supply to the fetus. In this study, maternal plasma fatty acid content was determined. After controlling for maternal fatty acid status as covariates, AADD remained independently associated with AA content in umbilical veins and arteries and with DHA in umbilical arteries. Therefore, differences in umbilical vessel fatty acid status among the different prenatal alcohol exposure groups are likely due to changes in fetal fatty acid metabolism and/or placental fatty acid metabolism or transfer rather than to differences in the supply from maternal blood. The influence of alcohol on fetal fatty acid metabolism has been discussed previously,[61] and the effect of alcohol on fetal metabolism seems to depend on the dosage and frequency of exposure.[59] Low doses may lead to stimulation of fatty acid metabolism, thereby increasing DHA concentration.[62]

Placenta function, as both a structural connection and communication platform between mother and fetus, can be directly affected by alcohol. Studies on reperfused placenta clearly show a preferential transport of DHA over AA, followed by LNA and LA. However, in the presence of alcohol, DHA transport moves to the end of this cascade.[30] Although this model could explain elevated percentages of AA in fetal tissue, it is inconsistent with the higher percentages of DHA also found here. In addition, the notion of alcohol-induced shifts in the selectivity for placental fatty acid transport provides no explanation for the fact that the major alcohol effect was found in the

arterial and not in the venous tissue. If the dominant effect of alcohol was on placental fatty acid transfer, one would expect the vessel afferent to the fetus (the umbilical vein) to be more affected by alcohol than the efferent vessel (the umbilical arteries). We observed the opposite: arteries were more affected by alcohol than the veins.

In addition to alcohol, other factors must be considered that might contribute to these findings. Gestational age and infant birth weight were positively associated with umbilical tissue DHA and AA in both bivariate correlations and multiple linear regression modeling but seem independent of the effects of AAD and AADD. Alcohol intake correlated positively with smoking. Maternal periconceptional smoking showed a significant bivariate association and persisted as a significant effect in the parsimonious multivariable regression analyses with DHA and AA content in umbilical vessels. The effect of smoking seems to mirror the effect of alcohol consumption. Because smoking and alcohol consumption are cluster behaviors in humans it is difficult to separate their individual effects in the present study.

Energy intake and BMI tended to be higher in the heavy drinkers and tended to positively correlate with AAD and AADD. However, energy intake and BMI did not correlate positively with umbilical tissue DHA and AA (total kilojoules was negatively correlated to venous AA), and neither persisted in the multiple regression modeling. The reason for the different energy intake between the groups may be a result of additional energy intake from alcohol or group differences in underreporting in the food frequency questionnaire. A large recent study shows that the average energy intake in pregnant, black women is ~13 389 kJ.[63] Underreporting of food intake in questionnaires is a common problem in nutritional studies. Many different factors are related to underreporting such as age and gender, but populations with lower health consciousness actually underreport food intake less.[64]

Results comparable to ours have been reported in 2 other studies.[61,65] In a small study including 13 subjects and 12 controls, alcohol consumption during pregnancy led to an increased proportion of DHA in umbilical plasma.[61] In another clinical trial with 20 very low birth weight infants from South-Africa, alcohol intake and smoking during pregnancy were associated with higher DHA percentages in red blood cells compared with 20 controls.[65]

CONCLUSIONS

Maternal alcohol intake during pregnancy in an urban, black population leads to elevated DHA and AA concentrations in umbilical vessel walls. This effect was related overall to the average "dose" expressed as grams of AAD but was even more pronounced when related to binge patterns of alcohol intake, expressed as grams of AADD. The effects of alcohol on the fetal fatty acid status were independent of maternal plasma fatty acid percentage and largely found in the umbilical arteries. Thus, there seems to be a direct influence of alcohol on the fetus. The increase of DHA and AA in umbilical cord vessels may or may not correspond to the direction of DHA or AA concentration changes in other fetal tissues and umbilical cord plasma or erythrocytes. As a consequence, fatty acid concentrations measured in umbilical cord tissues might not reflect the overall essential fatty acid status of an infant. Higher DHA and AA levels in cord tissue alone as a result of alcohol intake during pregnancy are not necessarily positive signs, nor do they imply a sufficient availability of these fatty acids for fetal growth and metabolism. Additional studies are necessary to investigate possible dose-dependent alcohol effects and to compare umbilical cord tissue to other fetal tissues.

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