THE IMPACT OF HYPOGLYCEMIA AND EPA AND DHA SUPPLEMENTATION ON BRAIN-DERIVED NEUROTROPHIC FACTOR LEVEL IN PREGNANT WOMEN WITH TYPE 1 DIABETES: A PROSPECTIVE COHORT STUDY

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SUMMARY

Background: *In addition to its neuroprotective effect, Brain-derived neurotrophic factor (BDNF) also plays a role in glucose and lipid metabolism. This study aims: a) to find changes in the BDNF concentration during pregnancy in type 1 diabetes. b) to prove the effect of DHA and EPA supplementation on changes in BDNF concentrations c) to investigate the impact of hypoglycemia on BDNF concentration.*

Subjects and methods: *The data from this study were from the PRE-HYPO cohort study. Twenty-one of them were on a standard diabetic diet enriched with EPA and DHA (EPA 120 mg/day and DHA 616 mg/day; Exposed group), and nineteen pregnant diabetic women were on the standard diabetic diet without EPA and DHA supplementation (Non-exposed group). In the first trimester of pregnancy, fifteen pregnant women developed hypoglycemia episodes (≤3.9 mmol/L; HYPO+ group), and twenty-five pregnant women did not have hypoglycemia episodes (HYPO- group).*

Results: *BDNF concentration significantly decreased during pregnancy from the first to the third trimester, in Non-exposed from 25.1 (22.0-30.2) to 22.1 (16.3-28.2), P<0.05, in the Exposed group from 22.1 (19.8-25.9) to 18.1 (14.8-18.9), P<0.01. Pregnant patients with hypoglycemia episodes (HYPO+ subgroup) had significantly higher BDNF in the third trimester of pregnancy [22.5 (20.6-28.4)] when compared with patients who did not develop hypoglycemia [16.3 (14.3-18.8), P<0.001]. In the third trimester of pregnancy, BDNF and n-6 PUFAs were associated with hypoglycemia (OR 1.818 95 % CI 1.079-3.003, P=0.025; OR 1.103 95 % CI 1.001-1.217, P=0.048). Total F.A.s were inversely associated with hypoglycemia (OR 0.969 95% CI 0.939-0.998, P=0.048).*

Conclusion: *Pregnant women with hypoglycemia (HYPO+ group) had higher concentrations of BDNF in the first and third trimesters of pregnancy compared to those without hypoglycemia. An increase in body weight during pregnancy leads to a decrease in BDNF concentration.*

Key words: *Brain-derived neurotrophic factor - diabetes mellitus type 1 – EPA – DHA – hypoglycemia- pregnancy*

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INTRODUCTION

Diabetes in pregnancy creates many problems for both mother and child (Persson et al. 2009). Poor metabolic control in pregnant women with type 1 diabetes mellitus is associated with an increased risk of spontaneous abortion, preeclampsia, congenital malformations, asphyxsia, macrosomia and neonatal morbidity and mortality (Djelmis 2005). If one aims for a successful pregnancy outcome and a healthy newborn, an intensive approach to achieving normoglycemia in the preconception period and during pregnancy is obligatory. Adequate nutrition and intensified insulin therapy lead to the desired optimal glucose control. Tight glycemic control improves maternal and perinatal outcomes, prevents microvascular complications, improves cardiovascular health, and saves lives, but creates a significant risk of hypoglycemia. Pregnant women with type 1 diabetes have frequent hypoglycemia and hyperglycemia episodes, which affect brain activity and decrease cognitive function (Rozanska et al. 2020).

The International Hypoglycemia Study Group (2017) considers glucose concentration levels of < 3.0 mmol/L unequivocally hypoglycemic values, which are detected by self-monitoring of plasma glucose, continuous glucose monitoring (for at least 20 minutes) or laboratory measurement of plasma glucose. The glycemic threshold for cognitive impairment is < 2.8 mmol/L, which considers a glucose concentration < 3.0 mmol/L low enough to indicate severe, clinically significant hypoglycemia. The same group suggested that a glucose value of 3.9 mmol/L or less should only show possible

hypoglycemia. Severe hypoglycemia is defined as a hypoglycemic episode requiring external assistance for recovery.

Brain-derived neurotrophic factor is a member of the nerve growth factor (NGF) family (Binder et al. 2004). BDNF plays a role in neurogenesis, regulates synaptic transmission, maintains adult synapses in the CNS, and improves cognitive function (Binder et al. 2004, Acheson et al. 1995). BDNF is found in the brain and the periphery (Trajkovska et al. 2007). It helps maintain and survive neurons and supports the growth and differentiation of new neurons and synapses. In the brain, it is active in the hippocampus, cerebral cortex, and in the brain's basal parts that serve for learning, memory, and thinking.

BDNF stimulates angiogenesis, placental development, and fetal growth (Golden et al. 2010, D'Souza et al. 2014). BDNF regulates glucose and energy metabolism and prevents beta-cell depletion (Das 2010). Hence, BDNF is useful in the prevention and treatment of neurodegenerative diseases but also in diabetes mellitus.

BDNF treatment of diabetic animals resulted in decreased plasma glucose, non-esterified fat, phospholipids, and liver weight, along with an increase in β-oxidation, peroxisome proliferator-activator receptor (PPAR-α) activation, and level of fibroblast growth factor (Tsuchida et al. 2002).

There are reports on dietary n-3 fatty acids normalizing BDNF levels, reducing oxidative damage, and improving learning ability after traumatic brain injury (Wu et al. 2004). These results emphasize the possible interaction between dietary polyunsaturated fatty acids and brain BDNF, hence its potential relevance to energy homeostasis.

This study aims:

- to find changes in the BDNF concentration during type 1 diabetic pregnancy,
- to prove the effect of DHA and EPA on changes in the concentration of BDNF,
- to investigate the impact of hypoglycemia on BDNF concentration.

SUBJECTS AND METHODS

Ethics approval

The Ethics Committee School of Medicine, the University of Zagreb (No. 380-59-10106-19-111/26), approved the scientific project PRE-HYPO No. IP-2018-01-1284 of which this study is a part. All women included in the study provided written informed consent for themselves and their newborns.

Study design

A prospective cohort study was undertaken at Referral Center for Diabetes in Pregnancy Ministry of the Health Republic of Croatia, Department of Obstetrics and Gynecology, Zagreb, University Hospital Center, Zagreb, Croatia, from February 1, 2019, and March 31, 2020. Data from this study were from the PRE-HYPO cohort study (https://mef.unizg.hr/znanost/istrazivanje/web-stranice-projekata/projekt-hrzz-pre-hypo). Forty pregnant women with T1DM were included in the prospective cohort study. Twenty-one of them were on a standard diabetic diet with prenatal vitamin used enriched with EPA and DHA (EPA 120 mg/day and DHA 616 mg/day; Exposed group), and nineteen T1DM pregnant women were on the standard diabetic diet with prenatal vitamin used without EPA and DHA supplementation (Non-exposed group). The duration of T1DM in all participants was between 5 and 30 years.

In the prospective cohort study, we included 40 women with type 1 diabetes mellitus before completed eight gestational weeks with a single living fetus during the study period from February 1, 2019, to July 31, 2020. All participants were admitted to the Department of Obstetrics and Gynecology at least once or repeatedly in each trimester. The daily glucose profiles of patients with type 1 diabetes were determined, and capillary plasma glucose (9/day) was monitored for 2-3 days. Glucose was measured in capillary plasma at the following time intervals: 7, 10, 13, 16, 19, 22, 1, 4, and 7 hours. Mild to moderate hypoglycemia severity was defined as a glucose concentration between ≤ 3.9 and ≥ 2.8 mmol/L detected by laboratory measurement of capillary plasma glucose in one or more experience. None of the pregnant participants in this study experienced a hypoglycemic coma or needed third party assistance during hypoglycemia neither glucagon/intravenous glucose during the hypoglycemic event. No episodes of severe hypoglycemia were reported for the whole pregnancy. To present the hypoglycemia risk factors in each trimester of pregnancy, we divided the participants into two subgroups: into the subgroup of participants who did not have hypoglycemia (HYPO- group) and into the subgroup that had hypoglycemia (HYPO+ group). We included 40 women with type 1 diabetes and singleton pregnancy who received insulin therapy for $\geq$ five years. The HbA1c was $\leq 8\%$ (≤ 64 mmol/mol) at gestation.

Blood samples were analyzed from all pregnant women for fasting plasma glucose (FPG), daily glucose profile (capillary plasma), HbA1c, the concentration of individual FAs in the first and third-trimester pregnancy.

The study included diabetic pregnant women who had no diabetes complications, and the pregnancy progressed neatly and resulted in the birth of healthy neonates. The exclusion criteria were spontaneous abortion, premature delivery, multiple pregnancies, preeclampsia, genetic abnormalities, or fetal malformations. Pregnant women were on intensified insulin therapy (a combination of short-acting and long-acting insulin) and had well-controlled glycemia.

Materials

The standards for gas chromatography (Supelco FAME MIX 37, PUFA No. 1, PUFA No. 3) are the product of the factories of Supelco Inc., Bellefonte, PA, USA. Organic solvents used for total lipid extraction were purchased from Sigma-Aldrich, Chemie GmbH, Steinheim, Germany, and internal standard heptadecanoic acid, anhydrous sodium sulfate, 2,6-di-tert-butyl-4-methyl phenol (BHT), and concentrated hydrochloric acid.

Blood samples were taken on fasting in the morning from the left cubital vein into tubes with serum separation gel (Vacuette® tube 5 mL Z Serum Separator Clot Activator). All samples were centrifuged at 2000 g within one hour of sampling for 10 minutes. The serum was separated, dissolved in two tubes (cryotube), and stored in a freezer at -75 °C until further analysis. Blood samples were taken on the first visit to the Clinic (1st trimester, between 10th to 12th week of pregnancy), then in the third trimester (31th-33rd week).

Blood glucose and glycated hemoglobin (HbA1c) content in maternal vein blood were determined on sampling, while total lipid analysis was subsequently performed.

Glucose concentration (mmol/L) was determined by an enzymatic UV test (reference method with hexokinase) on a Roche Cobas C301 analyzer using reagents from the same manufacturer (Roche Diagnostics, Switzerland) at the Clinic for Women's Diseases and Births. Analytical sensitivity, 0.056 mmol/L.

Determination of HbA1c (%) by turbidimetric inhibition immunoassay on a Roche Cobas C501 analyzer of the same manufacturer (Roche Diagnostics, Switzerland) was performed at Clinical Laboratory of Laboratory Diagnostics of Clinical Hospital Center Zagreb.

The extraction of total lipids and the conversion of fatty acids to methyl esters was carried out at the Department of Chemistry and Biochemistry, School of Medicine, University of Zagreb. Total lipids were isolated from serum samples by the Folch extraction method with organic solvents (Folch et al. 1957).

The analysis of the composition of fatty acid methyl esters was performed by gas chromatography using an Agilent 7890B chromatograph (Agilent Technologies, CA, USA) equipped with a flame ionization detector (FID) at a laboratory service company, Sample Control Ltd. For the separation of methyl esters, we used a capillary H.P. 88 column in the length of 100 m, an internal diameter of 0.25 mm, and an active layer thickness of 0.20 µm (Agilent J&W, USA). The same manufacturer's autosampler was used to apply a reduced sample volume (split/splitless system). The injector temperature was 200°C, and the detector temperature was 250°C.

Sample size

For sample size calculation, we tested the difference in the concentration of BDNF between the first and third trimester of pregnancy in the EPA and DHA group: first trimester 22.8 ± 4.6 pg/L vs. third trimester 17.7 ± 3.1 pg/L. For 80% power $P < 0.05$, 11 patients were needed in both groups. We also tested the difference in the percentage of n-3 PUFAs between the first and third trimester in the Exposed group (first trimester 3.2 ± 1.0 /100 g of fatty acid and the third trimester 4.3 ± 1.8 g/100 g of fatty acid). For 80% power $P < 0.05$, 14 patients were needed in both groups. Considering the inclusion criteria and the calculated sample size, 40 pregnant women with type 1 diabetes participated in the study.

Statistical analysis

Statistical analyses were performed using the statistical package SPSS version 24 (IBM, Armonk, NY, USA). Absolute and relative frequencies represent categorical data. Numerical data are described by the mean and standard deviation in the distributions following the normal, and in other cases by the median and the interquartile range's limits. The Shapiro-Wilk test was used to examine the normality of the distribution of numerical variables. Student's t-test tested group differences between normally distributed continuous variables, and the Mann-Whitney U test tested differences between nonnormally distributed continuous variables. The correlation between normally distributed numerical variables was evaluated by Pearson's correlation coefficient (r). Data that were not normally distributed were log-transformed before analyses. Regression with the Spearman correlation coefficient (r_{rho}) was performed for nonnormal data. For repeated measurements of continuous data, the Wilcoxon signed-rank test was used. All P values are two-sided. The significance level was set at $P < 0.05$.

We studied whether hypoglycemia during pregnancy was associated with BDNF, total FAs, and n-6 PUFA with a logistic regression model. The significance level was set at $P < 0.05$.

RESULTS

Maternal and neonatal characteristics in Non-exposed and Exposed group

We found no difference between the studied groups comparing age, duration of type 1 diabetes, body height/weight, BMI before pregnancy, and pregnancy weight gain. There were no differences in the percentage of HbA1c between the studied groups. The concentration of BDNF in the first trimester did not differ between the groups, but a significant difference was found in the third trimester between them. A significant decline in the concentration of BDNF occurred between the first and third trimester of pregnancy in both groups (Wilcoxon test: $P < 0.05$, $P < 0.001$). The results are presented in Table 1.

Comparing gestational age at delivery, birth weight/length, ponderal index, Apgar index at 1 and 5 minutes, C-peptide concentration, IR HOMA 2, and macrosomia prevalence, we found no difference between the studied groups (Table 2).

Table 1. Maternal characteristics

	Non-exposed group (n=19)	Exposed group (n=21)	P
Age (yr)	29.3±4.7	29.2±5.0	0.987
Duration of T1DM (yr)	7.9±4.2	8.4±5.2	0.750
Weight (kg)	64.2±10.6	62.9±9.0	0.659
Height (cm)	167.3±7.0	167.0±6.0	0.864
Prepregnancy BMI (kg/m^2)	27.2±3.3	27.2±3.6	0.957
Weight gain in 1st trimester (kg)	0.8±3.6[a]	1.4±2.5[d]	0.551
Weight gain in 3rd trimester (kg)	10.5±4.1[a]	12.1±4.6[d]	0.272
BMI (kg/m2) in 1st trimester	23.0±3.2[b]	22.5±2.8[e]	0.765
BMI (kg/m2) in 3rd trimester	26.4±3.3[b]	26.7±3.5[e]	0.785
Maternal vein blood measurements			
Fasting glucose (mmol/L) in 1st trimester	5.2±1.6	4.5±1.4	0.118
Fasting glucose (mmol/L) in 3rd trimester	4.9±1.3	5.1±2.2	0.746
HbA1c (%) in 1st trimester	7.0±1.4[c]	6.6±1.6[f]	0.316
HbA1c (%) in 3rd trimester	6.1±0.8[c]	6.0±0.9[f]	0.551
BDNF in 1st trimester (ng/L)	25.1[h] (22.0-0.2)	22.1[g] (19.8-25.9)	0.080
BDNF in 3rd trimester (ng/L)	22.1[h] (16.3-28.2)	18.1[g] (14.8-18.9)	0.047

[a]P<0.001, [b,c,d,e,f,g]P<0.01, [h]P<0.05, Wilcoxon test

Table 2. Neonatal characteristics

	Non-exposed group (n=19)	Exposed group (n=21)	P
Gestational age at delivery (weeks)	38.1±1.2	38.4±1.0	0.288
Birth weight (g)	3544.0±631.9	3416.4±520.9	0.489
Birth length (cm)	49.4±2.3	49.2±2.4	0.809
Ponderal index (g) x 100/ (cm^3)	2.9±0.3	2.9±0.2	0.559
Fetal macrosomia > 4000g Yes/No (%)	7/12 (36.8/63.2)	2/19 (9.5/90.5)	0.060
Apgar score at 1 min	9.9±0.3	9.9±0.2	0.307
Apgar score at 5 min	9.9±0.2	9.8±0.2	0.278
Umbilical vein serum measurements			
C-peptide (pmol/L)	850.0 (330.0-1360.0)	610.0 (480.0-1170.0)	0.908
Umbilical vein plasma glucose (mmo/L)	5.1±1.7	5.4±2.6	0.604
IR HOMA 2	1.8 (0.7-3.2)	1.5 (1.0-3.3)	0.776

Table 3. Maternal characteristics according to subgroups

	HYPO- group (n=25)	HYPO+ group (n=15)	P
Maternal characteristics 1st trimester			
Age (years)	28.4±5.1	30.7±4.2	0.135
Maternal height (cm)	165.7±6.7	166.3±5.8	0.573
Maternal weight (kg)	64.8±8.8	61.4±11.0	0.292
Pre-pregnancy body mass index (kg/m^2)	22.8±3.5	21.8±3.4	0.338
Duration of type 1 diabetes mellitus (years)	7.1±4.4	9.9±5.0	0.076
Age of onset type 1 diabetes (years)	21.2±8.5	20.9±7.1	0.856
Gestational weight gain (kg) 1st trimester	0.8±2.4	0.9±1.7	0.803
Gestational weight gain (kg) 3rd trimester	11.3±4.3	11.4±4.7	0.946
Total insulin dose (IU/kg) 1st trimester	0.7±0.2	0.7±0.2	0.195
Total insulin dose (IU/kg) 3rd trimester	0.8±0.2	0.7±0.2	0.145
Fasting glucose (mmol/L) in 1st trimester	5.1±1.5	4.3±1.4	0.098
Fasting glucose (mmol/L) in 3rd trimester	5.4±2.0	4.4±1.3	0.113
Mean value capillary glucose (mmol/L) in 1st trimester	6.2±0.8	5.2±1.2	0.030
Mean value capillary glucose (mmol/L) in 3rd trimester	6.1±0.8	5.3±0.7	0.020
HbA1c (%) in 1st trimester	7.0±1.0	6.5±1.4	0.238
HbA1c (%) in 3rd trimester	6.2±0.9	5.8±0.8	0.106
BDNF (ng/L) in 1st trimester	22.0 (19.8-25.1)	26.3 (23.4-32.6)	0.004
BDNF (ng/L) in 3rd trimester	16.3 (14.3-18.8)	22.5 (20.6-28.4)	<0.001

Maternal characteristics in HYPO- and HYPO+ subgroups

Table 3 shows the comparison between the first and third trimester of pregnancy in age, duration of type 1 diabetes, body height/weight, BMI, and pregnancy weight gain. No difference was found between the studied groups. The percentages of HbA1c between the groups studied did not differ. The concentration of BDNF was significantly higher in the first and third trimester of pregnancy in the HYPO+ group than in the HYPO- group. Mean glucose concentration was significantly lower in the first and third trimester of pregnancy in the HYPO+ group than HYPO- group.

Concentration of fatty acids in Non-exposed group and Exposed group

The concentration of fatty acids in the studied groups is shown in table 4. Elevated concentrations of total FAs, MUFAs, n-3 PUFAs, and DHA, were found in the Exposed group compared to the Non-exposed group in the third trimester of pregnancy. Concentrations of all F.A.s increased significantly in the third compared to the first trimester in both groups.

Concentration of fatty acids in HYPO- and HYPO+ subgroups

The concentration of fatty acids in the HYPO- and HYPO+ subgroups is shown in table 5.

Elevated concentrations of total FAs, SFAs, n-6 PUFAs, and AA were found in the HYPO+ group, and lower concentrations of EPA in the third trimester compared to HYPO-. A significant increase in the concentration of total FAs, SFAs, MUFAs, n-6 PUFAs, n-3 PUFAs, AA, and DHA between the first and third trimesters were measured in both subgroups (HYPO+ and HYPO-), as shown in Table 5.
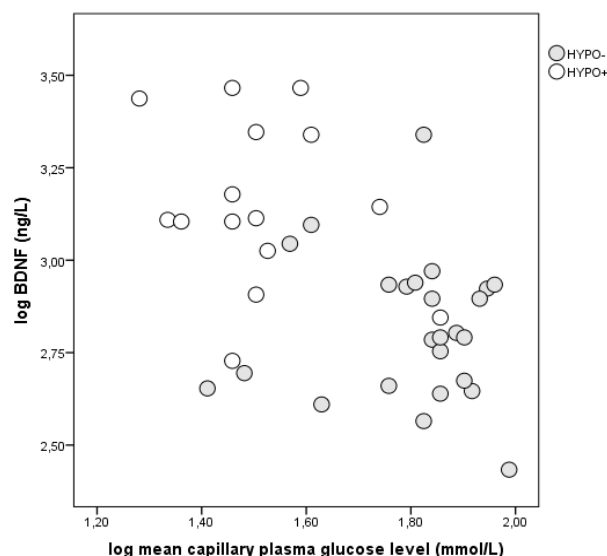
Figure 1. Nonparametric inverse correlation between mean glucose concentration in maternal capillary plasma and BDNF in the third trimester of pregnancy (r_{rho}=-0.502, P=0.001)

A positive nonparametric correlation was obtained between hypoglycemia and BDNF, EPA, total FAs, n-3 and n-6 PUFAs, and negative correlations between hypoglycemia and mean CPG (mean capillary glucose plasma), and between BDNF and CPG. Negative correlations were between CPG and EPA, DHA, A.A., total FAs, SFAs, n-3, and n-6 PUFAs.

A significant negative nonparametric correlation was established between mean glucose and BDNF concentrations in the third trimester of pregnancy. Lower average glucose values are associated with an increase in BDNF concentration (Figure 1).

The adjusted ORs for hypoglycemia by the duration of type 1 diabetes, BDNF, total FAs, and n-6 PUFAs at the first and third trimester of pregnancy are visualized in Table 7.

Table 4. The concentration of fatty acids in non-exposed and exposed pregnant women with type 1 diabetes mellitus

The concentration of fatty acids (g/L)	Non-exposed group (n=19)	Exposed group (n=21)	P
Total FAs in 1st trimester	202.7[a] (140.0-256.1)	250.1[b] (182.5-374.2)	0.077
Total FAs in 3rd trimester	422.6[a] (289.1-586.1)	505.5[b] (357.9-636.4)	0.048
SFAs in 1st trimester	61.9[c] (39.3-103.6)	87.5[d] (71.0-134.5)	0.051
SFAs in 3rd trimester	144.0[c] (97.0-232.1)	184.0[d] (133.0-247.3)	0.128
MUFAs in 1st trimester	27.3[e] (11.3-53.7)	48.7[f] (31.4-77.8)	0.128
MUFAs in 3rd trimester	70.1[e] (43.0-96.5)	85.2[f] (64.1-143.3)	0.043
n-6 PUFAs in 1st trimester	82.5[g] (56.4-107.6)	97.7[h] (74.9-151.6)	0.216
n-6 PUFAs in 3rd trimester	131.4[g] (99.2-250.5)	170.6[h] (118.2-228.6)	0.302
n-3 PUFAs in 1st trimester	6.6[i] (2.7-15.2)	8.3[j] (3.7-12.3)	0.077
n-3 PUFAs in 3rd trimester	13.4[i] (8.7-19.8)	20.8[j] (14.0-28.9)	0.002
AA in 1st trimester	16.1[k] (12.0-22.5)	20.3[l] (13.1-32.2)	0.410
AA in 3rd trimester	27.3[k] (17.5-40.9)	29.3[l] (20.6-39.6)	0.537
DHA in 1st trimester	3.4[m] (1.7-3.9)	4.2[n] (3.1-6.9)	0.077
DHA in 3rd trimester	7.4[m] (4.8-9.9)	13.4[n] (7.2-18.1)	0.001

[a,b,i,k,l,m]$P<0.01$; [c,d,e,f,g,h,j,n]$P<0.001$, Wilcoxon test; FAs - fatty acids; SFAs - saturated fatty acids; MUFAs - monounsaturated fatty acids; PUFA - polyunsaturated fatty acids; EPA - eicosapentaenoic acid; DHA - docosahexaenoic acid; AA - arachidonic acid

Table 5. The concentration of fatty acids in two subgroups (HYPO- and HYPO+) of pregnant women with type 1 diabetes in the first and third trimester of pregnancy

The concentration of fatty acids (g/L)	HYPO- group (n=25)	HYPO+ group (n=15)	*P*
Total FAs in 1st trimester	207.1[a] (151.0-287.1)	243.6[b] (179.5-419.9)	0.345
Total FAs in 3rd trimester	353.7[a] (270.8-544.5)	594.8[b] (408.0-856.2)	0.028
SFAs in 1st trimester	73.1[c] (55.6-105.0)	95.0[d] (70.6-164.1)	0.191
SFAs in 3rd trimester	138.3[c] (103.1-198.2)	334.4[d] (148.4-347.7)	0.034
MUFAs in 1st trimester	47.4[e] (31.4-77.8)	31.6[f] (11.5-55.5)	0.736
MUFAs in 3rd trimester	71.5[e] (50.5-108.4)	91.3[f] (73.1-170.4)	0.179
n-6 PUFAs in 1st trimester	88.4[g] (66.5-114.1)	96.8[h] (73.3-163.4)	0.363
n-6 PUFAs in 3rd trimester	136.0[g] (107.1-204.7)	226.4[h] (160.8-139.0)	0.028
n-3 PUFAs in 1st trimester	19.9[i] (13.1-29.1)	18.3[j] (12.3-27.3)	0.537
n-3 PUFAs in 3rd trimester	21.2[i] (19.8-31.0)	40.3[j] (28.8-61.6)	0.058
AA in 1st trimester	19.9[k] (13.1-29.1)	18.3[l] (12.3-27.3)	0.040
AA in 3rd trimester	21.2[k] (19.8-31.0)	40.3[l] (28.8-61.6)	0.005
DHA in 1st trimester	3.5[m] (2.4-4.5)	3.8[n] (2.7-6.1)	0.081
DHA in 3rd trimester	12.9[m] (7.0-16.5)	7.2[n] (4.6-11.5)	0.063
EPA in 1st trimester	1.9[o] (1.1-2.7)	2.2[p] (1.3-3.5)	0.511
EPA in 3rd trimester	3.4[o] (2.7-5.1)	2.9[p] (1.8-5.8)	0.031

[i]*P*<0.05; [m,o,p]*P*<0.01; [a,b,c,d,e,f,g,h,j,k,l,n]*P*<0.001; FAs - fatty acids; SFAs - saturated fatty acids; MUFAs - monounsaturated fatty acids; PUFA - polyunsaturated fatty acids; EPA - eicosapentaenoic acid; DHA - docosahexaenoic acid; AA - arachidonic acid; HYPO- (subgroup without hypoglycemia); HYPO+ (subgroup with hypoglycemia)

Table 6. Nonparametric correlation between Hypoglycemia and BDNF, mean value of glucose in capillary plasma, EPA, DHA, AA, total FAs, and m-6 PUFAs concentration in the third trimester of pregnancy

	HYPO+	BDNF	GCP mean	EPA	DHA	AA	Tot FA	MUFA	n-6 PUFA
BDNF	0.635**								
GCP mean	-0.665**	-0.502**							
EPA	0.376*	0.173	-0.451*						
DHA	0.326	0.123	-0.412*	0.890**					
AA	0.226	0.335	-0.409*	0.508**	0.530**				
Tot FA	0.383*	0.214	-0.420*	0.919**	0.858**	0.920**			
SFA	0.370*	0.172	-0.467**	0.912**	0.845**	0.620**	0.985**		
MUFA	0.238	0.315	-0.228	0.739**	0.565**	0.667**	0.851**		
n-6 PUFA	0.383*	0.202	-0.422*	0.924**	0.850**	0.610**	0.963**	0.906**	
n-3 PUFA	0.332*	0.124	-0.433*	0.907**	0.957**	0.565**	0.917**	0.723**	0.888**

*P<0.05; **P<0.01; BDNF - Brain-derived neurotrophic factor; GCP - glucose capillary plasma; EPA - eicosapentaenoic acid; DHA - docosahexaenoic acid; AA - arachidonic acid; tot FAs - total fatty acids; MUFA - monounsaturated fatty acids; n-6 PUFA - n-6 polyunsaturated fatty acids

Table 7. Risk of hypoglycemia by BDNF, total FAS, n-3 PUFAs in pregnant women with type1 diabetes mellitus according to each trimester of pregnancy

Adjusted for maternal age and duration of type 1 diabetes	OR	95% CI	*P* value
1st trimester of pregnancy			
BDNF (nmol/L)	1.324	1.050-1.668	0.017
Total FAs (g/L)	1.015	0.985-1.046	0.337
n-6 PUFAs (g/L)	0.964	0.888-1.047	0.388
3rd trimester of pregnancy			
BDNF (nmol/L)	1.818	1.079-3.053	0.025
Total FAs (g/L)	0.969	0.939-0.998	0.048
n-6 PUFAs (g/L)	1.103	1.001-1.217	0.048

BDNF concentration was associated with hypoglycemia (OR 1.324 95% CI 1.050-1.668, *P*=0.017) in the first trimester of pregnancy.

In the third trimester of pregnancy BDNF, and n-6 PUFAs concentration were associated with hypoglycemia (OR 1.818 95 % CI 1.079-3.053, *P* = 0.025; OR 1.103 95% CI 1.001-1.217, *P*= 0.048). Total FAs were inversely associated with hypoglycemia, OR 0.969 95% CI 0.939-0.998, P=0.048.

DISCUSSION

A significant decline in the concentration of BDNF during pregnancy

A significant decrease in BDNF concentration from the first to the third trimester of pregnancy in both studied groups is in line with other authors. Kim DR et al. (2012) found significantly lower serum BDNF in pregnant women versus non-pregnant women. D'Souza V et al. (2014) determined BDNF levels during pregnancy in pregnant women with preeclampsia and healthy pregnant women. They found a decline in the concentration of BDNF with the duration of pregnancy in both groups, and a significantly lower concentration of BDNF was found in pregnant women with preeclampsia compared to the healthy controls. The authors believe that BDNF plays an almost pivotal role in developing the maternal-fetal-placental unit during pregnancy. Decreased BDNF levels during pregnancy are due to abnormal placental development in preeclampsia, the authors conclude (D'Souza et al. 2014). Although the understanding of preeclampsia's pathogenesis is not entirely clear, the current theory explains its occurrence in two stages. The first phase is caused by a weak invasion of trophoblasts, which results in the inadequate reshaping of the spiral arteries, leading to the maternal response to endothelial dysfunction and an imbalance between angiogenic (PlGF) and antiangiogenic factors (sFlt-1), and at this point start the clinical symptoms of the disease. Since BDNF is also an angiogenic factor, it might involve placental development (Colorado-Barbposa et al. 2016).

Increased weight gain in pregnant women decreases BDNF levels

A negative correlation was found between BDNF and body weight gain in the third trimester of pregnancy (r_{rho}=-0.358, P=0.025). During pregnancy, women gain weight and become, to some extent, insulin-resistant. Elevated anti-insulin hormones, progesterone, cortisol, and human placental lactogen (HPL), lipids, and leptin increase insulin resistance, resulting in decreased BDNF concentration. Dietary restrictions stimulate the production of BDNF in brain cells. Reduced food intake reduces the concentration of glucose, insulin, and leptin, which increases the level of BDNF. Based on our results, we believe that an increase in insulin resistance in pregnant women with type 1 diabetes is responsible for decreasing BDNF concentration during pregnancy.

Comparing the mean capillary blood glucose values of pregnant women in the third trimester of pregnancy with BDNF, a negative correlation was found (r_{rho}=-0.502, P=0.001), which is in agreement with the results of Fujinami A et al. (2008), who found significantly lower BDNF values in patients with type 2 diabetes due to elevated glucose levels and insulin resistance. Tonoli et al. (2015) showed that high-intensity physical activity in patients with type 1 diabetes reduces glucose concentration and increases BDNF. BDNF reduces gluconeogenesis in the liver, blood glucose concentration, insulin, and leptin secretion (Morton et al. 2013). It reduces food intake, i.e., reduces appetite and increases insulin sensitivity.

Impact n-3 and n-6 PUFAs on BDNF concentration

This study shows that supplementation with 120 mg/day EPA and 616 mg/day DHA throughout pregnancy significantly alters the maternal vein serum's fatty acid concentration. Pregnant women in the exposed group had a higher concentration of total FAs, MUFAs, and n-3 PUFAs in the third trimester of pregnancy compared to the control group. The increased fatty acid concentration in the exposed group resulted in increased insulin resistance and, consequently, a decrease in BDNF concentration. During a third of gestation, the mother switches from the previous anabolic condition to a catabolic one permitting an enhanced transfer of nutrients through the placenta to sustain the rapid fetal growth. This catabolic condition is enhanced under the fasting condition. A special notice in terms of an improved breakdown of lipid stores by lipolysis in adipose tissue is facilitated by developing an overt insulin-resistant disease (Herrera 2005).

Many authors have found an association between n-3 PUFA intake and peripheral BDNF levels. A correlation was found between n-3 PUFA supplementation and BDNF levels in adolescents (Ferreira et al. 2014). DHA is essential for pre-and postnatal brain development, and EPA affects behavior and mood (Kidd 2007, Zaman et al. 2019). In double-blind, randomized, controlled trials, a combination of DHA and EPA is beneficial in attention-deficit/hyperactivity disorder, autism, dyspraxia, dyslexia, and aggression (Neuringer et al. 1994, Whalley et al. 2004). Accelerated cognitive decline and mild cognitive impairment correlate with decreased levels of DHA and EPA in tissue, and supplementation of these fatty acids improves cognitive functions (Edirappuli et al. 2020). A combination of n-3 PUFAs and pregnancy yields immunological tolerance and stimulates endogenous insulin production in women with T1DM (Horvaticek et al. 2017). Docosahexaenoic acid plays a crucial role in developing nervous system cells and reduces preterm birth frequency (Neuringer et al. 1994). The transfer of these fatty acids is markedly determined and directed from the mother to the fetus. The ability of fatty acids to pass through the placenta is vital for fetal brain development, fetal growth, and cardiovascular and pulmonary development. Proper transfer of fatty acids through the placenta in the fetus's direction plays a significant role in healthy fetal development. It is a significant energy source in constructing the cell membrane and is an essential signaling precursor of cellular molecules.

Arachidonic acid derivatives have an inflammatory effect, increase platelet aggregation, induce smooth muscle cell contraction, and are associated with many diseases. The increased proportion of n-3 PUFAs in cell membrane phospholipids reduces the availability of arachidonic acid and inflammatory metabolite production. EPA is a precursor for the synthesis of prostaglandins, thromboxanes, prostacyclin series three, and leukotrienes series five, which have anti-inflammatory activity. Additionally, EPA and DHA synthesize resolvins and protectins that have potent anti-inflammatory activity. Pregnant women in the exposed group had a significantly higher increase in the concentration of DHA and n-3 PUFAs in the third trimesters of pregnancy compared to the non-exposed group, which was expected given the supplementation of EPA and DHA in the diet. This study with the logistic regression model found that hypoglycemia during pregnancy was associated with BDNF and n-6 PUFAs, and inversely associated with total FAs.

The cognitive function of patients with type 1 diabetes is affected by the age of disease onset, disease duration, quality of glycemic regulation, and complications of diabetes. Frequent and prolonged hypoglycemia and hyperglycemia may affect pregnant women's cognitive function with type 1 diabetes (McCrimmon et al. 2012). Intense physical activity significantly increases BDNF levels in people with type 1 diabetes (Tonoli et al. 2015). Long-term hyperglycemia has been associated with microvascular complications such as proliferative diabetic retinopathy, nephropathy, and cognitive dysfunction (McCrimmon et al. 2012, Augustina et al. 2005). Reasonable glycemic control reduces the incidence of diabetic complications. Most patients with type 1 diabetes have mild to moderate cognitive function impairment manifested in the slowing of mental speed and reduced mental flexibility, while learning and memory are spared (Langan et al. 1991). Severe and frequent hypoglycemia in patients with type 1 diabetes is associated with lower cognitive test scores (Langan et al. 1991). Decreased serum BDNF levels correspond to the severity of cognitive impairment (Siuda et al. 2016). Reasonable glycemic control in pregnant women with type 1 diabetes often results in shorter and milder to moderate hypoglycemia episodes. Pregnant women with type 1 diabetes with mild to moderate hypoglycemia severity had lower mean capillary plasma glucose and higher BDNF values.

To the best of our knowledge, this is the first study that investigated the impact of hypoglycemia and EPA and DHA supplementation during pregnancy in women with type 1 diabetes on BDNF concentration. Because this is a prospective cohort study, the role of EPA and DHA supplementation and maternal hypoglycemia in determining BDNF concentration differences must be further investigated.

CONCLUSION

In both studied groups, BDNF concentration significantly decreased during pregnancy due to increased insulin resistance. EPA and DHA supplementation in pregnant women with T1DM did not increase BDNF concentration, probably due to elevated total fatty acid concentration. Pregnant women with lower glycemic values had higher concentrations of BDNF in the first and third trimesters of pregnancy.

Future research should focus on the impact of BDNF levels on cognitive function in pregnant women with T1DM who have frequent and severe hypoglycemia.

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