Original Article

Intrauterine nutrition and carotid intimal media thickness in young Thai adults

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Several studies show that intrauterine nutrition restriction is associated with vascular disease. Animal studies have shown that atherosclerosis can be affected by a constrained intrauterine diet, but this relationship in humans is controversial. The purpose of this study was to investigate the relationship between maternal intake during pregnancy and carotid intimal media thickness (CIMT). We measured CIMT in 565 twenty year old young adults whose mothers, while pregnant, participated in a nutritional study during 1989-1990 at two hospitals in Chiang Mai, Thailand. Maternal diet during pregnancy was assessed by two methods in each trimester, namely, the 24 hours food recall method and the food frequency method. Carotid intimal media thickness was greater in males and participants who showed higher blood glucose, higher body mass index and higher systolic blood pressure. Maternal protein intake during the first trimester was negative correlated with thickness of CIMT (p=0.02). The mean CIMT of participants whose mothers were in the lowest quarter of the distribution of protein intake in the first trimester was 0.45 mm (95% confidence interval (CI): 0.44-0.46) more than that of those whose mothers were in the highest quarter of the distribution (0.43 mm 95% CI: 0.42-0.44). In conclusion, lower maternal protein intake during early pregnancy may increase CIMT in adolescents.

Key Words: intrauterine nutrition, carotid intimal medial thickness, maternal diet, vascular disease, atherosclerosis

INTRODUCTION

Several studies have shown that intrauterine nutrition restriction is associated with vascular diseases such as coronary artery disease (CAD) or stroke in later life as review by Newnham *et al* [1] Animal studies have shown that atherosclerosis can be affected by a constrained intrauterine diet.[2,3] Adults exposed to famine during gestation in the Dutch Hunger Winter had a three-times higher rate of CAD fifty years later, than those in the non-exposed.[4] However, the mechanism in the relationship between intrauterine food restriction and vascular disease in later life is still unclear. Many biological risk factors for atherosclerosis and vascular disease are linked to intrauterine food restriction in the study of the Dutch Hunger Winter, such as hypertension, impaired glucose tolerance,[5] dyslipidemia[6] and higher level of obesity.[7] Therefore this relationship might be due to atherosclerosis. If that is the case, the surrogate marker of vascular diseases, namely, carotid intimal medial thickness (CIMT) in children[8] and young adults[9] might increase in people with lower intrauterine nutrition. The purpose of this study was to investigate this hypothesis with twenty year old Thai adolescents whose mothers had participated in a study on intrauterine nutrition. [10]

MATERIALS AND METHODS

Study population

In 1990, 2,184 pregnant Thai women with a gestational age of less than 24 weeks were invited to participate in a study at the Maharaj Nakorn Chiang Mai Hospital and The Maternal-Child Health Care Center, Chiang Mai, Thailand.[10] This study aimed to identify the risk factors associated with low birth weight. Excluded from the study were mothers who did not give birth in our centers, the birth of twins, stillbirth and abortions. Maternal diet intake was assessed by two methods. First, the 24-hour food recall method was used during the initial interview. Each mother was asked to recall all food consumed during the previous day and to estimate quantities in ordinary measures or servings. Then all details were calculated by using Thai Food Tables.[11] The amount of food was calcu-

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lated as energy, protein, fat and carbohydrate at each of the three trimesters: Weeks 10-12 (first trimester), weeks 22-24 (second trimester), and weeks 32-34 (third trimester). The food frequency questionnaire (FFQ) was used to assess the frequency of consumption of 34 foods in the previous month. Nutrient intakes in the FFQ were validated against the 24-hour food recall method.

In 2010, we contacted 2,184 mothers from the original study in the hopes of following up with their children by phone, mail and house visits. Five hundred and sixty four children (26%) agreed to participate. They did not differ significantly from the remainder of the participants in mean birth weight (2975 g versus 3011 g, p=0.09 or proportion of education in graduate level (4.96% versus 7.86%, p=0.25). The Local Research Ethics Committee approved the study and participants gave written consent. Participant anonymity was preserve.

Measurements

For at least 20 minutes, the children sat quietly in a room at the clinic. Their blood pressure was measured on the left arm at heart level, twice at an interval of 5-10 minutes. The mean of these two measurements were used for the analysis. Anthropometric measurements were performed. Participant's height, weight and waist-hip measurement were taken with indoor clothes, but no shoes. Each participant filled out questionnaires on various cardiovascular risk factors, such as family history of cardiovascular disease, smoking experience, past medical history and current drug habits.

While participants were recumbent, the certified investigator (KR) measured CIMT in the distal portion of the right common carotid artery using a Philip machine iE33 and a L10-4 MHz linear array transducer. Six longitudinal views including antero-posterior, lateral, antero-oblique were frozen at the end of the diastolic phase. Approximatly 10 mm proximal to the beginning of the bifurcation, we measured the CIMT of the far wall using Q-Lab Philip software, which was accompanied the Philip Machine. The mean of six CIMT measurements was used in the analysis.

Twelve hours before the clinical study, the participants fasted, then venous samples were collected. Total cholesterol, HDL cholesterol and triacylglycerol were measured using the Beckman Coulter analyzer (Unicel Dxc 800, Fullerton, California, USA).

Statistical analysis

To compare characteristics of the participants, we used ANOVA, t-test or Chi-squared tests. We used Pearson correlation coefficients tests to analyze the relationship between characteristics and CIMT. In the analysis of the relationship between the amount of maternal nutrient intake and CIMT, we grouped a distribution of maternal intake into quartiles. In linear regression, the mean value of CIMT according to these quartiles was calculated with adjustments of the potential confounders and then the significance of the trend of CIMT in these mean values was computed with the same adjustments. Variables were altered in dietary fat using logarithms to suit a normal distribution statistical assumptions. A *p-value* <0.05 was considered statistically significant.

RESULTS

There were 564 offspring participants, each 20 years old. There were 304 females and 260 males. The mean birth weight was 2.98 (2.94-3.01) kg. The mean CIMT was significantly greater in the males than the females (mean ±SD; males: 0.441±0.03 vs females: 0.436±0.03 mm, p=0.003). Subsequent analyses were presented for males and females, and adjusted for sex. Characteristics of the participants and their mothers according to sex of offspring are shown in Table 1. There were 12 females and 86 males with a history of smoking. Before the study began, a single investigator assessed the reproducibility of the CIMT measurements in twenty subjects using two repeated measurements a week apart. The intraclass correlation coefficient for repeated measurements was 0.91.

The maternal age and BMI were not related with CIMT (Table 2). Carotid intimal media thickness was positively correlated with: higher BMI, higher systolic blood pressure and higher fasting glucose. Consequence, further analysis adjusted with all factors that were positively associated and correlated with CIMT, namely: gender, fast-

Table 1: Characteristics of participants at birth and age 20 years

Characteristics	Mean value (95% confidence interval)	
	Male offspring	Female offspring
Off springs' data		
Birthweight (g)	3043 (2989-3096)	2935 (2889-2981)
Weight (kg)	63.4 (61.8-65.0)	52.5 (51.1-53.8)
Body mass index(kg/m^2)	21.8 (21.3-22.4)	20.9 (20.4-21.4)
Systolic blood pressure (mmHg)	122 (121-123)	108 (107-110)
Diastolic blood pressure (mmHg)	76.4 (75.2-77.7)	71.9 (70.7-73.0)
Plasma cholesterol (mmol/L)	4.33 (4.22-4.44)	4.37 (4.27-4.47)
Plasma glucose (mmol/L)	4.65 (4.60-4.70)	4.58 (4.51-4.65)
Plasma HDL (mmol/L)	1.38 (1.34-1.42)	1.53 (1.49-1.57)
Plasma triacylglycerol (mmol/L)	1.07 (1.00-1.15)	0.89 (0.84-0.95)
Maternal data		
Mean age (years)	26.0 (25.5-26.5)	26.5 (26.0-27.0)
Weight (kg)	56.3 (55.2-57.3)	57.1 (56.9-57.8)
Body mass index (kg/m^2)	21.3 (21.0-21.6)	21.4 (21.1-21.6)

Table 2. Linear regression coefficients (β) for carotid intimal medial thickness (CIMT) and characteristics of mothers and offspring adjusted for gender

Characteristics	Pearson correlation coefficient	*p*-value
Maternal characteristics		
Age at recruitment	-0.04	0.37
Gestational age at first attending study	0.06	0.15
Gestational age at deliver	-0.01	0.98
Body mass index	-0.04	0.31
Parity	0.02	0.60
Offspring characteristics		
Birth weight	0.06	0.16
Education	-0.09	0.24
Smoking history	0.02	0.62
Body mass index	0.17	<0.001
Plasma cholesterol	0.02	0.58
Plasma fasting glucose	0.09	0.04
Plasma triacylglycerol	0.04	0.28
Plasma high density lipoprotein	0.03	0.55
Systolic blood pressure	0.12	0.02
Diastolic blood pressure	0.04	0.34

Table 3. Mean (95% CI) of carotid intimal medial thickness (mm) by quartiles of nutrient intakes

Maternal nutrient intake	First trimester	Second trimester	Third trimester
Protein			
Q1	0.452 (0.440-0.463)	0.435 (0.4281-0.442)	0.436 (0.429-0.442)
Q2	0.443 (0.437-0.449)	0.437 (0.434-0.441)	0.437 (0.433-0.440)
Q3	0.435 (0.429-0.441)	0.439 (0.436-0.443)	0.437 (0.434-0.441)
Q4	0.426 (0.415 -0.438)	0.442 (0.435-0.448)	0.438 (0.432-0.444)
p-value for trend †	0.02	0.27	0.69
Fat			
Q1	0.447 (0.437-0.456)	0.436 (0.431-0.441)	0.435 (0.430-0.440)
Q2	0.442 (0.436-0.447)	0.438 (0.434-0.441)	0.436 (0.433-0.440)
Q3	0.437 (0.431-0.442)	0.439 (0.436-0.442)	0.438 (0.434-0.441)
Q4	0.432 (0.422-0.441)	0.440 (0.435-0.446)	0.439 (0.434-0.444)
p-value for trend †	0.06	0.33	0.44
Carbohydrate			
Q1	0.433(0.418-0.448)	0.442(0.434-0.451)	0.441 (0.433-0.449)
Q2	0.437(0.430-0.444)	0.440(0.436-0.443)	0.438 (0.435-0.442)
Q3	0.441(0.434-0.448)	0.437(0.433-0.441)	0.436 (0.432-0.439)
Q4	0.445(0.430-0.460)	0.434(0.426-0.443)	0.433 (0.425-0.441)
p-value for trend †	0.39	0.34	0.27

† by linear regression with adjustments of the potential confounders: sex, body mass index, systolic blood pressure fasting glucose and the amount of energy.

ing blood sugar, body mass index and systolic blood pressure. Since the amount of maternal energy intake was also calculated based on the amount of protein, fat and carbohydrate, we included the amount of energy in the same trimester as another confounder. Following adjustment of these potential confounders, the amount of protein intake in the first trimester was negatively correlated with CIMT (Table 3). Mean CIMT of participants whose mothers were in the lowest quartile of protein intake in the first trimester was 0.45 mm (95% CI: 0.44-0.46) more than that of those whose mothers were in the highest quartile (0.43mm, 95% CI:0.42-0.44) (p=0.02). The amount of fat intake in the first trimester was marginally negatively correlated with the CIMT (p=0.06). When correlation between the amount of maternal protein intake in the first trimester and current cardiovascular risk factors (namely waist circumference, fasting blood sugar and oral glucose tolerance test, plasma LDL, cholesterol and blood pressure) was made, there was statistically significant negative correlation only with diastolic blood pressure (p=0.02).

DISCUSSION

We found that lower maternal protein intake was associated with higher CIMT in twenty- years-old offspring. It is important to note that this appeared to be a similar trend for maternal fat intake in the first trimester as well. As is well known, CIMT is widely used as a marker of early atherosclerotic lesions in adult.[9,12] This supported the hypothesis that the mechanism in the relationship be-

tween intrauterine food restriction and vascular disease is through atherosclerosis. Our result is supported by a study in Southampton, England, which found that lower maternal protein, fat and energy intake during pregnancy predicted increased CIMT in 216 nine-year-old offsprings.[13] Interestingly, the amount of carbohydrate intake was not significantly associated with CIMT in either study. To our knowledge this is the first observational study between intrauterine nutrition and CIMT in later life with an Asian population, which has shown a different natural history in vascular disease compared with a Caucasian population.[14,15] Since this relationship was similar to the results of the Southampton study, this might reflect a correlation across racial lines. For example, intrauterine nutrition restriction might be associated with CAD or stroke in later life with thick CIMT. This is particularly important because in Thailand the incidence of low birth weight is high (9.2%) and vascular diseases, namely stroke and CAD, are two of the top ten causes of death.[10,16]

The mechanism of relationship between low maternal protein intake and high CIMT of twenty-year-old offspring has been controversial. Our study found that lower maternal protein intake in the first trimester was correlated with higher diastolic blood pressure. Langley-Evans investigated the effects of low maternal protein intake on blood pressure during gestation in rats. This study suggested that restricted maternal protein intake might program hypertension.[17] Therefore the relationship between lower maternal protein intake and higher CIMT of twenty-year-old offspring was partially explained through high blood pressure. To our knowledge, this is the first human study to confirm this explanation. Szitanyi and colleagues suggested that restricted energy intake resulted in hypercholesterolaemia based on their rat study and that this might be the relationship between low maternal protein intake and high CIMT found in twenty-year-old offspring, however our study did not find any correlation between lower maternal protein diet and any lipid markers namely cholesterol, HDL or triacyglycerol.

Limitation

Although we believe our results are valid, our study might have limitations. We lacked sufficient data on lactation, which might affect the outcome.[18] However, this relationship is not found in the nine-year-old participants study.[13] Though our study only represents 25% of the original study population, the birth weight and the education of the mothers between the previous and current study were similar, so we still believe our findings have merit. Although the wealth of data show the association between CIMT and CAD.[9,12] CIMT is not synonymous to atherosclerotic lesions. Moreover, from the pathological point of view, CIMT might not mirror atherosclerosis in coronary arteries. CIMT was measured in common carotid arteries, but most atherosclerosis happens downstream in the internal carotid artery.[19] CIMT is the thickness of intima and muscle, which is not the same as the known origin of atherosclerosis, i.e. pathological intimal thickening and lipid necrotic core.[19]

Conclusion

We found that lower maternal protein intake during early pregnancy may increase CIMT in adolescents. Further studies are needed to replicate this finding in older age groups and to clarify the mechanism.

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AUTHOR DISCLOSURES

None of the authors had any conflict of interest

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泰國年輕成人子宮內營養與頸動脈中層厚度的關聯

數個研究顯示子宮內營養限制與成長後血管疾病具有相關性。動物實驗顯示受限的妊娠期飲食可影響後代的動脈硬化，但是這個相關性在人體仍具爭議。此研究旨在研究母親懷孕期間的飲食攝取與其子女頸動脈中層厚度(CIMT)之相關性。我們測量 565 名 20 歲年輕成人的 CIMT，他們的母親在懷孕期間參加 1989-1990 年在泰國清邁兩間醫院執行的一個營養研究。母親孕期的飲食是以 24 小時飲食回憶法及食物頻率法兩種方法評估。參與者是男性或有較高的血糖值、身體質量指數及收縮壓者，呈現較高的頸動脈中層厚度。母親在第一孕期的蛋白質攝取量與 CIMT 的厚度為負相關(p=0.02)。這些參與者，若母親在第一孕期的蛋白質攝取量分佈在最低四分位者，其 CIMT 平均為 0.45 mm(95%CI: 0.44-0.46)，高於那些分佈在最高四分位者(0.43 mm; 95%CI: 0.42-0.44)。總而言之，母親在懷孕初期較低的蛋白質攝取量可能增加青少年的 CIMT。

關鍵字：子宮內營養、頸動脈中層厚度、妊娠飲食、血管疾病、動脈硬化