Research Article

Fetuin-A and its Relation with Methyl Group Metabolism in Children with Congenital Heart Defects and their Mothers

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ABSTRACT

Methyl group metabolism is related to energy metabolism, Derangements of both metabolisms are linked to the risk of Congenital Heart Defects (CHD). The study aimed at investigating the relationship between protein fetuin-A known for its role in energy metabolism and insulin resistance, and methyl group metabolism in children affected with (CHD) and their mothers. Fetuin-A was measured in serum samples of 37 children (age<3 years) affected with CHD and in 69 mothers of affected CHD children, including the 37 mothers of tested children. In addition, serum samples from 33 mothers of non-affected age comparable children served as controls. Serum fetuin-A levels were compared between the groups. The correlation of fetuin-A were studied with age, maternal body mass index (BMI), homocysteine (Hcy), methylmalonic acid (MMA), folate, cystathionine (Cys), S-adenosylmethionine (SAM), S-adenosylhomocysteine (SAH), choline, betaine, and dimethylglycine (DMG). Maternal fetuin-A was lower in mothers of children with single ventricular septal defect (VSD) as compared to mothers of children with VSD plus other heart defects (P = 0.025). Mean fetuin-A levels did not differ between mothers of CHD children and mothers of control children (mean (SD) 346(90) vs. 375(151) µg/mL). Levels of maternal fetuin-A in mothers of the CHD children showed negative correlation to maternal DMG (r = - 0.240, *P* = 0.049). Maternal fetuin-A showed also negative correlation to child DMG (R = - 0.363, *P* = 0.008). Child fetuin-A correlated negatively with child SAM (R = - 0.369, *P* 0.041). Fetuin-A correlated to intermediate metabolites in the methylation cycle in CHD children and their mothers, suggesting a common regulatory mechanisms.

Keywords: congenital heart defects, fetuin-A, methylation, dimethylglycine, S- Adenosyl methionine, choline.

INTRODUCTION

CHD are major birth defects with high economic and social burdens. The pathophysiology of CHD is poorly understood and there are currently no clear recommendations for prevention. Several human studies evoked possible derangement in the methylation pathway which involved many nutrients (as folate, vitamin B12, methionine, choline, betaine) in children affected with CHD[1-5].

Further mechanisms that might be involved in CHD are disturbed tissue calcification or energy metabolism, both of which have been related to phosphorylated glycoprotein fetuin-A (á2-HS-glycoprotein, AHSG)[6], that is synthesized and secreted primarily by the liver and increased in obese people[7], due to its metabolic role that may impact glucose transport and mitochondrial β-oxidation of fatty acids[8-9].

As the liver is the major site for fetuin-A and methyl group synthesis we hypothesized that fetuin-A metabolism may be related to the methylation process and its metabolic markers such as betaine and choline, that constitute a significant source of S–adenosyl methionine (SAM), the main methyl donor in the liver[10], the latter molecule and methyl donors contribute by other way in fetuin-A metabolism as SAM suppress adenosine monophosphate activated kinase (AMPK) the energy sensor regulator[11] responsible for fetuin-A inhibition in state of under-nutrition and enhancement in states of over-nutrition, while different methyl donors activate AMPK in obese mice[12].

Fetuin-A secretion increases secondary to decreased AMPK-activity. As such, fetuin-A can be regarded as a hepatic signal of energy excess, a relevant signal considering its role as a growth factor; but in states of over-nutrition[13] this signal may contribute to insulin resistance[14].

In the effort to provide additional information about the metabolic risk factors for CHD. We measured serum fetuin-A levels in mothers of children with congenital heart defects (CHD) and their affected children and study their correlation with previously measured methyl donors and related biomarkers.

SUBJECTS AND METHODS

Subjects

Women with previous pregnancy with CHD, and their children were recruited from 3 representative hospitals in Damascus (the University Hospital of Damascus, the Pediatrics' University Hospital, and the Heart Surge University Hospital). Control mothers and children were recruited from the nursery of the Paediatrics University Hospital of Damascus. The recruitment took place between August 2010 and June 2011.

Fetuin-A was measured in mothers of **69** of CHD children and in 37 of their children. All types of CHD were included (ventricular septal defects VSD, atrioventricular septal

defects ASD, transposition of the great arteries TGA, coarctation of the aorta, pulmonary valve stenosis, Tetrology of Fallot TOF, Pentology of Fallot).

The age of the CHD children was below 3 years and the affected pregnancy was within the last 3 years. The controls were mothers (n= 33) of healthy children (also < 3 years).

Exclusion criteria were, chromosomal and birth defects, recent operations, and kidney or hepatic diseases.

Exclusion criteria for the mothers were current pregnancy, diabetes mellitus, and recent operations.

All mothers were apparently healthy, None of the children or the mothers was taking vitamin supplements at the time of blood collection, the household income and the place of residency for the included mothers had not been changed since the concerned pregnancy and the dietary habits were relatively stable during the last 3 years.

A standardized interview and questionnaire were completed for each mother.

The complete medical history of the child and the mother, current medications, maternal health condition during the affected pregnancy (supplement, dietary habits) were documented.

All children with CHD were diagnosed by heart echocardiography performed by a cardiologist paediatrician.

The defect phenotype was documented. The study was approved by the ethical committee of Damascus University Hospital, and all participants signed a written consent form.

The study was performed in adherence with the guidelines of the Declaration of Helsinki.

Blood sampling and biochemical measurements

Venous blood (7 mL) was collected into dry tubes and those containing K+EDTA. K+EDTA tubes were chilled on ice and centrifuged within 40 minutes.

Several aliquots were prepared and stored at -70°C. Blood analyses were conducted as reported in an earlier report[15].

Fetuin-A was measured by an enzyme-linked immunosorbent assay ELISA kit (BioVendor R&D diagnostic, Czech Republic).

Statistical analyses were conducted using SPSS (version 19.0).

Results are shown as mean (SD, standard deviation).

Means or medians continuous variables were compared between two independent groups using ANOVA or Mann-Whitney tests, respectively. Correlations between continuous variables were conducted using Spearman's Rank test. P values below 0.05 were considered statistically significant.

Serum maternal fetuin-A levels were compared according to the mothers BMI, place of residency (rural and urban communities), weekly consumption of red meat (the serving size was considered approximately 85 g estimated on the average content of red meat in a meal according to the population culinary habits).

RESULTS

Maternal fetuin-A was lower in mothers of children with single VSD in comparison with mothers of children with multiple lesions (VSD) + one or more of other heart defects (ASD,TGA,PVS, PDA, Aorta malformation) (P= 0.025) (Table 1).

Table 1: Mean (SD) of serum maternal fetuin-A levels (µg/mL) according to type of CHD lesion in the affected child.

Type of CHD in the affected child	CHD mothers (n 69)
Single VSD (n=13)	309 (45)
Multiple lesions (VSD+ other lesions) ‡ (n=25)	350 (111)
P-value	0.025
Any single lesion* (n=28)	321 (46)
Any multiple lesions§ (n=41)	354 (111)
P-value	0.042

Data are means (SD). P-values are according to Mann-Whitney test.

‡ Other lesions were (auricular septal defects (ASD), transposition of the great arteries (TGA), Aorta malformation, Patent Ductus ateriosus (PDA), Pulmonary valve stenosis).

†TOF: Tetrology of Fallot.

* single lesions were (ventricular septal defects (VSD), auricular septal defects (ASD), transposition of the great arteries (TGA), Aorta malformation, Patent Ductus ateriosus (PDA), Pulmonary valve stenosis).

§multiple lesions were defined as the simultaneous presence of more than one single lesion.

Maternal fetuin-A levels were not different according to time to delivery (time between blood collection and birthday of the included child) of the affected children (P = 0.208) or the healthy children (p = 0.986).

No difference was found between mean maternal serum fetuin-A in control and that in the CHD group (mean= 375 vs. 346 µg/mL P = 0.601) (Table 2). Fetuin-A levels were higher in the overweight control women (BMI > 25 kg/m^2) compared with normal weight controls (P =0.045). In both CHD and control women, maternal fetuin-A levels did not differ according to residency, household income, or history of previous abortion (Table 2). Maternal fetuin-A levels were higher in CHD mothers who reported consuming more than 2 servings red meat per week (P = 0.016). This difference was not attenuated after adjustment for BMI (P = 0.019), education (P = 0.028), or household income (P = 0.022).

Table 2: Maternal fetuin-A concentrations (µg/mL) in the study groups according to health related information

	N	Control	n	CHD	*P-value*[*]
Mothers (all)	33	375 (151)	69	346 (90)	*0.601*
BMI, kg/m^2					
BMI >25	15	416 (179)	29	335 (55)	*0.251*
BMI ≤25	14	345 (129)	34	356 (113)	*0.464*
P-value†		*0.045*		*0.452*	
Residency					
Urban communities	17	434 (168)	30	339 (57)	*0.143*
Rural communities	16	315 (111)	39	350 (121)	*0.417*
P-value†		*0.101*		*0.956*	
Household income					
Household income <300$	7	291 (12)	41	375 (66)	*0.132*
Household income > 300$	20	391 (26)	23	353 (29)	*0.463*
P-value†		*0.311*		*0.880*	
Red meat consumption per week					
> 2 servings‡	20	373 (153)	29	376 (98)	*0.882*
≤ 2 servings	10	402 (201)	34	321 (77)	*0.782*
P-value†		*0.725*		*0.016*	
Presence of previous abortion					
Yes	7	363 (19)	19	345 (68)	*0.789*
No	18	395 (25)	44	349 (74)	*0.689*
P-value†		*0.929*		*0.577*	

Data are means (SD). * *P-value* between CHD and control groups; †P-value within groups are according to Mann-Whitney test. ‡ One red meat serving is considered approximately 85g depending on local culinary habits. [§] Available multivitamins formulas for adults that contain the daily allowances for different B group and fat soluble vitamins but do not contain choline or betaine.

Table 3 summarizes important correlations between fetuin-A and other biomarkers.

Table 3: Correlations between child fetuin-A and SAM and between maternal fetuin-A and other maternal and child biomarkers.

	R	P-value
Fetuin-A in CHD Children (n = 37)		
Child SAM	- 0.369	0.041
Fetuin-A in CHD Mothers (n = 69)		
With maternal biomarkers		
Cystathionine	-0.248	0.044
Betaine	-0.236	0.052
Choline	-0.307	0.011
DMG	-0.240	0.049
With child biomarkers		
DMG	-0.363	0.008
Betaine	-0.259	0.063

Correlation coefficient (r) is according to Spearman test. Only correlations with significant or marginally significant correlations are shown.

In the CHD group, there was a negative correlation between child fetuin-A and child SAM (P = 0.041). A negative correlation between maternal fetuin-A and that of maternal choline (P = 0.011), maternal DMG (p = 0.049) and maternal CYS (p = 0.044). Maternal fetuin-A tended to correlate negatively also to maternal betaine (p = 0.052). Maternal fetuin-A was negatively correlated with child DMG (R = -0.363, P =0.008) and tended to correlate negatively with child betaine (p = 0.063).

Feutin-A levels of the CHD mothers and their children were not correlated (P = 0.275), fetuin-A in CHD children were (mean (SD) = 322 (149) µg/mL).

DISCUSSION

Fetuin-A metabolic role conceivably will have more importance during pregnancy, as disturbance in glucose metabolism was associated with adverse pregnancy outcome including CHD[16-17], and energy restriction during pregnancy has also deleterious effect[18].

The novel findings in this study were; first, maternal feutin-A was negatively correlated to maternal plasma choline, betaine and DMG, and child plasma DMG.

Second, child fetuin-A was negatively correlated to child SAM. Third, maternal feutin-A concentrations were significantly higher when the CHD affected children had

multiple lesions compared to mothers with children with a single lesion.

In CHD mothers, negative correlation of maternal fetuin-A to maternal choline, betaine, and DMG suggests a role of choline metabolism as modifier for fetuin-A. In this context, higher fetuin-A levels were found when red meat consumption per week exceeds two servings (≥ 170 g) (table-2). This difference was not attenuated after adjusting for BMI, household income or maternal education. A higher meat intake is expected to provide higher methionine, B12, choline, serine, and glycine levels needed for trans- and re-methylation[19-20]. We therefore speculate that in the current study on a population with generally low vitamin B12 status (i.e., reflected by high MMA and Hcy)[15], the requirements for betaine and choline are higher in order to ensure adequate production of methionine and methyl group for protein synthesis. However although control mothers in our study reflected low vitamin B12 status also, maternal choline, betaine, DMG were negatively correlated to maternal fetuin-A in CHD mothers but not in control mothers suggesting a dysbalance in choline utilization.

DMG concentrations in plasma is a good indicator of betaine utilized as a methyl donor[21]. According to this, 74% of the folate-deficient subjects[22] showed elevated DMG, whereas only 36% showed increased betaine[22]. Fetuin-A correlated negatively to plasma DMG in CHD mothers and children and tended also to correlate to plasma betaine in children (p = 0.063). In apparently the same context, Fetuin-A in CHD children was also negatively correlated with SAM levels. These negative correlations between methyl donor compounds and fetuin-A may reflect a common regulatory mechanism for the methylation metabolism and fetuin-A levels in this group of patients (mothers and affected children). As stated earlier in the introduction, methyl donors activated AMPK (hence reduced fetuin-A levels) in a study on obese mice[12]. However, SAM levels have been shown to down regulate AMPK activity, depending on the cellular energy status[11], thus the founded negative correlation may reflect either ineffective regulation by SAM (Exceptionally high SAM levels were observed in a larger CHD group in our earlier report[15]), or the presence of a celluar over-nutrition signal in this group of patients.

Choline and betaine in B12 deficient milieu are increasingly needed and this can ameliorate insulin resistance[23]. In this same milieu, insulin resistance signal could be induced when a diet with higher content of fat (i.e., red meat) is consumed as this diet will induce fetuin-A[24], the state of adiponectin/AMPK axis depression and fetuin-A induction will enhance insulin resistance[25-26].

Fetuin-A correlate to BMI and has a role in modifying BMI too[27]. Additionally, BMI was inversely related to betaine and directly related to choline in one large population-based study[28].The risk for giving birth to a child with CHD was higher in obese (BMI ≥ 30 kg/m^2) women compared to normal-weight women (BMI= 19–24.9 kg/m^2)[29-31]. And with minor conflicting reports[29] the risk was also cited for over-weighted women (BMI=25-30 kg/m^2)[30-31] in comparison to normal weighted. However, the metabolic link between CHD and obesity that is currently not well defined, could be related to methyl donors as modifiers for energy metabolism.

As there is currently no molecular explanation for the potential differences in the metabolic profile according to the type of CHD lesion. The difference found in maternal fetuin-A levels according to the type of CHD lesion, and the observation of higher levels in multiple lesions (table-1), show the need for studies including large numbers of each type to assess possible role for fetuin-A and hence energy metabolism in CHD lesion type and severity.

The hypothesized link between the dietary pattern and CHD risk is summarized in the figure-1

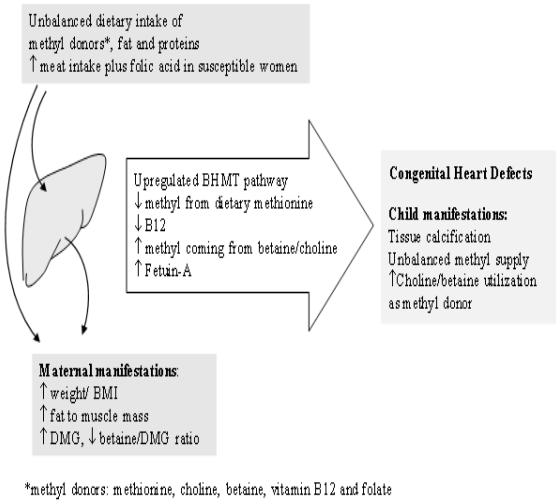

Figure 1: The hypothesized link between the dietary pattern and CHD risk.

Taken together, maternal fetuin-A is related to several component of the methylation cycle in CHD mothers, suggesting a potential dietary pattern in these women involving methyl donors (e.g., methionine, choline, betaine, folate, etc) and fetuin-A. The study warrants further investigation on dietary intake and the role of energy metabolism in the ethiology of CHD.

Conflict of interest: None declared.

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