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ORIGINAL ARTICLE

Tumor necrosis factor-alpha levels in blood cord is directly correlated with the body weight of mothers

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Summary

Background

Obesity has emerged as major public health problem leading to increased morbidity and mortality. Epidemiological studies indicate that in many regions of the world, children and teenagers are increasingly affected by obesity, which contributes for a pessimistic projection for the near future. Maternal obesity has been implicated in metabolic disorders of the offspring, but there are no biological markers that can be detected early on life that predict the development of obesity in the offspring.

Objective

To evaluate the expression of inflammatory markers in the umbilical cord blood of babies of mothers with obesity/overweight, and correlate these markers with the body weight at age 9 months.

Methods

Anthropometric data of mothers and babies were obtained during prenatal evaluation, at birth and 9 months after birth. Cord blood was collected during delivery of 54 babies from mothers with obesity/overweight and of 50 babies from lean mothers. Tumour necrosis factor-alpha (TNF-α), transforming growth factor 1 beta, monocyte chemoattractant protein-1 and 2 (MCP-1/MCP-2) were determined in serum samples using enzyme-linked immunosorbent assay methods. Correlations were evaluated using the Spearman correlation coefficient, and comparisons were evaluated using the non-parametric Mann–Whitney *U*-test.

Results

Cord blood TNF-α was positively correlated with maternal body mass index. There was an inverse correlation between cord blood transforming growth factor 1 beta and baby body weight at birth. There was no biological marker that predicted body weight at age 9 months.

Conclusion

Although we have not found a biological marker to predict increased body weight at 9 months of age, the study shows that maternal obesity exposes the baby to higher TNF-α level in the early stages of life, and this can affect metabolic and inflammatory parameters during adulthood.

Keywords: Inflammation, obesity, offspring, tumour necrosis factor alpha.

Introduction

Tumour necrosis factor-alpha was the first cytokine identified as a link between obesity and insulin resistance (1). Studies have shown that both macrophages present in the adipose tissue, and enlarged adipocytes can produce and secrete TNF-α, which acts as a paracrine and endocrine signal to induce insulin resistance in a number of tissues and cells that depend on insulin in order to control glucose uptake and metabolism (1–3). Upon binding to its receptor, TNF-α triggers cytosolic inflammatory pathways leading to the activation of c-Jun Kinase (JNK) and inhibitor of nuclear Kappa Kinase (IKK) (1,2,4). It has been shown that both JNK and IKK can catalyse the serine phosphorylation of the insulin receptor and at least two of its important substrates, insulin receptor substrate-1 and insulin receptor substrate-2, which results in the severe impairment of the insulin signal transduction (4,5). Other studies have identified additional components of the inflammatory network that connects obesity with insulin resistance (6–10). Currently, IL-6, IL-1β, PAI-1, TGF-1β and chemokines, such as MCP-1 and MCP-2, are all known to play important roles in the inflammatory phenotype of subjects with obesity (6–10).

Recent studies have shown that maternal obesity can lead to irreparable injuries in the metabolism of offspring (11,12). Dunn and Bale demonstrated that feeding pregnant rats with a high-fat diet resulted in an increase of body weight and a reduction of insulin sensitivity in the offspring and in the next generation (13). Another study demonstrated that maternal adiposity induces insulin resistance in the offspring and increased body weight that persists into adulthood (14). In addition, the induction of obesity in pregnant rodents result in a number of metabolic abnormalities in the offspring and mechanistic studies have identified changes in placental morphology, inflammation and epigenetic factors as important players in this scenario (11,15,16). Despite the fact that maternal obesity may increase the risk of obesity and other metabolic diseases later in life, there is no current method that allows the prediction of such an outcome. Here, we hypothesized that inflammatory markers in the cord blood could correlate with body-weight gain during the first year of life and, therefore, become useful markers to predict metabolic diseases in the offspring of mothers with obesity. In order to test our hypothesis, the levels of TNF-α, TGF-1β, MCP-1 and MCP-2 were determined in the cord blood of babies born from mothers with obesity/overweight or lean mothers and confronted with a number of clinical and anthropometric parameters from the mothers and babies, which were followed up for 9 months.

Methods

Patient selection and study design

Patients were selected from the obstetric clinic of the Women's Health Center at the University of Campinas. There were 54 patients with overweight/obesity (group 1) and 50 lean (group 2) included in the study. The inclusion criteria were pregnant women with obesity/overweight and appropriate/underweight according to Atalah *et al.* (17). The exclusion criteria were pregnancy of twins, use of steroids during pregnancy, use of non-steroidal anti-inflammatories, diagnosis of cancer during the 5 years that preceded pregnancy and diagnosis of any acute or chronic inflammatory diseases during pregnancy. Immediately after delivery, 2.0 mL of blood was collected from the umbilical cord blood, and serum was prepared and frozen at −80 °C. The Ethics Committee of the University of Campinas approved the study (no. 49.775), and all patients gave informed consent.

Laboratory assessment

The peptides TNF-α (Quantikine-R and D systems, Minneapolis, MN, USA), TGF-1β, MCP-1 and MCP-2 (Biolegend Legend Max, San Diego, CA, USA) were determined in serum samples using an enzyme-linked immunosorbent assay method according to the recommendations provided by the manufacturers.

Anthropometric evaluation

All the anthropometric data from the newborns were obtained from the medical records at birth and at age 9 months. Some babies could not be evaluated at 9 months because of discontinued follow-up.

Statistical analysis

Comparisons of blood levels of inflammatory markers were performed using the non-parametric Mann–Whitney *U*-test, whereas the correlations between proteins and anthropometric parameters were estimated using the Spearman correlation coefficient. All analyses had a significance level of 5%.

Results

The qualitative variables evaluated in the study are presented in Table 1. There were 61 men and 43 women newborns. At delivery, 100 newborns were at term whereas four were pre-term. Regarding the body weight of mothers at pregnancy detection, there were 29 obese,

Table 1 Qualitative variables evaluated in the study

Variable	*n* = 104	%
Gender		
Male	61	58.65
Female	43	41.35
GA at birth		
Preterm birth	4	3.85
Postmature birth	100	96.15
Group		
Obesity	29	27.88
Overweight	25	24.04
Adequate weight	40	38.46
Underweight	10	9.62
Group		
Obesity/Overweight	54	51.92
Adequate weight/Underweight	50	48.08

GA, gestational age.

25 overweight, 40 normal and 10 with underweight body mass index values (17). Table 2 depicts the distribution of mothers according to body mass index and age at delivery. In addition, Table 2 depicts data of body weight and body weight variation (from birth to age 9 months) in the babies.

Table 3 presents the concentrations of inflammatory markers in cord blood comparing samples from groups 1 and 2. TNF-α was significantly higher in the cord blood of newborns from group 1 mothers. None of the other peptides were significantly different in the groups.

After 9 months, we retrieved data from 78 infants. Table 4 shows the correlation analysis between cord blood inflammatory peptide levels and anthropometric parameters of mothers (body mass index and body weight) and infants (birth weight and weight/BMI at 9 months of age). The analysis confirmed the existence of a direct correlation between TNF-α concentration in the umbilical cord blood and maternal body mass index as well as a direct correlation between TNF-α and body weight of the mother. The variables body weight and body mass index of the infants at age 9 months did not correlate with the levels of inflammatory peptides. However, there was an inverse correlation between the concentration of TGF-1β and the body weight of newborns at birth, and a trend towards a positive correlation between TGF-1β and the body weight of infants at age 9 months.

Discussion

In the present study, we evaluated inflammatory markers in cord blood in a cohort that shares similar characteristics with other cohort previously published {Challier, 2008 #29}. The babies were followed up for 9 months because at this time most, if not all infants were interrupting breastfeeding. The main objective of the study was to evaluate the impact of maternal body weight on inflammatory parameters of cord blood and its relation with early body weight gain of babies. We considered that the longer the period out of breastfeeding the more other environmental factors could affect the results. First, we hypothesized that the age of the mothers could influence inflammatory peptide concentrations in cord blood. In Table 2, we show the distribution of mothers according to age. Upon statistical analysis, we detected no correlation between the age of mothers and markers of inflammation in cord blood (data not shown).

Tumour necrosis factor-alpha has been previously evaluated in the placenta and umbilical cord blood. In a study with only 15 subjects, Varastehpour and colleagues (18) evaluated samples from 15 newborns and found a

Table 2 Comparison of selected parameters in mothers and babies from distinct groups: obesity/overweight (group 1) vs. appropriate body weight/underweight (group 2)

		n	Mean	*P*
Mother's BMI	Obesity/Overweight	54	34.95	<0.0001**
	Proper weight/Underweight	50	26.09	
Mother's age	Obesity/Overweight	54	28.04	0.1773**
	Proper weight/Underweight	50	25.98	
Baby's birth weight	Obesity/Overweight	54	3.35	0.8227*
	Proper weight/Underweight	50	3.33	
Baby's weight at 9 months	Obesity/Overweight	45	9.29	0.0677*
	Proper weight/Underweight	35	8.67	
Baby's body mass gain	Obesity/Overweight	45	5.94	0.0712*
	Proper weight/Underweight	35	5.34	

BMI, body mass index.
**P* value Student's *t*-test unpaired
***P* value Mann–Whitney *U*-test

Table 3 Comparisons of the concentrations of inflammatory peptides in the umbilical cord blood between the groups of mothers with obesity/overweight (group 1) vs. appropriate body weight/underweight (group 2). TNF-α, MCP-1, MCP-2, TGF-1β (pg mL^{-1}). Mann–Whitney *U*-Test

Variable	Group	*n*	Mean	Standard deviation	Minimum	Median	Maximum	*P*
TNF-α	Obesity/Overweight	54	4.26	6.07	0.83	2.52	43.37	0.0096
	Proper weight/Underweight	48	2.11	1.10	0.25	2.18	4.45	
MCP-1	Obesity/Overweight	54	94.86	71.93	21.13	79.28	431.04	0.4051
	Proper weight/Underweight	50	111.62	102.63	22.12	84.12	642.65	
MCP-2	Obesity/Overweight	54	25.57	11.07	6.54	24.90	71.65	0.3079
	Proper weight/Underweight	50	28.00	11.07	7.76	25.35	62.71	
TGF-1β	Obesity/Overweight	50	179.32	132.20	13.26	150.23	743.69	0.2939
	Proper weight/Underweight	48	200.09	120.22	22.22	178.65	429.84	

MCP-1, monocyte chemoattractant protein-1; MCP-2, monocyte chemoattractant protein-2; TGF-1β, transforming growth factor-1 beta; TNF-α, tumor necrosis factor-alpha.

Table 4 Correlations between TNF-α, MCP-1, MCP-2 and TGF-1β in umbilical cord blood vs. anthropometric parameters of mothers and babies at birth and at age 9 months. Spearman correlation coefficient, *P* value and *n*

	TNF-α	MCP-1	MCP-2	TGF-1β
Mother's BMI	C 0.2440	−0.0584	−0.0714	−0.0002
	P 0.0135	0.5560	0.4713	0.9982
	n 102	104	104	98
Mother's weight	C 0.2038	−0.0892	−0.0948	−0.0473
	P 0.0399	0.3679	0.3385	0.6441
	n 102	104	104	98
Baby's birth weight	C 0.1133	−0.1299	0.1300	−0.3077
	P 03232	0.2507	0.2503	0.0072
	n 78	80	80	75
Baby's weight at 9 months	C 0.1800	−0.0543	0.0421	0.1343
	P 0.1148	0.6325	0.7106	0.2506
	n 78	80	80	75
Baby's BMI at 9 months	C 0.1190	−0.0377	−0.0392	0.0957
	P 0.3126	0.7465	0.7366	0.4273
	n 74	76	76	71
Weight difference (9 months vs. birth)	C 0.1620	−0.0319	−0.0028	0.2086
	P 0.1565	0.7789	0.9805	0.0725
	n 78	80	80	75

BMI, body mass index; C, Spearman correlation coefficient; MCP-1, monocyte chemoattractant protein-1; MCP-2, monocyte chemoattractant protein-2; *n*, number; *P*, *P* value; TGF-1β, transforming growth factor-1 beta; TNF-α, tumor necrosis factor-alpha.

positive correlation between TNF-α and placental adiposity. In another study, Challier and colleagues (19) showed an increased concentration of TNF-α in the placenta of newborns from mothers with obesity compared with lean mothers. However, there was no correlation between inflammatory markers in cord blood and anthropometric parameters of the newborns. Finally, placental TNF-α levels correlated positively with maternal adiposity in a cohort of 60 mothers (20). Similarly to the present data, there was no correlation between placental TNF-α levels and newborn body weight at birth (20).

No previous studies have measured the concentration of TGF-1β in umbilical cord blood in children of mothers with obesity. In our study, we found an inverse correlation between the weight of the baby at birth and TGF-1β as well as a tendency towards a positive correlation between TGF-1β and body weight gain during the first 9 months of life. As babies born with low body weight tend to gain more weight in the first months of life (21), it is possible that this positive correlation between TGF-1β and body weight gain is a simple reflex of this phenomenon. Nevertheless, it is important to emphasize that the findings regarding TGF-1β are novel, and further studies should focus on its potential role as a marker of body mass variability during early life.

Finally, we detected no correlation between the chemokines MCP-1 and MCP-2 and the anthropometric parameters evaluated in the present study.

Our data confirms that the body weight of mothers positively impacts the concentration of TNF-α in the cord blood. Despite the fact that inflammatory peptide levels in the cord blood had no impact on baby body weight at birth or at age 9 months, it is important to consider that early exposition to TNF-α may affect metabolic and inflammatory parameters later in life.

Conflict of interest statement

No conflict of interest was declared.

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