Influence of family history of type 2 diabetes on leptin concentration in cord blood of male offspring with high birth weight

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Summary. To verify whether a diabetes family history might be a risk factor for the development, in adult age, of metabolic disorders, leptin, anthropometric and endocrine parameters were analysed in 95 babies with grandparents affected by type 2 diabetes (DF) and in 95 matched babies without diabetes family history (NDF). A sexual dimorphism for leptin was present in the NDF group (males: 6.7±4.1 ng/ml; females: 12.3±6.5; $p < 0.0001$) but not in the DF group (males: 9.0±5.5; females: 10.8±6.4), due to the significant increase in DF male leptin level, compared to that of NDF males ($p < 0.05$). In DF males only, leptin was positively correlated with body length, PI, C-peptide, IGF-1 and IGF1BP$_3$. These results suggest that the increase in DF male leptin could be a compensatory mechanism for reduced insulin sensitivity in a pre-clinical alteration of glucose metabolism.

Key words: family history, diabetes mellitus type 2, fetal blood leptin, male newborn, birth weight.

Riassunto (*Influenza della familiarità diabetica non insulino dipendente sulla concentrazione della leptina nel cordone ombelicale di neonati maschi con alto peso alla nascita*). Per verificare l'ipotesi che la familiarità diabetica possa essere un fattore di rischio per lo sviluppo, in età adulta, di disordini metabolici, sono stati valutati la leptina ed altri parametri antropometrici ed endocrini in 95 neonati con nonni affetti da diabete tipo 2 (DF), confrontati con altrettanti bambini senza familiarità diabetica (NDF). Un dimorfismo sessuale per la leptina era presente nel gruppo NDF (maschi: 6,7±4,1 ng/ml; femmine: 12,3±6,5; $p < 0,0001$) ma non nel gruppo DF (maschi: 9,0±5,5; femmine: 10,8±6,4), dovuto all'aumento significativo nel livello di leptina dei maschi DF, confrontato con quello dei maschi NDF ($p < 0,05$). Solo nei maschi DF, la leptina si correlava positivamente con la lunghezza, il PI, il C-peptide, IGF-1 ed IGF1BP$_3$. Questi risultati indicano che l'aumento della leptina nei maschi DF potrebbe essere un meccanismo compensativo per una ridotta sensibilità all'insulina, in un'alterazione pre-clinica del metabolismo del glucosio.

Parole chiave: storia familiare di diabete tipo 2, leptina nel sangue ombelicale, neonati maschi, peso alla nascita.

INTRODUCTION

The mechanisms by which fetal/neonatal weight is regulated during human pregnancy and in early postnatal life are poorly understood. Regulation of fetal growth is complex and multifactorial. Diverse factors, including intrinsic fetal conditions as well as maternal and environmental factors, lead to aberrant intrauterine growth. An interaction between placental and fetal endocrine factors is likely to govern partitioning of nutrients and the rate of fetal cell proliferation and maturation.

Several serum proteins and hormones have been strongly related to fetal growth [1, 2]. Insulin is believed to have an important growth-promoting function in uterus [1-4], but in some well control-led diabetic women in whom fetal insulin levels are presumably normal, fetal size is nevertheless excessive [3, 5, 6]. Therefore, other factors might explain abnormal fetal growth. For example, insulin like growth factors (IGFs) and their binding proteins (IGFBPs) have been shown to play important roles in mediating fetal and postnatal growth and development [1, 3, 7, 8].

Also leptin, a 16-kDa hormone secreted by adipocytes and placenta that plays a key role in the regulation of body fat mass and weight in the adult life [4-6, 9], has recently been positively associated with intrauterine growth [1, 2]. Moreover, some peripheral actions of leptin have been recently investigated. One of these was the regulation of insulin

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secretion by leptin direct action on pancreatic β-islet, suggesting a positive interaction between leptin and insulin [3-5].

A number of studies have reported the presence of correlation between cord leptin and body mass index and birth weight [10-13].

Moreover in normal pregnancy a sexual dimorphism for leptin is present with higher values in newborn females then newborn boys [9, 10, 13-15].

However, in the offspring of type 1 diabetic and gestational diabetic mother versus control subjects, Persson *et al.* [16] and Maffei *et al.* [17] have reported cord leptin levels above the upper normal range, without gender differences.

Kostalova *et al.* [5] have suggested that increased plasma leptin levels in the offspring of diabetic mother could also represent a compensatory mechanism in a pre-clinical disturbance of glucose metabolism. This possibility is supported by the analysis of a healthy population, which revealed a negative correlation between plasma leptin and values of insulin sensitivity [9]. Vauhkon *et al.* [6] have reported that an insulin-resistant phenotype is associated with high serum leptin levels in the offspring of patients with type 2 diabetes. These authors have found defective insulin secretion in adult non-diabetic offspring of patients with type 2 diabetes mellitus and believe that latent autoimmune diabetes mellitus is a familial disease involving gene defects leading to progressive beta-cell destruction [18].

In an attempt to verify the hypothesis that even a family history of diabetes could be a risk factor for the development, in adult age, of metabolic disorders [19], newborns with grandparents affected by type 2 diabetes have been studied. These infants were matched with an equal number of newborns homogeneous for sex, weight and gestational age, without family history of diabetes.

METHODS

A group of 95 newborns with family history of diabetes (DF) and 95 newborns without family history of diabetes (NDF) were selected from a series of 747 consecutive deliveries by healthy women at the Civil Hospital of Urbino, Italy, from January 2000 to February 2001. At the moment of birth, questionnaires on matters concerning diabetes family history, previous pregnancies and possible complications during the pregnancy, use of medicines, food habits and tobacco use were compiled by all the mothers, who also signed a consent form.

Routine measurements of birth weight and length were made and, immediately after delivery, blood samples from umbilical cord were collected in heparinised syringes. The samples were stored at 4°C for up to 2 hours before centrifugation at 3000 rpm for 15 minutes. Cord plasma was stored at -80°C, until analyzed. All samples were sent, in ice dry, to the Italian National Institute of Health (Rome, Italy) for analysis.

Anthropometric measurements

Newborns were categorized as appropriate for gestational age (AGA, birth weight between 10th and 90th percentile), small for gestational age (SGA, birth weight lower than 10th percentile) and large for gestational age (LGA, birth weight more than 90th percentile). Newborn body mass index (BMI) was defined as weight (in kilograms) divided by length (in meters squared); ponderal index (PI) was calculated as birth weight divided by the cubed value of birth length ($100\times$ g/cm^3) and was used as an index of nutrition status for newborns.

Biochemical analysis

Total leptin concentrations in cord plasma were measured by radioimmunoassay using a commercially iodine-125-labeled human leptin radioimmunoassay kit (Linco Research Co, St. Charles, MO). Analyses were done in duplicate. Sensitivity was less than 0.5 ng/ml, inter-assay coefficient of variation was 4.5% and intra-assay coefficient of variation was less than 4.0%. A standard curve was generated with human leptin and fitted with an interactive non-linear curve-fitting program.

Levels of IGF1 and IGF1BP$_3$ were measured in cord plasma using a procedure employing two immuno-radiometric assay kits (IRMA) (Diagnostic Systems Laboratories, Inc, Webster, TX, and USA). Sensitivity was 0.8 ng/ml and 0.5 ng/ml, inter-assay coefficient of variation was 2.6% and 3.0%, and intra-assay coefficient of variation was 2.5% and 1% respectively.

Cord plasma levels of C-peptide were measured by a specific radioimmunoassay kit (Biochem ImmunoSystem Italia S.p.A, Bo, Italy). Sensitivity was < 0.1 ng/ml, inter-assay coefficient of variation was 4.6%, and intra-assay coefficient of variation was 3.3%.

Statistical analyses

Values were expressed as mean ± standard deviation (m ± SD). The Welch's approximate *t* test was used for comparison of anthropometric data and hormone levels between the two groups. Fisher's exact test was used for evaluating association between leptin levels and diabetes familiarity. The Pearson's correlation analysis was used to assess the relations between anthropometric parameters and cord plasma substrates in study subjects (DF group) and control subjects (NDF group). Statistical significance was assumed at p < 0.05. All data analysis was done using the SPSS, Inc. (Chicago, IL) 8.0 software for Windows.

RESULTS

The 190 newborns included in the study (101 males and 89 females) were born full-term (mean gestational age, 39.9 ± 1.0 weeks). Mean maternal age was 29.1 ± 5.3 yrs for both groups and cesarean delivery occurred in 34.2% of the study subjects

Table 1 | *Anthropometric parameters of the 190 newborns to term, divided in two groups with diabetic family history (Group DF) and without diabetic family history (Group NDF) (All: A) and sub-groups subjects higher to fifty percentile of gestational age (GA > 50th: B)*

| | SEX | Group DF | | Group NDF | | p values* | | | |
		Males	Females	Males	Females	Group DF Intra**	Males Inter***	Group NDF intra	Females inter
	All n.	50	45	51	44				
A	Weight (kg±SD)	3.469±0.5	3.206±0.4	3.481±0.51	3.288±0.3	< 0.01	ns	ns	ns
	Length (cm±SD)	50.78±1.6	50.04±1.4	50.19±1.5	49.98±0.9	< 0.05	ns	ns	ns
	PI (kg±SD)	2.64±0.2	2.55±0.2	2.74±0.2	2.63±0.2	n.s	< 0.05	ns	ns
	BMI (kg /m^2±SD)	13.42±1.4	12.76±1.2	13.77±1.6	13.16±1.2	< 0.05	ns	< 0.05	ns
	GA >50th n.	33	24	33	24				
B	Weight (kg±SD)	3.743±0.3	3.520±0.2	3.753±0.4	3.524±0.2	< 0.01	ns	< 0.05	ns
	Length (cm±SD)	51.52±1.3	50.92±1.2	50.73±1.4	50.12±1.0	< 0.05	< 0.05	ns	< 0.05
	PI (kg±SD)	2.71±0.2	2.67±0.2	2.87±0.2	2.80±0.2	ns	< 0.01	ns	< 0.05
	BMI (kg /m^2±SD)	14.00±1.1	13.58±0.8	14.57±1.4	14.03±0.8	ns	ns	ns	< 0.05

*Significance levels (Welch's approximate t test)
**Differences between sexes
***Differences between groups

and in 24.8% in the control subjects. The distribution of vaginal deliveries and cesarean sections was not significantly different between male and female newborns in both groups. Both parents of DF and NDF newborns did not accuse any sort of pathology.

Among all newborns selected, 76 infants with birth weight like or below the 50th percentile (8 small for gestational age only) were placed in the group GA ≤ 50th and 114 with birth weight above 50th percentile (of which only 24 large for gestational age, 11 LGA in DF group and 13 LGA in NDF group) were placed in the group GA > 50th.

In order to verify whether the family history of type 2 diabetes is an independent risk factor for high leptin values in the male babies, the Fisher's exact test was applied to all the newborn males taking into consideration leptin reference values obtained from 225 healthy male babies. The test has revealed that 32% of the DF male babies had significantly higher leptin values against 18% of the male NDF babies (p = 0.003; relative risk = 1.90). The DF female babies did not shown any difference with respect to NDF female babies (23% *vs* 29% respectively; p = 0.56; relative risk = 0.7)

No significant differences in the analyzed parameters were found between the DF group and the control group with birth weight below 50th percentile (data not shown).

Anthropometric measurements

Mean growth parameters were on the 50th percentile. For the DF group and NDF group the birth weight (BW) was 3.345 ± 0.47 and 3.393 ± 0.44 kg, the length was 50.43 ± 1.55 and 50.10 ± 1.26 cm, the body mass index (BMI) was 13.11 ± 1.32 and

13.49 ± 1.47 kg/m^2, and the ponderal index (PI) was 2.60 ± 0.2 and 2.69 ± 0.3, respectively.

When males and females were analyzed separately, significant differences for weight and length were found in the DF group only *(Table 1A)*.

The levels of ponderal index were statistically lower in males DF compared to the correspondent control group (PI: 2.64 ± 0.2 *vs* 2.74 ± 0.2; p < 0.05 respectively). This difference was significant not only in the DF boys but also in the DF girls with GA > 50th in respect to control groups *(Table 1B)*.

Biochemical analysis

DF males and females did not show sexual dimorphism for leptin (9.00 ± 5.5 ng/ml *vs* 10.84 ± 6.4 ng/ml) differently from what was found in the NDF group (6.68 ± 4.1 ng/ml *vs* 12.35 ± 6.5ng/ml, p < 0.0001, respectively) *(Table 2A)*.

The concentration of the leptin was more elevated in the females in both groups. In males with diabetes family history, leptin concentration was significantly higher (p < 0.05) in respect to NDF males. This difference was exclusively related to DF boys in the GA > 50th group *(Table 2B)*.

The complex IGFs showed a very significant difference among sexes, with prevalence for the DF girls in the GA > 50th group only.

IGF1BP3 concentration was significantly lower (p < 0.05) in DF boys in comparison with NDF boys, in the GA > 50th group.

The C-peptide concentrations were not statistically different in all groups and sexes.

Correlation

In DF newborns there was not correlation between sex and leptin, but leptin was significantly

Table 2 | *Hormonal concentrations of the 190 newborns to term, divided in two groups with diabetic family history (Group DF) and without diabetic family history (Group NDF) (All: A) and sub-groups subjects higher to fifty percentile of gestational age (GA>50th: B)*

			Group DF		Group NDF		p values*			
			Males	**Females**	**Males**	**Females**	**Group DF**	**Males**	**Group NDF**	**Females**
	All	**n.**	**50**	**45**	**51**	**44**	**Intra****	**Inter****	**intra**	**inter**
A	C-PEP (ng/ml ±SD)		1.28±0.7	1.48±0.8	1.24±0.61	1.59±0.7	ns	ns	ns	ns
	Lep (ng/ml ±SD)		9.00±5.5	10.84±6.4	6.68±4.1	12.35±6.5	n.s	< 0.05	< 0.0001	ns
	IGF1 (ng/ml ±SD)		38.26±36.2	62.21±46.2	36.67±30.9	57.84±33.6	< 0.01	n.s	< 0.005	ns
	IGF1BP$_3$ (ng/ml ±SD)		960.51±151.0	1109.47±227.7	1007.52±234.8	1116.21±265.9	< 0.001	n.s	< 0.05	ns
B	GA >50th	n.	33	24	33	24				
	C-PEP (ng/ml ±SD)		1.37±0.7	1.61±0.7	1.24±0.6	1.51±0.6	ns	n.s	ns	ns
	Lep (ng/ml ±SD)		10.74±5.7	12.90±7.2	7.93±4.5	12.8±4.9	ns	< 0.05	< 0.001	ns
	IGF1 (ng/ml ±SD)		46.78±40.4	83.54±45.4	42.87±34.1	68.73±35.3	< 0.005	ns	< 0.01	ns
	IGF1BP$_3$ (ng/ml ±SD)		975.57±151.4	1171.91±222.4	1072.23±220.4	1163.17±286.5	< 0.001	< 0.05	ns	ns

*Significance levels (Welch's approximate t test)
**Differences between sexes
***Differences between groups

correlated with: weight, length, PI, C-peptide, IGF1 and IGF1BP$_3$ *(Table 3)*. These very significant correlations were found only in the DF males in the GA > 50th subgroups *(Table 4B)*.

Moreover, in the group of DF offspring, in addition to leptin, the biochemical parameters C-peptide, IGF1 and IGF1BP$_3$, involved in the fetal increase, were significantly correlated among them and were also correlated with the anthropometric parameters *(Table 3)*.

DISCUSSION

This study has shown that in males and females with diabetes familiarity (DF group), the sexual dimorphism for leptin, present in newborn of both sexes without diabetes familiarity (NDF group) [9, 10, 13-15], is abolished. Moreover in DF group leptin was significantly correlated, not only with anthropometric data, but also with ponderal index, C-peptide, IGF1 and IGF1BP$_3$. In addition, the biochemical parameters, involved in the fetal increase,

Table 3 | *Univariate correlation among anthropometric data and serum hormones in relatives (Group DF) and control (Group NDF)*

Group DF (n = 95)	**Sex**	**Weigh**	**Length**	**PI**	**BMI**	**C-peptide**	**Lep**	**IGF1**
Weight	**-0.282***							
Length	*-0.238°*	**0.809**						
PI	*-0.199*	**0.770**	*0.258*					
BMI	*-0.252*	**0.925**	**0.530**	**0.950**				
C-peptide	0.131	*0.235*	0.089	**0.276**	**0.277**			
Lep	0.154	**0.391**	*0.255*	**0.369**	**0.407**	**0.342**		
IGF1	**0.281**	**0.329**	0.193	**0.327**	**0.348**	**0.389**	**0.262**	
IGF1BP$_3$	**0.366**	**0.240**	0180	0.192	*0.229*	**0.270**	*0.200*	**0.569**
Group NDF (n. = 95)								
Weight	*-0.216*							
Length	-0.086	**0.609**						
PI	0.196	**0.818**	0.048					
BMI	*-0.210*	**0.929**	**0.274**	**0.971**				
C-peptide	**0.264**	-0.086	-0.013	-0.102	-0.102			
Lep	**0.471**	0.178	0.090	0.172	0.178	*0.207*		
IGF1	**0.314**	*0.209*	0.138	0.175	0.189	*0.246*	*0.253*	
IGF1BP$_3$	*0.236*	*0.214*	0.067	*0.229*	*0.232*	0.066	*0.215*	**0.520**

*P < 0.001 (boldface numbers)
°P < 0.05 (italic numbers)

Table 4 | *The coefficient correlations r and P values of leptin versus growth and biochemical parameters in all (A) girls and boys from DF and NDF subjects and subgroups GA >50th (B)*

		Boys				Girls			
		Group DF		Group NDF		Group DF		Group NDF	
	All	50		51		45		44	
		r	p	r	p	r	p	r	p
A	Length	0.389	0.005	0.306	0.03	0.219	n.s	-0.009	ns
	PI	0.400	0.004	0.317	0.02	0.446	0.002	0.367	< 0.05
	C-peptide	0.492	< 0.001	0.012	ns	0.183	ns	0.156	ns
	IGF1	0.468	< 0.001	0.168	ns	0.054	ns	0.100	ns
	$IGF1BP_3$	0.512	0.0001	0.224	ns	-0.069	ns	0.050	ns
	$GA>50^{th}$	33		33		24		24	
		r	p	r	p	r	p	r	p
B	Length	0.102	n.s	0.145	ns	-0.068	ns	0.121	ns
	PI	0.382	0.03	0.091	ns	0.260	ns	0.441	0.03
	C-peptide	0.499	< 0.005	0.041	ns	0.302	ns	-0.113	ns
	IGF1	0.403	< 0.05	0.099	ns	-0.071	ns	-0.338	ns
	$IGF1BP_3$	0.591	< 0.001	0.182	ns	-0.111	ns	-0.358	ns

were significantly correlated among them and were also correlated with the anthropometric parameters. However, the biochemical and anthropometric parameters were significantly correlated with leptin only in newborn DF males, with birth weight above 50th percentile. In these babies, $IGFBP_3$ and ponderal index values were significantly lower than control, whereas the leptin concentration was significantly higher. Our data confirm the result obtained by Persson *et al.* [16] who have reported cord leptin levels above the upper normal range in the offspring of type 1 diabetic and gestational diabetic mother versus control subjects, without gender differences.

Furthermore, the $IGFBP_3$ and ponderal index values lower than normal suggest that there has been a nutritive suffering during the pregnancy [22, 23] with adipose tissue accumulation to the detriment of the muscular tissue, due, probably, to a genetic susceptibility to the insulin insensibility [12]. Thus it was recently established [3-5], a positive interaction between leptin and insulin (the adipoinsular axis hypothesis). This interaction is designed to maintain nutrient balance and the dysregulation of adipoinsular axis may contribute to obesity and the development of hyperinsulinemia associated with diabetes [20, 21]. Recently [25] it has been shown intergenerational associations between type 2 diabetes in one generation and birth weight in the subsequent two generations. Therefore, the increased plasma leptin in the newborn males with diabetes family history, could probably represent a compensatory mechanism in a preclinical disturbance of glucose metabolism [6, 26].

Although the parental obesity is a factor of risk for the development of hiperleptinemia in all newborns [27], this parameter has not been taken into consideration in this work, since all newborns parents did not have any sort of pathology.

The hypothesis is confirmed by the work of Jansson *et al.* [28] who found that the male subjects, which are genetically predisposed for type 2 diabetes, display several endocrine abnormalities, including that of leptin hormone. Moreover other authors reported that children with family history of type 2 of diabetes manifest evidence of insulin resistance early in the first decade of life [29] or are less insulin sensitive compared with a control group of subjects without familial history of type 2 diabetes [30].

In conclusion, since our work has demonstrated that newborn with grandparents affected by diabetes mellitus have increased plasma levels of leptin, independently from other anthropometric factors, and since this hormone is involved in the regulation of insulin secretion, we could formulate the hypothesis that the hormone increase occurs as a compensatory mechanism for reduced insulin sensivity, due to a dysregulation of adipoinsular axis.

A follow up study involving a selected number of original group DF and NDF males studied for some years after birth, will probably answer the question. New information could also be gained about the influence of genetic background on the risk of the obesity and/or diabetes in male offspring, with high birth weight and family history of diabetes mellitus.

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