Abundance of Circulating Preadipocyte Factor 1 in Early Life

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OBJECTIVE—Soluble preadipocyte factor 1 (Pref-1) inhibits adipocyte differentiation. We tested whether circulating levels of soluble Pref-1 are higher in smaller fetuses.

RESEARCH DESIGN AND METHODS—We performed longitudinal assessments of circulating Pref-1 in infants born appropriate for gestational age (AGA) or small for gestational age (SGA) and also in late-gestational women and in newborns on days 2 and 3.

RESULTS—At birth, Pref-1 levels were ~100-fold higher than in adults, being in SGA fetuses ~50% higher than in AGA fetuses. By age 4 months, Pref-1 had reached near-adult levels and the original AGA versus SGA difference had disappeared. Pref-1 levels were low in late-gestational women and were still elevated in newborns.

CONCLUSIONS—Pref-1 is abundantly present in the fetus, is higher in SGA than in AGA fetuses, and is likely to be of fetal origin. We speculate that Pref-1 in early life contributes to variation in postnatal adipocyte numbers, in the subsequent expandability of adipose tissue, and thus in the susceptibility to diabetes in later life.

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The number of subcutaneous adipocytes wherein fat can be stored during adulthood is essentially determined in two windows of adipogenesis, namely, fetal life and puberty (1,2). For example, in monozygotic twins discordant for growth before birth, the smaller twin continues to have a lower number of subcutaneous adipocytes after birth (3). It is still poorly understood how a transient restraint of fetal growth can be linked to a persistent lowering of adipocyte number. We propounded that one of the potential links is a downregulation of adipogenesis in a critical window of growth restraint before birth. In a first test of this concept, we studied at birth whether circulating preadipocyte factor 1 (Pref-1) is higher in smaller fetuses. Pref-1 is a transmembrane protein that is encoded by an imprinted (paternally expressed) gene on chromosome 14q32 and that contains an extracellular domain with epidermal growth factor–like repeats; juxtamembrane cleavage generates a soluble 50-kDa form, which is the Pref-1 form that inhibits adipocyte differentiation by upregulating Sox9 in preadipocytes (4,5). Thus far, the circulating levels of Pref-1 have not been studied in human fetuses, newborns, infants, or pregnant women.

RESEARCH DESIGN AND METHODS

—Pref-1 was measured longitudinally (at birth and at 4 months) in serum from 72 infants (42 born appropriate for gestational age [AGA] [19 girls and 23 boys] and 30 born small for gestational age [SGA] [16 girls and 14 boys]) who had been recruited into a study that was initiated in 2007 and that assesses longitudinally the endocrine-metabolic state and the body composition of SGA infants compared with breastfed AGA control subjects across the first postnatal years (6,7). For the present substudy focusing on Pref-1, specific inclusion criteria were as follows: *1*) birth at Hospital Sant Joan de Déu after an uncomplicated, term (38–40 weeks), and singleton pregnancy (no maternal hypertension and no gestational diabetes); *2*) birth weight between 3.0 and 3.8 kg for AGA control subjects (birth weight range between −1 and +1 SD), and birth weight <2.6 kg for SGA infants (below −2 SD); *3*) cord serum available to measure Pref-1; and *4*) written informed consent in the Catalan language. Specific exclusion criteria were complications at birth (need for resuscitation or for parenteral nutrition), congenital malformations, or an extremely low birth weight (below −3.0 SD).

Gestational age was calculated according to the last menses and confirmed by first-trimester ultrasound. The prevalence of delivery by caesarean section was 12%. A total of 17 mothers smoked during pregnancy; they delivered 7 AGA and 10 SGA infants.

Pref-1 was also measured in 11 healthy AGA newborns (mean birth weight 3.3 kg) sampled on postnatal day 2 or 3 (mean age 44 h [range 30–57]) and from 18 women in late gestation (mean 35 weeks [32–38]) who subsequently delivered healthy infants (mean birth weight 3.3 kg [2.7–4.0]) after uncomplicated singleton pregnancies (mean duration 39 weeks [37–41]).

Body weight was measured at birth with a beam balance (Seca, Hamburg, Germany). Blood from the longitudinally studied infants was sampled at birth (from the umbilical cord before placental separation) and in the prefeeding state at age 4 months. All samples were centrifuged and frozen at −80°C until analysis. Body composition was assessed by absorptiometry at age 2 weeks (mean ± SEM 17 ± 1 days) with a Lunar Prodigy, coupled to Lunar software (version 3.4/3.5; Lunar, Madison, WI) adapted for infants (6,7). Soluble 50-kDa Pref-1 was assessed by ELISA (R&D Systems, Minneapolis, MN) (intra- and interassay coefficients of variation 3.6 and 6.2%, respectively; detection limit 0.01 μg/L).

Statistical analyses were performed using SPSS 12.0 (SPSS, Chicago, IL). Results are expressed as means ± SEM. Comparisons between groups were performed by *t* test. Skewed data were log transformed prior to comparison. $P < 0.05$ was considered significant.

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Table 1—*Results from AGA versus SGA infants*

	AGA	SGA*	*P***
N (% female)	42 (45)	30 (53)	
At birth			
Weight (kg)	3.4 ± 0.1	2.4 ± 0.1	(<0.0001)
Weight *Z* score	0.0 ± 0.1	−2.2 ± 0.1	(<0.0001)
Pref-1 (μg/L)	19 ± 1	27 ± 3	0.004
Lean plus fat plus bone (kg)#	4.0 ± 0.1	3.2 ± 0.1	<0.0001
Lean mass (kg)#	3.0 ± 0.1	2.5 ± 0.1	<0.0001
Fat mass (kg)#	0.88 ± 0.04	0.53 ± 0.05	<0.0001
Bone mineral content (kg)#	0.11 ± 0.00	0.09 ± 0.00	<0.0001
At age 4 months			
Pref-1 (μg/L)	1.3 ± 0.1	1.3 ± 0.1	NS
Lean plus fat plus bone (kg)	7.5 ± 0.2	6.6 ± 0.2	0.003
Lean mass (kg)	4.3 ± 0.1	4.0 ± 0.1	NS
Fat mass (kg)	3.0 ± 0.1	2.5 ± 0.1	0.001
Bone mineral content (kg)	0.20 ± 0.01	0.18 ± 0.01	0.002

Data are means ± SEM unless otherwise indicated. ***P* values are for comparisons between AGA and SGA infants and are adjusted for sex and maternal smoking. #Absorptiometry was performed at age 2 weeks. *All results were comparable in breastfed (*N* = 16) and formula-fed (*N* = 14) SGA infants. All AGA control subjects were breastfed (see RESEARCH DESIGN AND METHODS).

All assessments were approved by the institutional review board of Barcelona University Hospital. Written (parental) consent was an inclusion criterion.

RESULTS—Pref-1 levels in late-gestational women were 0.7 ± 0.1 μg/L (range 0.02–1.6), which were comparable with those reported in nonpregnant women (8). Circulating Pref-1 was readily detectable in all infants. Pref-1 levels were comparable in girls and boys, and the results of both sexes were therefore pooled (Table 1).

At birth, Pref-1 levels ranged from 6 to 100 μg/L and were thus one to two orders of magnitude higher than in the maternal circulation. Pref-1 levels were ~50% higher in SGA than in AGA fetuses. By age 4 months, Pref-1 concentrations had reached a near-adult level and the original AGA versus SGA difference in Pref-1 levels had disappeared. No close associations were observed between Pref-1 concentrations and body composition either at birth or at age 4 months. On day 2 or 3, serum concentrations of Pref-1 in AGA newborns were still 14 ± 2 μg/L (range 8–26), suggesting that prenatal Pref-1 has a fetal rather than a placental or maternal origin.

CONCLUSIONS—In the first months after birth, SGA infants prioritize the recovery of lean mass to that of fat mass and of bone mineral content. Soluble Pref-1 is thought to reduce adipogenesis as well as bone formation by inhibiting the differentiation of multipotent mesenchymal cells into adipocytes, chondrocytes, and osteoblasts (5,9,10). Here, we show that Pref-1 is abundantly present in the circulation of the human fetus and that SGA fetuses have higher Pref-1 levels than do AGA control subjects.

Growth restraint in early life is a major risk factor for diabetes in later life (11,12). Our finding that fetal growth restraint is associated with markedly high Pref-1 levels suggests that Pref-1 may be among the mediators of a reduced adipocyte differentiation in growth-restrained fetuses, and thus of a reduction in their life-long lipid-storage capacity and also of their adult vulnerability to metabolic disease, once lipid storage becomes an issue.

In conclusion, Pref-1 is abundantly present in the circulation of the human fetus and is likely to be of fetal rather than maternal or placental origin. Circulating Pref-1 levels are higher in SGA than in AGA fetuses.

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