High-sensitivity c-reactive protein concentration in young adults of the Helsinki Study of Very Low Birth Weight Adults

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Short Title: Very low birth weight adult and c-reactive protein

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Low birth weight and chronic low-grade inflammation have been significantly associated with an increased risk of cardiovascular disease (CVD) (1,2). C-reactive protein (CRP) is a highly sensitive biomarker used to detect inflammation associated with predictive factors related to CVD, such as atherogenesis or atherosclerosis (3). Adults born preterm at a very low birth weight (VLBW) of less than 1,500 g constitute the low end of the low birth weight spectrum and have increased levels of CVD risk factors (4). Follow-up population studies have reported higher CRP concentrations in low birth weight children and adults (5–7). However, the effect of VLBW on CRP in young adulthood can be further explored. The present analysis examined the effect of VLBW on CRP concentration in a cohort of VLBW and control subjects and considered whether conditions that occurred prenatally, at birth or during later life contributed to inflammation in young adulthood.

The Helsinki Study of Very Low Birth Weight Adults included VLBW participants born between January 1978 and December 1985 who were treated at the Children's Hospital at Helsinki University Central Hospital in Uusimaa, Finland (8). Recruitment methods, collection of clinical measurements, assays, cohort characteristics and exercise data had been previously described (9,10). This study and its protocols conform to the Declaration of Helsinki and were approved by institutional ethics committees, and written informed consent was provided by each participant. The analysis included 329 subjects with birth, adult and exercise data. High-sensitivity CRP was measured by photometric immunochemical assay using a 917 Analyzer (Hitachi Ltd, Liaoning, China.; Ultrasensitive CRP Kit, Orion Diagnostica, Espoo, Finland), with a detection limit of 0.05mg/L, from plasma samples collected during clinical follow-up. CRP concentration above 10 mg/L may indicate acute inflammation, and nine subjects were excluded from the analyses (11). A restricted analysis examined the effect of pre-eclampsia and whole body fat percentage (WBFP), assessed by DEXA (Discovery A, Hologic, Massachusetts, USA), was performed using a sub-sample of 284 subjects with 149 VLBW and 135 term-born control.

Statistical analyses were performed using Statistical Package for Social Sciences (SPSS, version 23, Chicago, IL). VLBW were compared with term-born controls. Birth weight standard deviation scores were calculated using Finnish growth standards (12). Continuous CRP was non-normally distributed and

$\log_{10}$ base transformed values were used in the analyses. Subjects were categorized as having low (<1.0 mg/L), moderate (1.0-3.0 mg/L) or high (>3.0 mg/L) CVD risk based on CRP measurements following recommendations by the American Heart Association and Center for Disease Control and Prevention (13). Upper limit for normal systolic blood pressure was 120 mmHg. Unadjusted comparisons were made using Student's t-test for continuous measures and Pearson's chi-square test or Fisher's exact test for proportions. Adult CRP was fitted to multivariable linear regression models. A basic model considered the effects of VLBW and sex on adult CRP. A second series of models, Model 2, separately considered the effects of prenatal and adult characteristics on CRP whilst controlling for VLBW and sex (Table 1). A third series of models, Model 3, separately examined the added contributions of pre-eclampsia and WBFP to adult CRP. Significance level for all tests were set at $p<0.05$.

Prenatal, birth and adult characteristics were described in Table 1. Subjects were 21.9 years of age at follow-up ($p=0.84$). In adulthood, VLBW subjects were lighter ($p<0.001$) than control subjects. Control subjects had a higher BMI score than VLBW, by 1.1kg/m^2 ($p=0.003$), although their WBFP were similar ($p=0.37$) (Table 1) (9). VLBW subjects engaged in less intense forms of exercises and exercised for shorter time periods than control subjects (Table 1). We did not find a significant difference ($p=0.16$) between the CRP concentration of VLBW and control subjects, whose median CRP values were 4.70 mg/L and 5.10 mg/L, respectively. No correlation was found between CRP and birth weight in grams ($r=0.05$, $p=0.35$). Further, there were no significant differences in the proportion of VLBW and control subjects with low, moderate and high CVD risk ($p=0.31$).

In examining the effect of VLBW, sex and prenatal characteristics, we found that males had a 37% lower CRP than females ($p<0.001$, 95% CI 23%-52%). An additional model further examined the contribution of fetal growth, by the inclusion of birth weight standard deviation scores with a mean value of -1.3 (95% CI -1.55-1.07), on adult CRP. This model was restricted to VLBW subjects and produced similar results. Secondly, we examined the associations between VLBW, sex and adult characteristics, and the results are described under Model 2 (Table 2). Birth weight was not a significant contributor to adult CRP. However, compared with subjects with normal BMI, underweight (<18.5 kg/m^2) subjects had

a 39% lower CRP (p=0.001, 95% CI 17%-61%) and overweight (≥25.0kg/m^2) subjects had 50% higher CRP (p<0.001, 95% CI 34%-65%). Subjects who exercised at least once a week had a 27% lower CRP than subjects who did not exercise at all (p=0.02, 95% CI 4%-49%), and adults who exercised by running briskly had a 21% lower CRP than subjects who walked as their primary form of exercise (p=0.03, 95% CI 2.6%-40%). In an analysis restrict to a sub-sample of 149 VLBW and 135 control subjects, the added effects of pre-eclampsia and WBFP to adult CRP was assessed. Although pre-eclampsia was not significantly associated with adult CRP, each WBFP unit increase in adulthood was associated with a 4% higher CRP concentration (p<0.001, 95% CI 2%-5%). In this model, sex, BMI, exercise frequency and exercise intensity were no longer significant contributors.

Although VLBW adults had lower CRP than control adults, this association was not statistically significant and suggested similar levels of inflammation in VLBW and control adults. We found that later life characteristics had significant effects on young adult CRP and inflammation. Specifically, higher WBFP was associated with higher CRP. This association between body fat and CRP was consistent with previously published works that describe the inflammatory processes related to adiposity (2,14,15). However, although not statistically significant, lower CRP in VLBW does not follow the trend of longitudinal population studies that reported higher CRP associated with lower birth weights (5,16,7). This difference may be related to study design, as our study focused on those born preterm at VLBW while population-based studies included the whole range of birth weights but included very few VLBW individuals by design. Future works may consider assessing VLBW and CRP in conjunction with other inflammatory biomarkers, searching for differences in developmental pathways, and variations in lifestyle and environment elements to further investigate the relationship between VLBW and adult inflammation.

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CONFLICTS OF INTEREST STATEMENT

The authors have no conflicts of interest to declare.

ABBREVIATIONS

CRP, C-reactive protein; CVD, Cardiovascular disease; VLBW, Very low birth weight; WBFP, Whole body fat percentage

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TABLES

Table 1. Birth and Adult Characteristics of the Helsinki Study of Very Low Birth Weight Adults

	VLBW (*n*=160)	Control (*n*=169)	*P*-value	Missing
A. Cohort Characteristics				
1. Birth				
Birth Weight (g)	1121.8 (1087.1 – 1056.6)	3591.6 (3519.9 – 3663.3)		
Gestational Age (weeks)*	29.2 (28.7 – 29.6)	40.1 (40.0 – 40.3)	***<0.001***	
2. Adult				
Age (years)†	21.9 (3.5)	21.9 (3.8)	0.84	
*Weight (kg)**	60.8 (59.0 – 62.8)	67.9 (66.1 – 69.7)	***<0.001***	
Females	57.2 (55.0 – 59.5)	63.2 (61.2 – 65.2)	***<0.001***	
Males	66.0 (63.0 – 69.1)	75.4 (72.7 – 78.1)	***<0.001***	
BMI ‡				
Underweight (<18.5)	23 (14.4)	8 (4.7)	***0.01***	
Normal (18.5-24.9)†§	105 (65.6)	118 (69.8)		
Overweight (≥25.0)	32 (20.0)	43 (25.4)		
*Whole Body Fat Percentage**	24.5 (23.2 – 25.9)	25.4 (24.0 – 26.8)	0.37	48
Females	29.4 (28.1-30.7)	30.1 (28.9 – 31.3)	0.43	
Males	17.9 (16.3 – 19.5)	18.1 (16.7 – 19.6)	0.81	
Systolic Blood Pressure *‡				
≤120 mmHg	143 (89.4)	158 (93.5)	0.18	
>120 mmHg	17 (10.6)	11 (6.5)		
Exercise Frequency‡			0.20	3
Not at all§	10 (6.4)	3 (1.8)		
Not even once a month	21 (13.4)	19 (11.2)		
Once or twice a month	20 (12.7)	16 (9.5)		
Once a week	31 (19.7)	32 (18.9)		
2-3 times/week	42 (26.8)	64 (37.9)		
4-5 times/week	18 (11.5)	18 (10.7)		
Daily	15 (9.6)	17 (10.1)		
Intensity of Exercise‡			***<0.001***	7
Walking§	47 (30.5)	19 (11.3)		
Walking or light running	44 (28.6)	45 (26.8)		
Light Running	42 (27.3)	49 (29.2)		
Brisk running	21 (13.6)	55 (32.7)		
Average Duration‡				6
<30 minutes§	26 (16.8)	9 (5.4)	***<0.001***	
30-60 minutes	58 (37.4)	41 (24.4)		
1-2 hours	66 (42.6)	103 (61.3)		
2 or more hours	5 (3.2)	15 (8.9)		
B. Prenatal Characteristics				
Pre-eclampsia‡	34 (22.8)	13 (8.2)	***<0.001***	22
Gestational Hypertension‡	7 (4.4)	25 (14.9)	***0.001***	2
Detected Hypertension‡			***0.001***	
None	107 (66.9)	139 (82.2)		
At Least One Parent	53 (33.1)	30 (17.8)		
Mother Smoking‡	30 (20.0)	28 (17.0)	0.49	14
Parental Education‡‖			***0.04***	3
Elementary§	17 (10.8)	11 (6.5)		
High School	33 (20.9)	30 (17.9)		
Intermediate	63 (39.9)	54 (32.1)		
University	45 (28.5)	73 (43.5)		

*Geometric mean (95% Confidence Interval), unadjusted P-values
†Median value (Interquartile Range)
‡Frequency (Column Percent)
§Reference category
‖P-value calculated using Fisher's Exact Test

Table 2. Modeling Associations Between CRP Concentration and Adult Characteristics

	Basic Model (n=329)			*Model 2 (n=329)*			*Model 3 (n=284)*		
	B*	**s.e.**	β	**B***	**s.e.**	β	**B***	**s.e.**	β
Birth Weight Group†	0.09	0.07	0.07	0.02	0.07	0.02	0.07	0.07	0.05
Sex	**-0.39Δ**	0.07	-0.30	**-0.36Δ**	0.07	-0.27	0.03	0.12	0.03
BMI									
Underweight				**-0.39‖**	0.11	-0.17	-0.17	0.12	-0.08
Normal†									
Overweight				**0.50Δ**	0.08	0.32	0.16	0.10	
Systolic Blood Pressure				-0.15	0.12	-0.07	-0.10	0.12	0.11
Exercise Frequency									
Not at all‡									
Not even once a month				.004	0.22	0.001	-0.07	0.26	-0.02
Once or twice a month				0.07	0.12	0.04	0.12	0.13	0.06
Once a week				**-0.27§**	0.11	-0.13	-0.19	0.13	-0.09
2-3 times/week				0.03	0.09	0.02	0.05	0.09	0.03
4-5 times/week				0.04	0.11	0.02	0.16	0.11	0.08
Daily				-0.23	0.12	-0.10	-0.09	0.13	-0.04
Intensity of Exercise									
Walking‡									
Walking or light running				0.06	0.11	0.03	-0.05	0.12	-0.03
Light Running				0.04	0.09	0.03	0.02	0.09	0.02
Brisk running				**-0.2§**	0.09	-0.14	-0.17	0.10	-0.11
Average Duration									
<30 minutes‡									
30-60 minutes				-0.16	0.14	-0.08	-0.06	0.15	-0.03
1-2 hours				-0.11	0.08	-0.08	-0.12	0.08	-0.09
2 or more hours				0.12	0.14	0.04	0.09	0.14	0.03
WBFP (%)							**0.04Δ**	0.01	0.47

Marks variable hierarchical grouping in respective models

B Reflects relative change in CRP value

† Birth Weight Status code: 0=VLBW; 1=Term-Born Control

‡ Reference category

§ $P<0.05$

‖ $P<0.005$

Δ $P<0.001$