ASSOCIATION OF SERUM C-REACTIVE PROTEIN AND INDICES OF BODY FAT DISTRIBUTION AND OVERWEIGHT IN MEXICAN AMERICAN CHILDREN

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Background: Few data have been published on the association of indices of body fat distribution and components of the insulin resistance syndrome and serum C-reactive protein (CRP), an acute phase protein and putative risk factor for cardiovascular morbidity, in representative samples of total populations of children or in Hispanic Americans, who have a high prevalence of obesity and diabetes as adults.

Objective: To evaluate the association of waist-to-hip ratio (WHR) and body mass index (BMI) and components of the insulin resistance syndrome with CRP in Mexican American children and to assess the independence of the association.

Design: Cross-sectional survey of a large national sample, the Third National Health and Nutrition Examination Survey.

Participants: Mexican American children aged 6-11 years.

Measurements: Body circumferences, skinfold thickness, BMI, blood pressure, and serum CRP and lipid concentrations.

Results: Overall, 11% of children had detectable CRP (> 0.21 mg/dL). CRP was not associated with age, gender, or birth weight. WHR showed significant positive associations with serum CRP concentration independent of BMI. BMI was also significantly associated with CRP independent of WHR. CRP was significantly associated with HDL cholesterol but not triglyceride, or systolic blood pressure, concentration after controlling for BMI.

Conclusion: Further research is needed on the associations of serum CRP concentration with WHR and other indices of body fat distribution and obesity to elucidate the mechanisms and significance of these associations. (*J Natl Med Assoc.* 2003;95:545-552.)

Key words: C-reactive protein ♦ Hispanics ♦ adipose tissue ♦ child ♦ obesity

Few studies have examined the relationship of acute phase proteins and indices of body fat distribution and overweight, despite the relationship postulated for these variables with coronary heart disease and atherosclerosis.[1-6] Studies of adults have reported independent associations of serum C-reactive protein (CRP) with body mass index (BMI), waist-to-hip ratio (WHR) and subscapular-to-triceps ratio (SFR), as well as fasting serum insulin, insulin sensitivity, but not fasting serum glucose.[7] Recently adipose tissue, (in addition to activated leukocytes,) has been shown to be a source of cytokines tumor necrosis factor-alpha (TNF-alpha) and interleukin-6

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(IL-6), the latter being the main regulator of synthesis of CRP by the liver.[7, 8] These cytokines have been postulated to mediate insulin resistance in skeletal muscle and adipose tissue.

Well established are associations of body circumference measures and skinfold measures of body fat distribution and fatness with insulin sensitivity, non-insulin dependent diabetes, and other cardiovascular risk factors of the insulin resistance syndrome.[4, 9, 10] Inflammation is hypothesized to play a role in the initiation of atherosclerosis, as well as in promoting its progression and precipitating acute events.[1] Since adverse patterns of blood lipids and atherosclerosis itself begin in childhood, studies of population and individual differences in the early onset and progression through adolescence of possible initiating risk factors are important.[9, 11-14] Tracking of blood lipids, obesity, and body fat distribution over long periods has been demonstrated.[14-17] In order to test the hypothesis that obesity and circumference and skinfold indices of body fat distribution are significantly associated with acute phase proteins as indicated by serum CRP concentration in children independent of gender, ethnicity, age or maturity level, data from the Third National Health and Nutrition Examination Survey (NHANES III) were examined. Mexican Americans were selected for study because of the reported higher prevalence of obesity, diabetes, and insulin-resistance compared to non-Hispanic whites or blacks.[18, 19] The study was restricted to ages 6-11 years to eliminate or reduce confounding by age, puberty, pregnancy, parity, hormone use, smoking and age-related chronic metabolic and inflammatory diseases.

METHODS

The Third National Health and Nutrition Examination Survey (NHANES III) was conducted in 1988-1994 on a nationwide multistage probability sample of approximately 40,000 persons from the civilian, non-institutionalized population aged two months and over of the United States, excluding reservation lands of American Indians. Of these, 31,311 were examined. The descriptive analyses of serum CRP concentration and WHR in this report are restricted to 996 Mexican American children aged 6-11 years examined with valid serum CRP, height, weight, waist, and hip circumference measurements in the survey. Numbers of persons in various cross-tabulation and regression analyses that follow may vary slightly due to differing numbers with missing values on selected other variables. Details of the plan, sampling, operation, and response have been published as have procedures used to obtain informed consent and to maintain confidentiality of information obtained.[20, 21]

Demographic, medical history, and behavioral information was collected prior to the examination by household interview. Race and Mexican American ethnicity were determined by self report.[20] Examinations were carried out in a mobile examination center. Blood samples were obtained at the examination center. Blood in a red-top Vacutainer tube was allowed to stand for 45 minutes at room temperature to allow complete clotting and clot retraction. Samples were centrifuged at 1500 x g for 30 minutes at 4° C. Samples were frozen at -20° C.

CRP concentration in serum, a marker of inflammation, was measured at the Immunology Division of the University of Washington (Seattle, WA) with latex-enhanced nephelometry using a Behring Nephelometer Analyzer System (Behring Diagnostics Inc., Somerville, NJ).[22] The standard was prepared by Behring Diagnostics and standardized against the World Health Organization International Reference Preparation of CRP serum, an internationally recognized source of purified human CRP. Diluted standards were run daily. Bench quality controls were used and long term quality control charts consulted for each analytic run as detailed elsewhere.[22] Although this is a quantitative CRP assay, the percentage of persons in this healthy sample with detectable CRP levels > 0.21 mg/dL was low; therefore, CRP is treated as a dichotomous (detectable/not detectable) variable in the present study. Frozen plasma was sent to the Missouri Diabetes Diagnostic Laboratory for determination of glycated hemoglobin (HbA1c) in whole blood using a high-performance liquid chromatographic assay on the Diamat automated HPCL system, model 723 (Bio-Rad Laboratories, Hercules, CA). The upper limit of normal for HbA1c in this system has been defined as 6.1%.[22] Samples were shipped on dry ice to the Johns Hopkins University Lipoprotein Analytic Laboratory in Baltimore,

Maryland. Serum high-density lipoprotein cholesterol (HDL) was measured in serum following the precipitation of other lipoproteins with a polyanion/divalent cation mixture (Hitachi 704 Analyzer/Boehringer-Mannheim Diagnostics, Indianapolis, IN).[22]

Technicians measured height to the nearest 0.1 centimeter, weight to the nearest 0.01 kg, triceps, subscapular, suprailiac and mid-thigh skinfold thickness to the nearest 0.1 millimeter and waist and

Table 1. DETECTABLE C-REACTIVE PROTEIN* AND MEAN INDICES OF BODY FAT DISTRIBUTION, OBESITY, AND SELECTED CARDIOVASCULAR RISK FACTORS BY AGE IN MEXICAN AMERICAN CHILDREN, AGED 6-11 YEARS: THIRD NATIONAL HEALTH AND NUTRITION EXAMINATION SURVEY, 1988-1994

Variable	Age, years					
	6	7	8	9	10	11
C-reactive protein (%)	12.6	13.6	8.2	7.0	9.3	14.4
WHR	0.91	0.90	0.89	0.88	0.87	0.88
WTR	1.68	1.67	1.66	1.64	1.64	1.65
SFR	0.71	0.71	0.78	0.79	0.74	0.85
CPR	0.64	0.66	0.72	0.73	0.74	0.84
Waist (cm)	56.7	59.8	62.0	64.6	65.9	74.3
Hip (cm)	62.2	66.1	69.4	73.1	75.2	84.1
SSF (mm)	42.1	43.9	47.1	50.5	50.3	60.9
BMI (kg/m^2)	16.8	17.5	17.9	18.6	18.7	21.6
HbA1c (%)	5.0	5.0	5.0	5.1	5.0	5.1
HDL (mg/dL)	52.2	50.7	55.5	54.1	51.5	51.1
TG (mg/dL)	98.5	101.5	100.2	106.2	102.5	113.2
SBP (mmHg)	93.7	94.8	97.8	99.2	100.9	103.1
DBP (mmHg)	48.3	49.8	52.0	53.2	54.6	57.7
Age (months)	77.5	89.5	101.8	113.6	125.1	137.2

** C-reactive protein > 0.21 mg/dL*
WHR, waist-to-hip ratio; WTR, waist-to-thigh ratio; SFR, subscapular to triceps skinfold ratio; CPR, (subscapular + suprailiac)/(triceps + thigh); WAIST, waist circumference; HIP, hip circumference; SSF, sum of 4 skinfolds; BMI, body mass index, kg/m^2; HbA1c, glycated hemoglobin; HDL, serum HDL cholesterol; TG, serum triglycerides; SBP, systolic blood pressure; DBP, diastolic blood pressure.

buttocks circumference to the nearest 0.1 centimeter as described in detail elsewhere.[20, 23-25] With the sample person standing at minimal respiration, waist circumference was measured in a horizontal plane at the level of the high point of the iliac crest to the nearest 0.1 cm. This method was chosen after consultation with experts in the field to maximize reproducibility. Hip circumference was measured in a horizontal plane at the maximum extension of the buttocks. Thigh circumference was measured at mid-thigh in a plane perpendicular to the long axis of the thigh. The following were computed: waist-to-hip circumference ratio (WHR), waist-to-thigh circumference ratio (WTR), ratio of subscapular to triceps skinfold thickness (SFR), central-peripheral skinfold ratio (CPR=(subscapular skinfold + suprailiac skinfold)/(triceps skinfold + thigh skinfold); sum of the four skinfolds (SSF); and body mass index (BMI=weight /height^2, kg/m^2).

Previously it has been reported that 17.4% (SE 2.4) of Mexican American boys and 14.3% (SE 2.4) of Mexican American girls aged 6-11 were overweight based on revised NCHS growth charts.[23] Therefore, in the present study, children exceeding the 85th percentile of age-, sex-specific BMI were considered overweight. By analogy, children exceeding the 85th percentile of age-, sex-specific WHR were arbitrarily considered to have elevated WHR. Extensive descriptive data on height, weight, BMI and obesity prevalence in the NHANES III population are being published elsewhere and will not be duplicated here.[23, 25] Blood pressure was measured using standardized methods as described elsewhere; the mean of all available readings was used in this analysis.[20, 21]

Statistical analysis. The plan of the present analyses was as follows. Detailed descriptive statistics and measures of association were computed using the Statistical Analysis System (SAS).[27] Data presented here were computed using techniques that incorporated sampling weights and design features of the survey.[28] Population estimates for percentiles of BMI and WHR and frequencies were produced using weighted SAS or SUDAAN procedures.[29] Logistic regression results of serum CRP with other variables were confirmed and statistical testing and variance estimation were performed using the PROC LOGISTIC procedure for linear regression models in the SUDAAN system.[28-29] All data presented were computed using sampling weights.

RESULTS

The weighted percentages of children with detectable levels of CRP by age are shown in Table 1. Detectable levels of CRP were found in 11% of children overall and were not significantly associated with years of age (p=0.78), sex (p=0.69), or birthweight category (p=0.48). Also shown in Table 1 are mean levels of indicators of body fat distribution, overweight, and selected cardiovascular risk factors associated with the insulin resistance syndrome.

Detectable CRP was seen in 34.7% of overweight children but only 6.8% of other children (p=0.0006, RR=5.12, 95% CI 3.32-7.90). Controlling for sex or year of age by stratification did not change this result. Similarly, detectable CRP was seen in 26.5% of children with WHR above the 85th percentile but only 8.3% of those with lower WHR (p=0.0015). The association persisted after controlling for sex or year of age by stratification.

The weighted association was tested of WHR greater than the 85th percentile (yes/no) with detectable CRP (yes/no). The odds ratio for high WHR ("yes") compared to "no" was 4.00, 95% CI 2.47-6.51, p<0.001). After controlling also for BMI, the odds ratio was 1.97, 95% CI 1.25-3.12, p=0.004). Also tested was the weighted association of overweight (BMI > 85th percentile)(yes/no) and dectible CRP (yes/no). The odds ratio for high BMI yes compared to no was 7.31, 95% CI 3.89-13.72, p<0.001).

Table 2 shows weighted mean levels of indicators of body fat distribution and overweight by level of CRP. The mean of each indicator was significantly higher among children with dectable levels of CRP. Table 2 also shows weighted mean levels of selected cardiovascular risk factors generally considered to be components of the insulin resistance syndrome by level of CRP. Children with detectable CRP had significantly lower mean HDL and significantly higher mean triglyceride concentrations in serum and higher systolic blood pressure. No signifi-

cant difference between groups was seen for glycated hemoglobin percent or mean diastolic blood pressure. The differences for HDL and systolic blood pressure but not triglycerides remained significant though somewhat diminished after controlling for BMI.

Weighted logistic regression models were fit with CRP (detectable/not detactable) as the dependent variable and WHR as the exposure variable controlling for age, gender, or BMI, to determine whether WHR was significantly associated with CRP independent of overall fatness, age or sex. WHR was significantly associated with CRP when entered alone (beta 9.85, SE 2.55, $p<0.001$) or after controlling for age in months (beta 10.12 SE 2.59, $p<0.001$) or BMI

Table 2. MEAN LEVELS OF INDICATORS OF BODY FAT DISTRIBUTION AND OBESITY AND SELECTED CARDIOVASCULAR RISK FACTORS BY LEVEL OF C-REACTIVE PROTEIN IN SERUM IN MEXICAN-AMERICAN CHILDREN AGED 6-11 YEARS: THIRD NATIONAL HEALTH AND NUTRITION EXAMINATION SURVEY

	C-reactive protein		
	<=0.21 mg/dL	>0.21 mg/dL	
WHR	0.89	0.92	*
WTR	1.65	1.69	*
SFR	0.75	0.85	+
CPR	0.71	0.80	+
WAIST (cm)	62.92	72.99	*
HIP (cm)	70.92	79.37	*
SSF (mm)	47.74	61.74	*
BMI (kg/m^2)	18.14	22.14	*
Hb A1c (%)	5.03	5.12	
HDL(mg/dL)	53.28	45.21	*
TG(mg/dL)	101.59	121.73	+
SBP(mmHg)	97.97	98.31	*
DBP(mmHg)	51.99	53.42	
Age(months)	107.76	108.08	

** P<0.01, +P<0.05*

WHR, waist-to-hip ratio; WTR, waist-to-thigh ratio; SFR, subscapular to triceps skinfold ratio; CPR, (subscapular + suprailiac)/(triceps + thigh); WAIST, waist circumference; HIP, hip circumference; SSF, sum of 4 skinfolds; BMI, body mass index, kg/m^2; HbA1c, glycated hemoglobin; HDL, serum HDL cholesterol; TG, serum triglycerides; SBP, systolic blood pressure; DBP, diastolic blood pressure.

(beta 4.33, SE 0.02, p<0.001). In the latter model, BMI was also a significant predictor of CRP (beta 0.17, SE 0.02, p<0.001). There was no significant WHR x BMI interaction. When entered alone, the model R-square for WHR was 0.04, that for BMI was 0.12, and that for both entered together was 0.13. Further, in models with WHR or BMI and a quadratic term, the quadratic term was not significant. Neither age in months nor gender was significantly related to CRP, nor were there significant interactions of age or sex with WHR. Hence both body fat distribution (WHR) and overweight (BMI) were independently associated with CRP.

Of other measured components of the insulin resistance syndrome, only HDL, TG, and systolic blood pressure showed significant univariate associations with CRP. In weighted logistic regression models with no co-variables or with BMI controlled for, HDL remained significantly associated with serum CRP (unadjusted beta -0.06, SE 0.02, p=0.001; BMI-adjusted beta -0.04, SE 0.01, p=0.005). No significant HDL x BMI interaction term was seen. TG was not significantly associated with CRP after controlling for BMI (unadjusted beta 0.005, SE 0.002, p=0.015; BMI-adjusted beta 0.001, SE 0.002, p=0.546). Systolic blood pressure was not significantly associated with CRP after controlling for BMI (unadjusted beta 0.035, SE 0.012, p=0.004; BMI-adjusted beta -0.013, SE 0.014, p=0.362).

DISCUSSION

WHR, WTR, SFR, CPR, waist and hip circumferences, SSF, BMI, triglycerides, and systolic blood pressure showed consistent positive associations with serum CRP levels in a national sample of Mexican American children aged 6-11 years; HDL was negatively associated. When age and gender were controlled for, WHR remained significantly associated with serum CRP; the association weakened but remained significant after controlling for BMI. BMI was significantly positively associated with CRP after controlling for WHR. When BMI was controlled for, HDL remained significantly associated with serum CRP.

Mechanisms

CRP is an acute phase protein that rises quickly after an inflammatory event and returns to normal within eight days. It is a polymeric protein (molecular weight 120,000) composed of five identical non-glycosylated subunits bound together by non-covalent bonds.[30-34] Recently, adipose tissue, (in addition to activated leukocytes,) has been shown to be a source of cytokines TNF-alpha and IL-6, the latter being the main regulator of synthesis of CRP by the liver.[7, 8, 34, 35] IL-6 is released into the systemic circulation by subcutaneous adipose tissue.[8] In vitro, omental adipose tissue released two-to-three times more IL-6 than did subcutaneous adipose tissue.[35] These cytokines have been postulated to mediate insulin resistance in skeletal muscle and adipose tissue. TNF-alpha is expressed at high levels in the enlarged adipose tissue on obese humans as well as rodent models of genetic obesity. TNF-alpha reduces insulin-induced glucose uptake and impairs insulin receptor tyrosine kinase activity in adipocytes. In animal models of obesity, absences of TNF-alpha or its actions improves insulin sensitivity but does not restore it to normal. TNF-alpha may work in synergy with other cytokines to produce insulin resistance in skeletal muscle.[35] Thus, the association of CRP with indices of obesity and body fat distribution and with components of the insulin resistance syndrome may reflect the role of cytokines in mediating the metabolic effects of obesity, even in the absence of an inflammatory process involving leukocytes.

Comparisons with Previous Reports

Only a few studies have assessed associations of obesity and central or abdominal fat distribution with serum CRP concentration in children. Elevated BMI was associated with elevated serum CRP in earlier analyses of NHANES III data.[36, 37] A similar association was also noted in English children aged 10-11.[38]

In adults, CRP has been found to be associated with obesity, central obesity and insulin sensitivity.[7, 39, 40] One study of English men reported independent associations of serum C-reactive protein (CRP) with body mass index (BMI), waist-to-hip ratio (WHR) and subscapular-to-triceps ratio (SFR) as well as fasting serum insulin, insulin sensitivity, but not fasting serum glucose.[7] A study of adults aged 17 and over in NHANES III revealed positive associa-

tions of CRP with BMI and WHR that persisted after controlling for age, race, gender, smoking, inflammatory disease, and estrogen use.[39] Another report from NHANES III showed associations of elevated CRP with diabetes, impaired fasting glucose, and higher serum concentrations of insulin, glycosylated hemoglobin and glucose.[40]

Limitations of the present study include possible bias arising from survey non-response and from missing values for some variables. Several special studies of earlier HANES and NHANES III data have indicated little bias due to non-response.[23,25,28,39] Day-to-day variability in serum CRP is due to circadian variation. Children generally were not examined when acutely ill or febrile. Further, CRP is rarely elevated in uncomplicated upper respiratory infections.[32] The limited sensitivity of the nepholometric CRP assay used, precluded using results as a continuous variable in this healthy population as has been possible in studies using ELISA assays; under normal conditions CRP is present in very low concentration.[7,30-32] IL-6 was not measured in NHANES III. Blood collection conditions in NHANES III were standardized with regard to body position and vein constriction. Although WHR may not accurately reflect intra-abdominal fat mass in children,[40-42] SFR and CPR may reflect distribution of subcutaneous fat, which is also related to cardiovascular disease occurrence in adults.[43-47] The lack of a single, generally accepted measurement protocol for body circumferences remains a problem for inter-study comparisons, perhaps explaining in part inconsistencies among studies. However, a standardized measurement protocol was followed by trained technicians in NHANES III.[20, 24] Confounding by variables not controlled for cannot be excluded. The large sample size in NHANES III provided good statistical power. Since the number of tests was restricted to those of regression models and p-values for WHR and BMI were generally <0.01, chance is an unlikely explanation of findings. The representativeness of the sample and the use of sample weights provides wide generalizability of the results to Mexican American children of the same ages.

Future research should include longitudinal studies of body fat distribution and serum CRP in non-Hispanic white and black and Hispanic children and adults to determine temporal sequence of the relationship.[48-50] Dual-energy x-ray absorptiometry or other techniques for accurate body fat measurement and assessment of regional fat distribution should be used to determine whether adipose tissue in various depots varies in its association with serum CRP, IL-6 and TNF-alpha. Body fat distribution, obesity and serum CRP, IL-6 and TNF-alpha should be assessed jointly as risk factors for development of non-invasively measured atherosclerosis (e.g. carotid intima-medial thickness) and non-insulin dependent diabetes.

ACKNOWLEDGEMENTS

We acknowledge the staff and contractors of the Division of Health Examination Statistics of the National Center for Health Statistics, Centers for Disease Control and Prevention, who conducted the survey and prepared the data for analysis.

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