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Longitudinal changes in C-reactive protein, proform of eosinophil major basic protein, and pregnancy-associated plasma protein-A during weight changes in obese children

Abstract

Background: Childhood obesity is associated with several complications, including cardiovascular comorbidity. Several biomarkers, such as high-sensitive C-reactive protein (hs-CRP), proform of eosinophil major basic protein (Pro-MBP) and pregnancy associated plasma protein-A (PAPP-A), have equally been linked to increased cardiovascular susceptibility. This study investigates these biomarkers during weight loss and regain in obese children.

Materials and methods: A longitudinal study during a 12-week weight loss program with a 28 months follow-up was conducted. Anthropometrics and plasma concentrations of hs-CRP, Pro-MBP, and PAPP-A were measured at baseline; at days 14, 33 and 82 during weight loss; and at months 10, 16, and 28 during follow-up.

Results: Fifty-three boys and 62 girls aged 8–15 years with a median body mass index (BMI) standard deviation score (SDS) at baseline of 2.78 (boys), and 2.70 (girls) were included. Ninety children completed the weight loss program and 68 children entered the follow-up program. Pro-MBP and PAPP-A, but not hs-CRP, exhibited individual-specific levels (tracking) during weight loss and regain. The PAPP-A/Pro-MBP correlation was strong, whereas the hs-CRP/PAPP-A correlation was weak during weight fluctuations.

Conclusion: Hs-CRP changes reflect weight changes. PAPP-A and Pro-MBP exhibited tracking during weight perturbations and may contribute as early risk markers of cardiovascular susceptibility.

Keywords: BMI SDS; childhood obesity; hs-CRP; PAPP-A; Pro-MBP; weight loss; weight regain.

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Introduction

Childhood obesity is associated with a wide variety of comorbidities (1). Of particular interest is the strong association between cardiovascular disease and obesity (2). For this reason, it is important to risk stratify obese individuals to reduce future cardiovascular morbidity and mortality (3). Several biomarkers may aid herein, but their relationship with weight changes in a pediatric setting has yet to be fully elucidated.

Childhood obesity has been shown to be accompanied by elevated concentrations of high-sensitivity C-reactive protein (hs-CRP) (4–6). In adults, hs-CRP has been found to be closely associated with early atherosclerotic changes represented by carotid plaque formation (7). Hs-CRP may also serve as a complementary quantitative inflammatory biomarker for clinical practice in acute coronary syndrome (8) and hypertension (9).

High pregnancy associated plasma protein (PAPP-A) has been shown to be significantly correlated to cardiovascular risk factors (10). PAPP-A has also been reported as a biomarker of acute coronary syndrome (11) and acute

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ST elevation myocardial infarctions (12, 13). Free PAPP-A seems to be superior than total PAPP-A concentrations in predicting non ST-elevation acute coronary syndrome (14). PAPP-A concentrations are associated with poor prognosis during long-term follow-up in patients with chronic stable angina pectoris (15), and seems to be associated with atherosclerotic plaque instability (16, 17). However, a recent study has reported that PAPP-A seems to increase after the administration of heparin, a finding that may influence the presumed association between PAPP-A and acute ST elevation myocardial infarctions (18).

Proform of eosinophil major basic protein (Pro-MBP) has been found to function as a proteinase inhibitor of PAPP-A and bind into a PAPP-A/Pro-MBP complex (19), which may implicate Pro-MBP in pathways that link PAPP-A with atherosclerotic development. However, the extent to which hs-CRP, Pro-MBP, and PAPP-A concentrations are defined by genetic factors has yet to be assessed. If the genetic influence is considerable, concentrations of hs-CRP, Pro-MBP, and PAPP-A would exhibit tracking over time.

A genetically based risk factor, which can exhibit tracking over time, is potentially a good biomarker in the early identification of individuals with potential susceptibility to later cardiovascular disease. Such early identification of potential severe cardiovascular disease would be valuable in health-care preventive strategies.

In the present longitudinal study, concomitant concentrations of hs-CRP, Pro-MBP, and PAPP-A were analyzed during weight perturbations in severely obese children, so as to evaluate whether these atherosclerotic risk factors were affected with a lowered or regained degree of obesity.

Materials and methods

Design and subject

Obese children were examined on days 1 (baseline) 14, 33, and 82 during a 12-week weight loss program (20, 21), and at months 10, 16, and 28 during the follow-up program. The Scientific Ethical Committee of Copenhagen approved the study, and written informed consent was obtained from all participants and their parents.

Procedures

Height was measured by stadiometer to the nearest 5 mm. Weight was measured to the nearest 0.1 kg on a SECA Delta Scale (model 707, SECA, Bradford, MA, USA). Body mass index standard deviation score (BMI SDS) was calculated using growth data generated in Danish children (22). The developmental pubertal stage was rated according to Tanner after evaluating pubic hair, breasts, and testicular size by Prader's orchidometer.

After an overnight fast, venous blood samples were collected from each child between 7:00 and 8:30 h during the weight loss program, and between 8:00 and 9:00 h during follow-up. The serum was stored at −80°C until analysis.

Hs-CRP was measured in sera by the ultra-sensitive CRP immunofluorescent assay (Brahms, Hennigsdorf, Germany) and run on the Kryptor analyser (Brahms, Hennigsdorf, Germany) using time-resolved amplified cryptate emission technology. The analyses were performed as single measurements on automated equipment, and outliers were automatically re-measured. The within- and between-assay variations were 5.1% and 14.2%, respectively, in the low concentration area. The quantification limit of the assay was 0.06 μg/mL CRP. The measured hs-CRP values below the detection limit were given the value 0.06 μg/mL during analysis. Hs-CRP values >20 μg/mL were excluded from statistical analysis due to suspicion of infection in these children (viral infections were prevalent in Denmark at that time).

PAPP-A was quantified essentially as previously described (23) by use of a biotin-tyramide amplified enzyme immunoassay, with a polyclonal anti-PAPP-A polyclonal antibody (24) as capture antibody and a combination of monoclonal antibodies (25) for detection. The detection limit was 0.03 mIU/L and the intra- and inter-assay variabilities were 10% and 15%, respectively. The assay was calibrated against the World Health Organization's international reference standard 78/610.

Pro-MBP was quantified as total pro-MBP as previously described (24). Briefly, Pro-MBP complexes were quantified by an enzyme immunoassay using a polyclonal rabbit anti PAPP-A/Pro-MBP developed at Statens Serum Institute, Copenhagen, as catching antibody, and a mouse monoclonal anti-Pro-MBP (HYB 234-10, Statens Serum Institute, Copenhagen) as detection antibody (25). The functional sensitivity was 3.9 mIU/L, and the intra- and inter-assay variations were both <5%. The assay was calibrated against the World Health Organization's international reference standard 78/610.

Statistical methods

Given that the distribution of hs-CRP, Pro-MBP, and PAPP-A concentrations were right-shifted, a log-transformation was performed in order to achieve approximate normality and variance homogeneity. Tracking was assessed using Pearson correlation coefficients between the first measurement and later measurements of the same variable.

In order to perform longitudinal analysis, multivariate linear regression models were applied (26). We chose an unstructured model for the covariance (27) in order to avoid bias originating from the missing observations during the longitudinal analysis.

Results

During the study, 232 children were identified as eligible and 115 children aged 8–14 years agreed to participate. Ninety children completed the weight loss program, and 68 engaged in follow-up. Of these, only 44 children completed all examinations in the follow-up program, yielding

retention rates of 78% during weight loss and 65% during follow-up.

At baseline, the median BMI SDS was 2.79 in boys and 2.70 in girls. The median BMI SDS was reduced by –1.0 BMI SDS in boys ($p<0.0001$) and by –0.9 BMI SDS in girls ($p<0.0001$) during weight loss. During follow-up, BMI-SDS increased by 0.6 BMI SDS in boys ($p<0.0001$) and by 0.8 BMI SDS in girls ($p<0.0001$) (Table 1). Children dropping out were no different with respect to baseline age (median age of 12.1 years in boys and girls), but those dropping out were heavier with a baseline mean BMI SDS of 2.91±0.60 compared with those completing the study who had a mean BMI SDS of 2.62±0.57 ($p=0.023$).

In both genders, the hs-CRP concentrations were slightly elevated at baseline, and the degree of hs-CRP elevation was correlated to BMI SDS ($r=0.31$, $p=0.05$ in boys and $r=0.56$, $p<0.0001$ in girls) (Table 1). During weight loss, hs-CRP had a tendency to decrease in boys and girls and then increase during regain. The hs-CRP were dependent on BMI SDS during both weight loss and regain and a multiple regression analysis showed a positive association between BMI SDS and hs-CRP, i.e., an increase of 1 SD in BMI was associated with an increase in hs-CRP by 60% (CI 12%–128%, $p=0.01$) in boys, and an increase in hs-CRP by 88% (CI 26% to 179%, $p=0.002$) in girls.

Multiple regression analysis revealed an inverse association between Pro-MBP and BMI SDS in boys, i.e., an increase of 1 SD in BMI was associated with a decrease in Pro-MBP by 9.9% (CI 2.2%–18.3%, $p=0.01$), whereas no relationship was established between Pro-MBP and BMI in girls, and an increase of 1 SD in BMI was associated with a 3.2% (CI 3.9%–10.9%, $p=0.39$) higher Pro-MBP level. No relationships were observed between BMI SDS and PAPP-A during either weight loss or follow-up (data not shown).

The correlation coefficients and associated p-values between hs-CRP and PAPP-A and between Pro-MBP and PAPP-A are shown in Table 2. There was a strong correlation between Pro-MBP and PAPP-A, whereas the correlation tended to be inverse between hs-CRP and PAPP-A, but was weak overall.

Table 3 shows partial correlation coefficients of repeated measures of hs-CRP, Pro-MBP and PAPP-A where the baseline measurement is compared with a later measurement (e.g., baseline hs-CRP compared with month 16 hs-CRP in each child, etc.), which revealed strong tracking of Pro-MBP and PAPP-A during both weight loss and regain. Tracking indicated that in any given individual, Pro-MBP and PAPP-A had a tendency to remain in the same specific layer in the distribution of that variable during weight loss and regain. Initially, hs-CRP also exhibited a strong degree of tracking without adjustment for BMI SDS (data not shown), but when BMI SDS and pubertal development were adjusted for (partial correlation coefficients) tracking of hs-CRP became less obvious (Table 3). Such a tendency (dependent on BMI SDS and pubertal development) was not observed with regards Pro-MBP or PAPP-A (data not shown).

Discussion

The present longitudinal study of obese children showed that hs-CRP declined during weight loss and increased

Table 1 BMI SDS, hs-CRP, Pro-MBP, and PAPP-A expressed as means and corresponding standard deviations during weight loss (days 1–82) and follow-up (day 82–28 months).

	Days				Months		
	1	14	33	82	10	16	28
Boys, n	56/41	45/38	44/33	42/30	29/27	26/23	24/17
BMI SDS	2.8±0.7	2.5±0.6	2.4±0.6	1.8±0.6	2.1±0.8	2.3±0.7	2.4±0.7
Hs-CRP, µg/mL	3.2±4.0	1.2±1.3	1.6±3.0	0.7±0.9	1.5±1.8	1.6±3.3	2.0±2.4
Pro-MBP, IU/mL	1.7±0.5	1.7±0.6	1.6±0.5	1.6±0.5	1.5±0.5	1.5±0.5	1.5±0.4
PAPP-A, mIU/mL	6.3±2.4	6.2±2.4	5.9±1.8	5.9±1.9	5.7±1.7	5.3±1.7	5.1±1.6
Girls, n	61/46	52/41	48/40	46/32	33/29	33/29	22/11
BMI SDS	2.7±0.7	2.3±0.7	2.1±0.7	1.8±0.7	2.1±0.6	2.3±0.7	2.3±0.9
Hs-CRP, µg/mL	3.5±4.0	1.7±3.2	1.6±2.9	1.8±3.0	1.7±2.6	1.5±1.8	2.2±3.7
Pro-MBP, IU/mL	1.3±0.4	1.4±0.4	1.4±0.4	1.4±0.4	1.4±0.6	1.2±0.5	1.3±0.7
PAPP-A, mIU/mL	5.1±3.4	5.5±3.6	5.3±1.7	5.3±1.8	6.1±5.0	4.8±2.0	4.6±1.5

BMI SDS, body mass index standard deviation scores; hs-CRP, high sensitive C-reactive protein; PAPP-A, pregnancy associated plasma protein A; Pro-MBP, proform of eosinophil major basic protein. Numbers shown are for those having measures of BMI SDS over the slash, and for those with additional measures of hs-CRP, Pro-MBP, and PAPP-A under the slash.

Table 2 Correlation coefficients between hs-CRP and PAPP-A and between Pro-MBP and PAPP-A during weight loss (days 1–82) and follow-up (day 82–28 months).

	Days				Months		
	1	**14**	**33**	**82**	**10**	**16**	**28**
Boys, n	41	38	33	30	27	23	17
Hs-CRP/PAPP-A	−0.12	−0.21	−0.19	0.16	0.32	−0.50	−0.50
p-Value	0.45	0.20	0.29	0.41	0.11	0.02	0.04
Pro-MBP/PAPP-A	0.55	0.60	0.49	0.68	0.46	0.59	0.37
p-Value	0.0002	[a]	0.004	[a]	0.02	0.003	0.15
Girls, n	46	41	40	32	29	29	11
Hs-CRP/PAPP-A	0.01	−0.004	−0.33	−0.29	−0.26	−0.41	−0.80
p-Value	0.93	0.98	0.04	0.11	0.18	0.03	0.003
Pro-MBP/PAPP-A	0.45	0.31	0.34	0.49	0.48	0.54	0.48
p-Value	0.002	0.045	0.03	0.004	0.008	0.002	0.13

hs-CRP, high sensitive C-reactive protein; PAPP-A, pregnancy associated plasma protein A; Pro-MBP, proform of eosinophil major basic protein; [a]Indicates a p-value below 0.0001.

during weight regain, whereas concentrations of PAPP-A and Pro-MBP were relatively stable. The hs-CRP concentrations were associated with BMI SDS, i.e., a difference of 1 SD in BMI was associated with a 60% increase in hs-CRP in boys and an 88% increase in hs-CRP in girls. Further, Pro-MBP and PAPP-A both exhibited a strong degree of tracking during both weight loss and regain in boys and girls.

A previous report has suggested that it is not the elevated hs-CRP per se that is linked to ischemic heart disease, because high CRP concentrations seen in subjects with four CRP polymorphisms are not associated with an increased risk of ischemic heart disease (28). Instead, the high CRP concentrations seen in obese children (4–6), along with the finding that elevated CRP concentrations are associated with ischemic vascular disease (29–31), suggest that the changes in hs-CRP shown in the present study may indicate two things: hs-CRP is a marker of the non-specifically inflammatory process seen in the development of obesity, and there is a possible associated development of atherosclerosis. In the current study, the hs-CRP concentrations seen at 10 and 16 months during weight regain were comparable with the hs-CRP concentrations at the last

Table 3 Partial correlation coefficients between baseline hs-CRP, Pro-MBP, and PAPP-A compared with later measurements of hs-CRP, Pro-MBP, and PAPP-A, respectively, during weight loss during weight loss (day 1–82) and follow-up (day 82–28 Months). Partial inclined adjustment for BMI SDS and pubertal development.

	Days				Months		
Day 1 vs.	**1**	**14**	**33**	**81**	**10**	**16**	**28**
Boys, n	41	30	26	26	21	16	11
Hs-CRP	1.0	0.59	0.14	0.06	0.40	0.64	0.73
p-Value		0.0009	[a]	[a]	[a]	0.01	0.02
Pro-MBP	1.0	0.84	0.58	0.71	0.65	0.83	0.59
p-Value		[a]	0.002	[a]	0.002	[a]	[b]
PAPP-A	1.0	0.88	0.86	0.71	0.76	0.77	0.88
p-Value		[a]	[a]	[a]	[a]	0.0009	0.0007
Girls, n	46	34	32	26	23	23	9
Hs-CRP	1.0	0.40	0.36	0.19	0.17	0.53	−0.10
p-value		0.02	0.048	[b]	[b]	0.01	[b]
Pro-MBP	1.0	0.85	0.85	0.87	0.74	0.77	0.57
p-value		[a]	[a]	[a]	[a]	[a]	[b]
PAPP-A	1.0	0.96	0.68	0.50	0.26	0.51	0.79
p-Value		[a]	[a]	0.01	[b]	0.02	0.02

hs-CRP, high sensitive C-reactive protein; PAPP-A, pregnancy associated plasma protein A; Pro-MBP, proform of eosinophil major basic protein. [a]Indicates a p-value below 0.0001. [b]Indicates a p-value above 0.05. Numbers of children were lower, since the tracking analyses required that the same individual had two consecutive measures of the same biomarker in each interval.

measurement at weight loss. This finding suggests that it takes some time to rebuild such an inflammatory state during continuous weight regain after a significant weight loss in children.

During weight loss and regain, concentrations of PAPP-A and Pro-MBP were relatively stable in the present study. An inverse association between Pro-MBP and BMI SDS in boys was shown, whereas no such association was shown in girls or between PAPP-A and BMI SDS. On the one hand, the correlation was strong between PAPP-A and Pro-MBP. This may reflect indicate that Pro-MBP functions as a proteinase inhibitor of PAPP-A, and binds into a PAPP-A/Pro-MBP complex (19). These findings may imply that pro-MBP indirectly interact in atherogenic development, as suggested by several recent studies regarding PAPP-A (11–18). However, similar associations between Pro-MBP and atherosclerosis have not been established.

On the other hand, the correlations between hs-CRP and PAPP-A were weak. This suggests that hs-CRP and PAPP-A function in separate and independent biological pathways, i.e., they do not seem to interact in the same processes linking childhood obesity and atherosclerosis. Further, tracking of hs-CRP declined when BMI SDS and pubertal development were adjusted for in the tracking analyses. This finding also suggests that a considerable proportion of the hs-CRP changes recorded during changes in weight may be dependent on the concomitant changes in BMI SDS and pubertal development, and thus, may seem less associated with atherosclerosis and PAPP-A.

A high degree of PAPP-A and Pro-MBP individual specific values persisted during both weight loss and regain in the present study despite concomitant adjustment for BMI SDS. This finding indicates a strong degree of tracking, possibly reflecting a considerable genetic influence over time in these obese children.

Considering the strong associations between hs-CRP and PAPP-A on the one hand, and cardiovascular disease on the other hand (7–9, 11–18), it is intriguing that especially PAPP-A concentrations were stable during perturbations in weight, which may render PAPP-A as a candidate for a biomarker of later cardiovascular disease.

Some possible confounding influences on the findings in the present study should be acknowledged. As an example, the common cold is prevalent in Denmark; therefore, concentrations of hs-CRP >20 μg/mL were excluded from statistical analysis in order to eliminate the influence of infections in the obese children.

Much effort was done to increase retention, but the drop-out rate and resulting attrition of children participating in the present study may have weakened the established relationships and associations among changes in BMI SDS, hs-CRP, PAPP-A, and Pro-MBP. Those dropping out had the same age, but were heavier than those children who completed the study. More importantly, it is not uncommon for pediatric weight management studies to report retention rates ranging from 20 to 50% (32), which is why our retention rate of 78% during weight loss and 65% during follow-up can be comparatively considered as acceptable.

Both the ultra-sensitive CRP immunofluorescent assay and the assay for PAPP-A (biotin-tyramide amplified enzyme immunoassay with a polyclonal anti-PAPP-A polyclonal antibody) exhibited some inter-assay variability, whereas the intra-assay variability was low. Any variation in the assay results would lead to an underestimation of the observed relationships, because greater variability would most likely have weakened the relationships established in the present study.

According to earlier cross-sectional studies, hs-CRP and PAPP-A may have a prognostic value of atherosclerotic vascular disease (7–9, 11–13, 15–17, 29–31). In addition, tracking of PAPP-A during weight perturbations may render PAPP-A as a potential marker of depicting younger individuals at risk for developing later cardiovascular disease. However, larger long-term prospective studies are required to determine the efficacy of PAPP-A in providing early identification of individuals with an increased risk for cardiovascular disease.

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References

1. Haslam DW, James WP. Obesity. Lancet 2005;366:1197–209.
2. McGill HC, McMahan CA, Gidding SS. Preventing heart disease in the 21st century: implications of the pathobiological determinants of atherosclerosis in youth (PDAY) study. Circulation 2008;117:1216–27.

3. Balakrishnan PL. Identification of obesity and cardiovascular risk factors in childhood and adolescence. Pediatr Clin North Am 2014;61:153–71.
4. Reinehr T, Stoffel-Wagner B, Roth CL, Andler W. High-sensitive C-reactive protein, tumor necrosis factor alpha, and cardiovascular risk factors before and after weight loss in obese children. Metabolism 2005;54:1155–61.
5. Soriano-Guillén L, Hernández-García B, Pita J, Domínguez-Garrido N, Del Río-Camacho G, et al. High-sensitivity C-reactive protein is a good marker of cardiovascular risk in obese children and adolescents. Eur J Endocrinol 2008;159:R1–4.
6. Skinner AC, Steiner MJ, Henderson FW, Perrin EM. Multiple markers of inflammation and weight status: cross-sectional analyses throughout childhood. Pediatrics 2010;125:e801–9.
7. Makita S, Nakamura M, Hiramori K. The association of C-reactive protein levels with carotid intima-media complex thickness and plaque formation in the general population. Stroke 2005;36:2138–42.
8. Otake H, Shite J, Shinke T, Watanabe S, Tanino Y, et al. Relation between plasma adiponectin, high-sensitivity C-reactive protein, and coronary plaque components in patients with acute coronary syndrome. Am J Cardiol 2008;101:1–7.
9. Kozan O, Buyukozturk K, Ilerigelen B, Kabakci G, Koylan N. The impact of plasma high-sensitivity C-reactive protein levels on cardiovascular risk stratification of hypertensive patients: results of the ICEBERG study. J Clin Hypertens 2007;9:500–5.
10. Woelfle J, Roth CL, Wunsch R, Reinehr T. Pregnancy-associated plasma protein A in obese children: relationship to markers and risk factors of atherosclerosis and members of the IGF system. Eur J Endocrinol 2011;165:613–22.
11. Liu Z-Y, Zhang J-Y, Sun T-W, Zhang Y-J, Zhang L, et al. Levels of pregnancy-associated plasma protein A in patients with coronary artery disease. Clin Invest Med 2008;31:E85–9.
12. Iversen KK, Dalsgaard M, Teisner AS, Schoos M, Teisner B, et al. Usefulness of pregnancy-associated plasma protein A in patients with acute coronary syndrome. Am J Cardiol 2009;104:1465–71.
13. Iversen KK, Teisner AS, Teisner B, Kliem A, Thanning P, et al. Pregnancy associated plasma protein A, a novel, quick, and sensitive marker in ST-elevation myocardial infarction. Am J Cardiol 2008;101:1389–94.
14. Lund J, Wittfooth S, Qin Q-P, Ilva T, Porela P, et al. Free vs total pregnancy-associated plasma protein A (PAPP-A) as a predictor of 1-year outcome in patients presenting with non-ST-elevation acute coronary syndrome. Clin Chem 2010;56:1158–65.
15. Consuegra-Sanchez L, Petrovic I, Cosin-Sales J, Holt DW, Christiansen M, et al. Prognostic value of circulating pregnancy-associated plasma protein-A (PAPP-A) and proform of eosinophil major basic protein (pro-MBP) levels in patients with chronic stable angina pectoris. Clin Chim Acta 2008;391:18–23.
16. Heider P, Pfäffle N, Pelisek J, Wildgruber M, Poppert H, et al. Is serum pregnancy-associated plasma protein A really a potential marker of atherosclerotic carotid plaque stability? Eur J Vasc Endovasc Surg 2010;39:668–75.
17. Li X, Liu Q, Zhou T, Zhao S, Zhou S. PAPP-A: A possible pathogenic link to the instability of atherosclerotic plaque. Med Hypotheses 2008;70:597–9.
18. Terkelsen CJ, Oxvig C, Nørgaard BL, Glerup S, Poulsen TS, et al. Temporal course of pregnancy-associated plasma protein-A in angioplasty-treated ST-elevation myocardial infarction patients and potential significance of concomitant heparin administration. Am J Cardiol 2009;103:29–35.
19. Overgaard MT, Sorensen ES, Stachowiak D, Boldt HB, Kristensen L, et al. Complex of pregnancy-associated plasma protein-A and the proform of eosinophil major basic protein. Disulfide structure and carbohydrate attachment. J Biol Chem 2003;278:2106–17.
20. Holm J-C, Gamborg M, Kaas-Ibsen K, Gammeltoft S, Ward L, et al. Time course and determinants of leptin decline during weight loss in obese boys and girls. Int J Pediatr Obes 2007;2:2–10.
21. Holm J-C, Gamborg M, Ward L, Ibsen KK, Gammeltoft S, et al. Longitudinal analysis of leptin variation during weight regain after weight loss in obese children. Obes Facts 2009;2:243–8.
22. Nysom K, Mølgaard C, Hutchings B, Michaelsen KF. Body mass index of 0 to 45-y-old Danes: reference values and comparison with published European reference values. Int J Obes Relat Metab Disord 2001;25:177–84.
23. Bayes-Genis A, Conover CA, Overgaard MT, Bailey KR, Christiansen M, et al. Pregnancy-associated plasma protein A as a marker of acute coronary syndromes. N Engl J Med 2001;345:1022–9.
24. Christiansen M, Jaliashvili I, Overgaard MT, Ensinger C, Obrist P, et al. Quantification and characterization of pregnancy-associated complexes of angiotensinogen and the proform of eosinophil major basic protein in serum and amniotic fluid. Clin Chem 2000;46:1099–105.
25. Qin QP, Christiansen M, Oxvig C, Pettersson K, Sottrup-Jensen L, et al. Double-monoclonal immunofluorometric assays for pregnancy-associated plasma protein A/proeosinophil major basic protein (PAPP-A/proMBP) complex in first-trimester maternal serum screening for Down syndrome. Clin Chem 1997;43:2323–32.
26. Diggle P, Zeger SL, Liang K-Y, Heagerty P. Analysis of longitudinal data, 2nd ed. Oxford: Oxford University Press, 2002: 379 pp.
27. Ibrahim JG, Chu H, Chen M-H. Missing data in clinical studies: Issues and methods. J Clin Oncol 2012;30:3297–303.
28. Zacho J, Tybjaerg-Hansen A, Jensen JS, Grande P, Sillesen H, et al. Genetically elevated C-reactive protein and ischemic vascular disease. N Engl J Med 2008;359:1897–908.
29. Ridker PM. Clinical application of C-reactive protein for cardiovascular disease detection and prevention. Circulation. 2003;107:363–9.
30. Danesh J, Wheeler JG, Hirschfield GM, Eda S, Eiriksdottir G, et al. C-reactive protein and other circulating markers of inflammation in the prediction of coronary heart disease. N Engl J Med 2004;350:1387–97.
31. Everett BM, Kurth T, Buring JE, Ridker PM. The relative strength of C-reactive protein and lipid levels as determinants of ischemic stroke compared with coronary heart disease in women. J Am Coll Cardiol 2006;48:2235–42.
32. Oude Luttikhuis H, Baur L, Jansen H, Shrewsbury VA, O'Malley C, et al. Interventions for treating obesity in children. Cochrane Database Syst Rev 2009;1:CD001872.