Huang, T., Larsen, K. T., Møller, N. C., Ried-Larsen, M., Frandsen, U., Andersen, L. B. (2015). Effects of a multi-component camp-based intervention on inflammatory markers and adipokines in children: a randomized controlled trial. *Preventive Medicine, 81*, 367-372.

Effects of a physical activity camp intervention on inflammatory markers and adipokines in children: A randomized controlled trial

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Objective: To examine the effects of a multi-component overweight intervention on inflammatory markers and adipokines in children.

Methods: One hundred and fifteen children were recruited in Odense, Denmark (2011-2014). The participants were randomly allocated to either the day camp intervention arm (DCIA) or the standard intervention arm (SIA). The intervention for the DCIA comprised a 6-week camp intervention and a 46-week family-based intervention. The SIA was offered one weekly physical activity session for 6 weeks and one educational meeting. C-reactive protein (CRP), monocyte chemoattractant protein-1 (MCP1), leptin, and adiponectin were measured in serum at baseline, 6 weeks and 52 weeks.

Results: Compared with the SIA, the reductions in CRP (P=0.003) and leptin (p<0.001) were larger in the DCIA at 6 weeks. The intervention effects on leptin were significantly mediated by the changes in body fat mass. No intervention effects on CRP and leptin were seen at 52 weeks. No between-group differences in changes in MCP1 and adiponectin were observed at 6 weeks or 52 weeks.

Conclusions: The 6-week camp intervention resulted in reductions in CRP and leptin. The intervention effects did not persist to 52 weeks. The intervention effect on leptin was explained by the changes in body fat mass.

Introduction

The prevalence of childhood obesity has increased markedly during recent decades (Ng et al., 2014). Obesity causes a low-grade inflammatory status (Ouchi et al., 2011). Inflammatory markers and adipokines contribute to insulin resistance, endothelial dysfunction, systemic inflammation, and atherosclerosis (Hotamisligil, 2006; Rocha and Libby, 2009). Just as in adults (Shoelson et al., 2006), elevated levels of pro-inflammatory markers, such as monocyte chemoattractant protein-1 (MCP1) (Breslin et al., 2012) and leptin (Valle et al., 2005), as well as acute-phase protein C-reactive protein (CRP) (Cook et al., 2000; Visser et al., 2001), have been found in obese children. The level of adiponectin, an anti-inflammatory marker, is decreased in obese children (Valle et al., 2005). Furthermore, studies show that circulating CRP in children is positively associated with individual components of metabolic syndrome and clustered cardiovascular disease (CVD) risk score (Andersen et al., 2010; Cook et al., 2000). In children, high leptin and low adiponectin levels are also found to be predictive of the accumulation of CVD risk factors (Yoshinaga et al., 2008).

However, adipose tissues have depot-specific physiological traits, and their relation to health risk vary among different fat depots (Karpe and Pinnick, 2015). Generally, central obesity (accumulation of visceral

fat and abdominal subcutaneous fat) is correlated with increased risk of CVD, and lower-body fat accumulation is inversely associated with risk of CVD (Lee et al., 2013). Meanwhile, evidence has shown a depot difference in the productions of inflammatory cytokines and adipokines. For example, the expressions of cytokines such as interleukin 6 (IL-6), MCP1, and adiponectin are higher in visceral fat tissue, whereas the expression of leptin is higher in subcutaneous adipose tissue (Tchkonia et al., 2013). A recent study demonstrated that gluteal subcutaneous adipose tissue (GSAT) had less inflammatory profiles than abdominal subcutaneous adipose tissue (ASAT). The release of IL-6 was higher from ASAT than from GSAT (Pinnick et al., 2014). The authors also observed that the android fat mass was positively associated with insulin resistance and CRP, whereas the opposite was seen for gynoid fat mass after adjusting for total fat mass. However, knowledge about how early in life these depot differences between ASAT and GSAT affect the metabolic health is scarce and needs further attention.

Studies have shown that increased physical activity and fitness protect against low-grade inflammation in young people (Platat et al., 2006; Steene-Johannessen et al., 2013). However, relatively few randomized controlled trials (RCTs) have investigated the effects of lifestyle interventions on low-grade inflammation in overweight and obese children. Mixed findings were reported in terms of the intervention effects of exercise and lifestyle intervention on CRP, leptin, and adiponectin (Chae et al., 2010; Kelly et al., 2007; Rosenbaum et al., 2007; Vos et al., 2011). No RCT studies have examined the effects of exercise or lifestyle intervention on MCP1 in children. In addition, most of the previous RCT studies had relatively small sample sizes or short follow-ups.

No previous studies have examined the effects of camp-based interventions on low-grade inflammation in children. The Odense Overweight Intervention Study (OOIS) was a one-year multi-component day camp intervention for overweight and obese children with an RCT design, which resulted in a significant reduction in body fatness (Larsen et al., 2015). We hypothesized that the OOIS intervention would lead to improvements in inflammatory markers and adipokines. Therefore, this study aimed at examining the effects of the intervention on CRP, MCP1, leptin and adiponectin in the OOIS. Given the potential association between body fatness and low-grade inflammation, we also aimed to study whether the intervention effects on inflammatory markers and adipokines were mediated by total body fat mass, android fat mass, and gynoid fat mass.

Methods

The study design, methods, and determination of sample size of the OOIS have been described in detail elsewhere (Larsen et al., 2014) and are summarized below. The study protocol was approved by the

regional scientific ethical committee for Southern Denmark (Approval number: S-20120015). The OOIS was registered with the Danish data protection agency and at clinicaltrial.gov (Registration identifier: NCT01574352). Written informed consents were obtained from children's parents or legal guardians.

Study design and participants

One hundred and fifteen children (11-13 years) were recruited during the mandatory annual examination of fifth-grade school children in 2011 and 2012 in Odense, Denmark. Children were eligible for participating in the OOIS if they exceeded the BMI cut-points for overweight from the International Obesity Task Force (IOTF) (Cole et al., 2000). However, after the completion of the project, we were aware that 6 children who were slightly below the IOTF cut-points at screening were also suggested by the school nurses to participate in the OOIS project. This was due to the fact that the nurses thought that the children were at risk of being overweight. Because the 6 children were included in the randomization, they were not excluded from the analyses. The participants were randomly allocated to either the day camp intervention arm (DCIA) or the standard intervention arm (SIA). Sex stratified concealed block randomization (1:1) with a block size of 2 to 6 (random permuted blocks) was used to ensure balance between groups. Because the study was a behavioral intervention, the blinding of participants and their parents were not possible. Researchers and measurement staff were blinded at all assessments.

Interventions

The intervention for the DCIA comprised two parts - a 6-week day camp intervention and a subsequent 46-week family-based intervention program. The day camps were located in the city of Odense, and took place from middle of May to the end of June in 2012 and 2013, respectively. Participants stayed at a day camp from 7.30 a.m. to 8.30 p.m. for 7 days per week. In the camp, the children were engaged in physical activity and sports as well as health classes. The scheduled exercise sessions were 3 hours per day. These intervention components were structured into four main teaching modules, 1) Your health; 2) Know your city; 3) Get in shape; 4) Run and jump (Table 1). During a camp day, three meals and three snacks were prepared and served according to the national Danish dietary recommendations (Astrup et al., 2005) without caloric restriction. After the 6-week intervention of day camp, a 46-week family-based lifestyle intervention was followed. The intervention consisted of one physical activity day and four parents-involved meetings targeting daily physical activity and dietary behavior. The content of the meetings included presentations given by the nurses or instructors, experience sharing between families, and problem-solving regarding the meeting's theme. The following four themes were addressed during the four meetings: 1) healthy cooking and grocery shopping, 2) networking, 3) everyday physical activity, and 4)

future challenges. An "activity and sports day" was arranged for the children by the camp instructors after the second meeting.

The standard intervention for the SIA consisted of one weekly fun-based physical activity session (approximately 2 hours of duration) for 6 weeks. One health and lifestyle educational session for the parents was delivered by a dietician and physical activity specialist.

Table 1 Teaching modules of intervention components (Denmark from March 2012 to April 2014)

Name of modules	Main purpose	Examples
Your health	Provide participants with systematic knowledge on physical activity, nutrition, and health.	Benefits of exercise, micro- and macro nutrients, Danish recommendations on food and physical activity, etc.
Know your city	Increase the participants' knowledge and interest regarding the city and its activity facilities.	Activities at local natural environments, visiting local sports clubs, etc.
Get in shape	Provide theoretical knowledge and practical experiences about how to improve physical fitness.	Circle training, flexibility training, strength training, and adventure race, etc.
Run and jump	Improve participants' motor skills, coordination, and physical fitness.	Ball games, gymnastics, dancing, and other games, etc.

Measures

Anthropometrics

Height was measured to the nearest 0.5 cm without footwear. Body weight was measured to the nearest 0.1 kg in underwear using a Soehnle professional medical electronic scale (Soehnle Industrial Solutions GmbH, Backnang, Germany). BMI was calculated as weight (kg) divided by height (m) squared. BMI z-score was calculated based on the reference data from 2000 CDC (Centers for Disease Control and Prevention, US) children's growth chart. Body composition was estimated by dual energy X-ray absorptiometry (DEXA). The scanning was performed by an experienced operator on a GE Lunar Prodigy (GE Medical Systems, Madison, WI, USA). The regional fat depots were defined by manufacturer's enCORE software (GE Medical Systems, Madison, WI, USA). Specifically, the android region is located in the area between the ribs and the pelvis. The lower limit of android region is at upper rim of the pelvis. The upper limit of android region is

above the upper rim of pelvis by 20% of the distance between the iliac crest and the base of skull. The upper limit of gynoid region is below the top of the iliac crest by 1.5 times of the android height. The height of gynoid region is two times the height of android region.

Blood measures

Venous blood samples were drawn in the morning after an over-night fast. Participants were lying supine during blood collection. Samples for serum were left at room temperature for 30 minutes. All samples were centrifuged at 2500G for 10 minutes at 4°C. Serum was stored at −80°C until analysis. Serum samples were analyzed using AlphaLISA® immunoassay kits (PerkinElmer, Waltham, MA, USA) for CRP, leptin, MCP1, and total adiponectin in 384 microplate platform. All intra-assay %CVs were less than 11. The AlphaLISA bead-based assays are homogeneous, no-wash immunoassays with high sensitivity and wide dynamic ranges (Bielefeld-Sevigny, 2009). In brief, serum samples (3 μL) were mixed with freshly prepared AlphaLISA anti-analyte acceptor beads (10 μg/mL) and biotinylated antibody anti-analyte (1 nM). After incubating for 60 minutes at room temperature in the dark, 10 μl of Streptavidin-donor beads (40 μg/mL) was added to each well and incubated for an additional 60 minutes in the dark. Data was generated by reading AlphaLISA counts using an Enspire 2300 multimode plate reader (PerkinElmer, Waltham, MA, USA). Background corrected data was analyzed using a sigmoidal nonlinear regression with variable slope and a $1/Y^2$ data weighting. Serum samples used for CRP and Adiponectin were pre-diluted (1:120) using AlphaLISA buffer. The observations of CRP≥10 mg/L were excluded from analyses, because the high values likely indicated acute clinical infection (Skinner et al., 2010). Therefore, 13 observations of CRP were excluded from analysis.

Physical activity during the camp

The physical activity was assessed for 7 consecutive camp days by accelerometers (Actigraph GT3X+, Pensacola, FL, USA). The data were analyzed using open-source software (Propero v. 1.7.4).

Parental educational level and ethnicity

Information on parental educational level and ethnicity was obtained by questionnaires at baseline. Parental educational level was used as an indicator of parental social economic status (SES). The parental education level was collapsed into 3 categories: (I) Basic school of no more than 10 years, (II) High school or non-university vocational programs, (III) College or university degrees. Ethnicity was categorized into Danish and non-Danish.
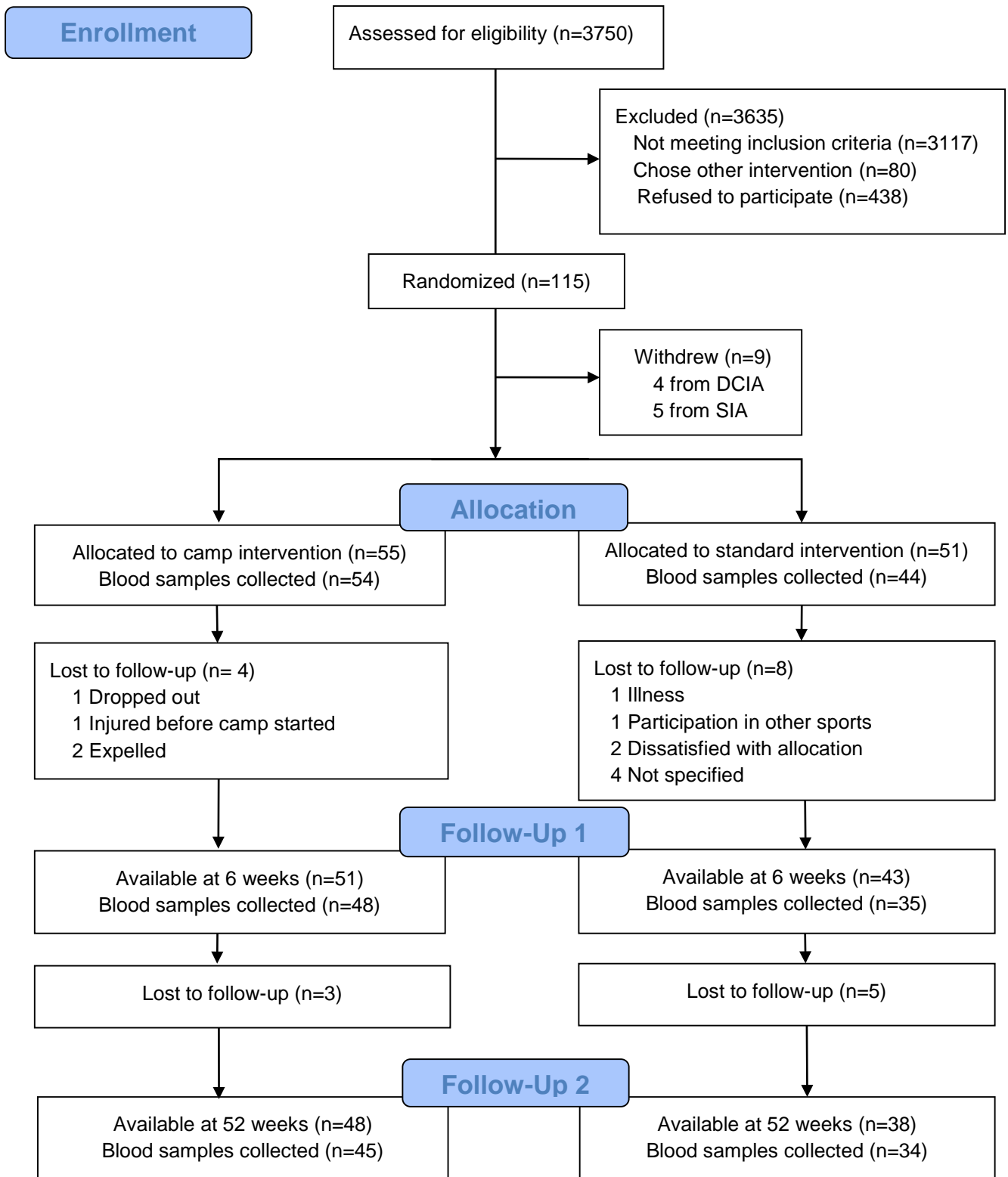
Fig.1 Participants flow chart (Denmark from March 2012 to April 2014)

Pubertal development

Pubertal development was assessed using the Tanner scale (Tanner, 1962). The children self-reported their sexual development by comparison with Tanner's drawings in a confidential room. For this study, girls were staged according to breast development. Boys were staged according to genital development.

Statistics

Statistical analyses were conducted with STATA 12 for Windows (StataCorp LP, College Station, TX, USA), and the level of significance was set at $P < 0.05$ (two-sided). Baseline descriptive characteristics were summarized by group. For continuous variables, the differences were evaluated using an independent sample t-test. Categorical variables were assessed using Chi-squared test. Correlations between baseline body fat mass and inflammatory markers were examined using Spearman's rank correlation. The intervention effects were analyzed with linear mixed effects modelling without data imputation. Linear mixed effects modelling allow the inclusion of partial data of participants who may have dropped out or who were unavailable at follow-ups. The initial analyses modeled for effects for time, group, and time by group interaction with sex and parental educational level as covariates. Because 6 participants did not provide information on parental education level and the effects of parental educational level were not significant for all models, parental educational level was removed from final models. For all linear mixed-model analyses, distributions of residuals at level 1 (repeated measures) and level 2 (measures nested in child) were checked graphically and natural logarithmic transformations were used to improve normality when necessary. The results (difference in changes) from log CRP and log MCP1 were exponentiated as ratios of geometric means of changes between two groups.

When a significant intervention effect was identified, we further examined whether the intervention effect was mediated by total body fat mass, android fat mass, and gynoid fat mass. According to the procedure outlined by Baron and Kenny (1986), a series of linear regression models were fitted on the changes of independent variable (intervention), dependent variables (inflammatory markers), and mediators (total or regional body fat mass). The first model regressed the mediator on the independent variable (path a). The second regressed the dependent variable on the independent variable (total effect, path c). The third regressed dependent variable on both independent variable (direct effect, path c') and mediator (path b). The path analyses were controlled for sex. The statistical significance of mediation effects was assessed by Sobel approach (Sobel, 1982). Furthermore, a bootstrapping approach (Mackinnon et al., 2004) (5000 bootstrap resamples) was used to obtain bias-corrected 95% confidence interval (CI) of the mediation

effect (a×b) and direct effect (path *c'*). The proportion of mediation was calculated by dividing the mediation effect (a×b) by the total effect (c).

Results

Participant flow is presented in Fig. 1. Nine children (4 from the DCIA and 5 from the SIA) withdrew after the randomization, which led to 51 children in the SIA and 55 children in the DCIA at baseline. Of the 106 children, 98 provided blood samples at baseline. Descriptive characteristics at baseline are shown in Table 2. The SIA had a larger proportion of higher parental educational level (P=0.04). No differences were found in other baseline characteristics.

Forty-nine children in the DCIA provided accelerometer data. The mean worn time was 12.7 (SD 0.2) hours per day. The mean physical activity level was 793 counts per minute (CPM) per day. According to Evenson's cut points (Evenson et al., 2008), on each camp day the children accumulated 91.6 (SD 20.4) minutes of MVPA, 238.9 (SD 43.2) minutes of light physical activity, and 432.8 (SD 55.1) minutes of sedentary time.

Table 3 summarizes the results for baseline, follow-ups and changes in CRP, MCP1, leptin and adiponectin. At baseline, CRP was positively correlated with total body fat mass (r=0.44, P<0.001), android fat mass (r=0.51, P<0.001), and gynoid fat mass (r=0.38, P<0.001). Leptin was positively correlated with total body fat mass (r=0.70, P<0.001), android fat mass (r=0.70, P<0.001), and gynoid fat mass (r=0.69, P<0.001). Adiponectin was negatively correlated with total body fat mass (r=-0.31, P=0.03), android fat mass (r=-0.34, P=0.01), and gynoid fat mass (r=-0.29, P=0.03) only in girls. MCP1 was not correlated with total body fat mass, android fat mass, and gynoid fat mass (all P>0.05).

The reduction in CRP was larger in the DCIA at 6 weeks (ration of geometric means in changes, 0.45, 95% CI, 0.26 to 0.76, P=0.003), which suggested that the CRP reduction in the DCIA was 55% higher than that in the SIA at 6 weeks. The reduction in leptin was larger in the DCIA at 6 weeks (difference in changes, -2.56, 95% CI, -3.43 to -1.69, P<0.001) compared with the SIA. The between-group differences in changes in CRP and leptin were not significantly different at 52 weeks (both P>0.05). The between-group differences in changes in MCP1 and adiponectin were not different at 6 or 52 weeks (all P>0.05).

We further examined whether the intervention effects on CRP and leptin could be explained by changes in body fat mass and regional fat mass (Table 4). Single mediation analysis demonstrated that the intervention effects on leptin were significantly mediated by total body fat mass (mediation effect, -2.08, 95% CI, -3.38 to -0.79), accounting for 83% of the total effect. Similarly, android fat mass could account for 73%

Table 2 Baseline participant characteristics (Denmark from March 2012 to April 2014)

Characteristics	No.	SIA (n=44)	DCIA (n=54)	Total
Age (year)	98	12.0 (0.5)	12.0 (0.3)	12.0 (0.4)
Sex (Girl/boy)	98	25/19	29/25	54/44
Parental educational level * (I/II/III)	92	9/12/20	15/24/12	24/36/32
Ethnicity (Danish/Non Danish)	98	30/14	33/21	63/35
Tanner stage (I/II/III/IV/V)	98	1/11/23/7/2	2/15/27/10/0	3/26/50/17/2
Weight (kg)	98	59.6 (9.1)	61.7 (8.7)	60.7 (8.9)
Height (cm)	98	156.1 (5.7)	156.3 (6.5)	156.2 (6.0)
BMI (kg/m^2)	98	24.4 (3.0)	25.2 (2.9)	24.8 (2.9)
BMI z-score	98	1.5 (0.4)	1.6 (0.4)	1.6 (0.4)
Total body fat mass (kg)	96	22.6 (6.5)	24.4 (6.6)	23.6 (6.6)
Android fat mass (kg)	96	1.9 (0.7)	2.1 (0.6)	2.1 (0.7)
Gynoid fat mass (kg)	96	4.3 (1.1)	4.5 (1.0)	4.4 (1.0)
Total body fat (%)	96	38.5 (6.3)	39.6(6.3)	39.1 (6.3)

Abbreviations: BMI, body mass index.

Data is expressed as mean and standard deviation (SD) for continuous variables and frequency for categorical variables.

Parental educational level were mainly based on mother's highest education (I = Basic school of no more than 10 years; II = High school or non-university vocational programs; III = College/university or higher degrees).

* Significant between-group difference was found on the parental educational level (p=0.04).

Table 3 Changes in inflammatory markers and adipokines in SIA and DCIA at 6 weeks and 52 weeks (Denmark from March 2012 to April 2014)

	Baseline	6 weeks	52 weeks	Within-group change at 6 weeks Mean/Ration (95 % CI)	Within-group change at 52 weeks Mean/Ratio (95 % CI)	Between-group differences in changes at 6 weeks Mean/Ratio (95 % CI)	P	Between-group differences in changes at 52 weeks Mean/Ratio (95 % CI)	P
CRP (mg/L)[a]									
SIA (n=44)	1.17 (1.42 to 2.76)	0.99 (0.65 to 1.52)	0.64 (0.40 to 1.01)	0.88 (0.59 to 1.32)	0.58 (0.39 to 0.88)*	0.45 (0.26 to 0.76)	0.003	0.87 (0.50 to 1.49)	0.61
DCIA (n=50)	1.49 (1.00 to 2.21)	0.55 (0.40 to 0.74)	0.74 (0.51 to 1.08)	0.39 (0.28 to 0.56)*	0.51 (0.35 to 0.72)*				
MCP1 (pg/mL)[a]									
SIA (n=44)	306.53 (259.82 to 361.63)	312.54 (236.05 to 413.82)	298.00 (249.81 to 355.48)	1.00 (0.82 to 1.22)	0.95 (0.78 to 1.16)	1.05 (0.80 to 1.36)	0.74	1.08 (0.83 to 1.41)	0.56
DCIA (n=54)	297.92 (254.11 to 349.28)	310.58 (271.86 to 354.81)	308.60 (275.72 to 345.40)	1.05 (0.88 to 1.24)	1.03 (0.86 to 1.22)				
Leptin (ng/mL)[b]									
SIA (n=44)	5.35 (2.72)	4.63 (2.56)	4.29 (2.61)	-0.61 (-1.27 to 0.05)	-0.96 (-1.62 to -0.30)*	-2.56 (-3.43 to -1.69)	<0.001	0.07 (-0.81 to 0.96)	0.87
DCIA (n=54)	5.77 (2.49)	2.53 (1.68)	4.59 (2.17)	-3.17 (-3.74 to -2.60)*	-0.89 (-1.47 to -0.31)*				
Adiponectin (µg/mL)[b]									
SIA (n=44)	40.09 (36.48)	31.90 (19.00)	29.56 (13.99)	-8.78 (-17.06 to -0.49)*	-10.10 (-19.88 to -0.32)*	5.95 (-5.06 to 16.96)	0.29	2.47 (-10.62 to 15.56)	0.71
DCIA (n=54)	40.40 (25.96)	37.88 (31.22)	33.02 (20.99)	-2.82 (-10.07 to 4.42)	-7.63 (-16.34 to 1.07)				

Abbreviations: CI, confidence interval; CRP, C-reactive protein; DICA, Day camp intervention arm; MCP1, Monocyte chemoattractant protein-1; SIA, Standard intervention arm.

[a] Natural logarithmic transformed CRP and MCP1 were used for the analyses. The values of baseline and follow-ups are expressed as geometric mean and 95% CI. The regression coefficients and 95% CI were exponentiated. Therefore, the within-group changes and between-group differences in changes of CRP and MCP1 represented the ratios of geometric means of changes between two groups.

[b] The values of baseline and follow-ups are expressed as mean and SD.

*indicates a significant within-group difference compared with baseline (p<0.05).

Table 4 Mediation analysis for changes in CRP and leptin in SIA and DCIA at 6 weeks (Denmark from March 2012 to April 2014)

Dependent variable	Mediator	Total effect (Path c)	Intervention effects on mediator (path a)	Direct effect (path c')[a]	Effects of mediator on dependent variable (path b)	Mediation effect (a×b)[a]
CRP	Total body fat mass	-2.02 (-3.12 to -0.92)*	-4.73 (-5.52 to -3.95)*	-2.19 (-4.31 to -0.07)*	-0.04 (-0.38 to 0.30)	0.17 (-1.58 to 1.92)
	Android fat mass	-2.02 (-3.12 to -0.92)*	-0.47 (-0.57 to -0.38)*	-1.74 (-3.68 to 0.20)	0.59 (-2.21 to 3.39)	-0.28 (-1.84 to 1.28)
	Gynoid fat mass	-2.02 (-3.12 to -0.92)*	-0.76 (-0.92 to -0.61)*	-2.06 (-3.70 to -0.43)*	-0.06 (-1.79 to 1.67)	0.05 (-1.44 to 1.53)
Leptin	Total body fat mass	-2.51 (-3.38 to -1.65)*	-4.53 (-5.28 to -3.78)*	-0.43 (-1.88 to 0.98)	0.46 (0.22 to 0.70)*	-2.08 (-3.38 to -0.79)*
	Android fat mass	-2.51 (-3.38 to -1.65)*	-0.45 (-0.54 to -0.36)*	-0.68 (-1.86 to 0.50)	4.09 (2.15 to 6.03)*	-1.84 (-2.88 to -0.79)*
	Gynoid fat mass	-2.51 (-3.38 to -1.65)*	-0.75 (-0.90 to -0.61)*	-0.96 (-2.33 to 0.41)	2.06 (0.79 to 3.34)*	-1.55 (-2.79 to -0.31)*

Abbreviations: CRP, C-reactive protein.

Data are expressed as estimates and 95% CI.

[a] Bootstrapped and bias-corrected estimates.

* P<0.05.

(mediation effect, -1.84, 95% CI, -2.88 to -0.79) of the total effect. Gynoid fat mass could account for 62% (mediation effect, -1.55, 95% CI, -2.79 to -0.31) of the total effect. Conversely, the intervention effects on CRP at 6 weeks were not mediated by the changes in total body fat mass, android fat mass, and gynoid fat mass.

Discussion

The major findings were that the DCIA had larger reductions in CRP and leptin at 6 weeks compared with the SIA. The improvement on leptin could be explained by changes of total body fat mass, android fat mass, or gynoid fat mass. However, the improvement on CRP was not explained by the changes in total body fat mass or regional fat mass. No intervention effects on CRP and leptin were observed at 52 weeks. No intervention effects were found in adiponectin and MCP1 at 6 or 52 weeks.

CRP is a common and sensitive inflammatory marker. To the best of our knowledge, this was the first report which examined the change in CRP after a camp-based intervention. Despite varying in intervention components, our results were consistent with the previous studies. For example, Chae et al. (2010) found that CRP and body weight were decreased in obese children after a 12-week structured exercise program including nutritional education. Rosenbaum et al. (2007) reported that a 3 to 4-month school-based intervention program (aerobic exercise and health education classes) significantly reduced body fatness and CRP in children. We extended the existing knowledge by examining the effects over one-year period of time. However, no intervention effects on CRP were observed at 52 weeks, although the intervention effects on body fatness persisted at 52 weeks (Larsen et al., 2015). Studies suggest that regular exercise may improve low-grade inflammation by reducing body fat mass and local inflammation in fat tissue (Gleeson et al., 2011; You et al., 2013). However, in this study, the reduction in CRP at 6 weeks was not mediated by the changes in total body fat mass or regional fat mass. Similarly, Okita et al. (2004) showed that two months of aerobic exercise resulted in reduced body weight and CRP in adults, though the changes in CRP were not proportionally associated with the extent of weight loss. One explanation for the disproportional changes in fat mass and CRP may be that exercise exerted its anti-inflammatory effects by other mechanisms. Evidence has suggested some potential mechanisms underlying the anti-inflammatory effects of exercise, including effects on muscle tissue to produce anti-inflammatory myokines, effects on endothelial cell to reduce leukocyte adhesion, and reducing pro-inflammatory cytokines (Gleeson et al., 2011; You et al., 2013). Nonetheless, the visceral fat mass was not quantified in our study. Therefore, our results did not preclude the possibility of a mediation effect of visceral fat mass.

The 6-week camp intervention resulted in a greater reduction in leptin compared with the SIA. In an RCT study, Balagopal et al. (2010) found a reduction in leptin and increase in soluble leptin receptor in obese children after a 3-month lifestyle intervention (physical activity, caloric restriction and limiting TV time). In an intervention study in obese children, Bluher et al. (2014) found that leptin decreased after a one-year lifestyle intervention (physical activity and educational classes for parents) compared with baseline. In the current study, we did not find intervention effects on leptin at 52-week follow-up. However, a *post-hoc* analysis showed that the changes in total body fat mass was positively correlated with the changes in leptin at 52 weeks (r=0.44, P<0.001). Furthermore, our mediation analysis demonstrated that the reduction in leptin at 6 weeks was mediated by the changes in body fat mass. Leptin is secreted by adipocytes and its level in blood is positively correlated with adipose mass (Ouchi et al., 2011). Accordingly, it is reasonable to speculate that the reduction in body fat mass led to decreased expression of leptin and improvement in leptin resistance. Our results are in accordance with previous studies which showed that exercise programs resulting in reduced fat mass usually decreased leptin concentration (Bouassida et al., 2010).

MCP1, a well-known inflammatory chemokine, plays an important role in regulating migration and infiltration of monocytes/macrophages, thereby contributing to initiation of inflammation (Deshmane et al., 2009). Breslin et al. (2012) found that monocytes and MCP1 were elevated in obese children. Althrough our intervention led to a greater weight loss in the DCIA compared with the SIA (Larsen et al., 2015), no intevention effects were observed on MCP1. Similarly, Roth et al. (2011) showed that MCP1 was not significantly changed in children who successfully lost weight after a lifestyle intervention (physical activity, nutrition education and behavior therapy). Adiponectin is an anti-inflammatory adipokine and plays a protective role against metabolic disorder (Ouchi et al., 2011). The adiponectin was significantly decreased in the SIA. However, consistent with the previous intervention studies in children (Chae et al., 2010; Rosenbaum et al., 2007), the changes in adiponectin in the current study did not differ between groups. In contrast, a study in obese adolescents showed increased adiponectin after a 3-month lifestyle intervention (physical activity, caloric restriction and limiting TV time) (Balagopal et al., 2005). Given the inconsistent findings, more research is needed to examine the changes in MCP1 and adiponectin in response to lifestyle interventions.

The strengths of the current study include its RCT design and one-year follow-up. It enabled us to look at the long-term changes in the inflammatory markers and adipokines following a camp-based intervention. We also acknowledge a number of potential limitations of this study. Although the participants were characterized by diversity of parental SES and ethnicity, only 115 children of fifth grade were enrolled in the study. Therefore, the generalizability of the findings may be limited. The blood samples were not available

from all of the participants, which may reduce the statistical power to detect the intervention effects at 52 weeks. It is also noteworthy that the blood samples were not analyzed immediately after sampling due to the multiple times of data collection. The serum samples were stored at -80°C for various periods of time (from 5 months to 30 months), but serum CRP was previously shown to be stable up to 11 years at -80°C (Doumatey et al., 2014).

In conclusion, the 6-week day camp intervention resulted in reductions in CRP and leptin. The intervention effect on leptin, but not CRP, was explained by the changes in body fat mass. The intervention effects on CRP and leptin did not persist to 52 weeks. No intervention effects on MCP1 and adiponectin were observed at 6 weeks or 52 weeks.

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