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ORIGINAL RESEARCH

Intensive Lifestyle Intervention Increases Plasma Midregional Proatrial Natriuretic Peptide Concentrations in Overweight Children

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BACKGROUND: Overweight adults have low circulating concentrations of ANP (atrial natriuretic peptide) and proANP fragments. We tested the hypothesis that an intensive lifestyle intervention with an intended weight loss would increase plasma concentrations of a proANP fragment in overweight children.

METHODS AND RESULTS: We measured MR-proANP (midregional proANP) concentrations in plasma from overweight children who participated in the OOIS (Odense Overweight Intervention Study). OOIS randomized 115 overweight children (11–13 years, 55% girls) to an intensive day-camp intervention arm with increased physical activity and healthy diet or to a less intensive standard intervention arm for 6 weeks. We used linear mixed-effects modeling for repeated measures to estimate the difference in the mean change with 95% CIs in fasting plasma MR-proANP concentrations between the 2 arms, and we used partial least squares regression analysis to identify candidate mediators. Differences in weight, fitness, and metabolic factors were also analyzed. At baseline, fasting plasma MR-proANP concentrations were (median [interquartile range]) 35.0 pmol/L (26.8–42.0) in the day-camp intervention arm and 37.2 pmol/L (31.7–44.7) in standard intervention arm participants, respectively. After 6 weeks intervention, children in the day-camp intervention arm had increased their MR-proANP (5.4 pmol/L [0.8–10.0], P=0.022) and their fitness (2.33 mL O_2/min per kg [0.52–4.14], P=0.012) and they had deceased their body mass index (–2.12 kg/m^2 [–2.59 to –1.65], P<0.001) as compared with children in standard intervention arm. In the partial least squares analysis, decreases in fasting insulin and in estimated insulin resistance were associated with the observed increase in MR-proANP concentrations.

CONCLUSIONS: An intensive lifestyle intervention increases plasma MR-proANP among overweight children.

REGISTRATION: URL: https://www.clinicaltrials.gov; Unique identifier: NCT01574352.

Key Words: atrial natriuretic peptide ■ children ■ fitness ■ overweight ■ weight loss

ANP (atrial natriuretic peptide) is well known as a natriuretic, diuretic, and vasodilatory hormone, but ANP has also metabolic actions and increases lipolysis and lipid oxidation.[1] It is also well known that higher circulating concentrations of ANP and its prohormone fragments are associated with a poor prognosis among patients with cardiovascular disease.[2,3] What may be less well known, and perhaps even counterintuitive, is that higher circulating concentrations of N-terminal proANP (proatrial natriuretic peptide) in Framingham Offspring Study participants free of cardiovascular disease were found to associate with an ideal American Heart Association Cardiovascular Health Score,[4] which includes an ideal body mass

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CLINICAL PERSPECTIVE

What Is New?

- In a randomized controlled trial in overweight children, an intensive lifestyle intervention, which effectively reduced weight and improved cardiorespiratory fitness, significantly increased plasma MR-proANP (midregional proatrial natriuretic peptide) concentrations as compared with a less intensive standard intervention.
- In mediation analysis, decreases in plasma insulin concentrations and in estimated insulin resistance were the 2 factors that explained most of the observed increase in plasma MR-proANP concentrations.

What Are the Clinical Implications?

- It is well established that a high plasma concentration of ANP and its prohormone fragments are associated with a poor prognosis among patients with cardiovascular disease.
- Nevertheless, there are several studies in adults showing that a proANP fragment concentration high in the normal range is a marker of a healthy lifestyle and cardiometabolic health.
- The results of this study extend the findings in adults to children that a healthier lifestyle with improved cardiometabolic health is associated with higher plasma concentrations of a proANP fragment and lend further support to the notion that close links exist between metabolism and the endocrine heart.

Nonstandard Abbreviations and Acronyms

DCIA	day-camp intervention arm
HOMA	homeostasis model assessment
MR-proANP	midregional proatrial natriuretic peptide
OOIS	Odense Overweight Intervention Study
PLS	partial least squares
proANP	proatrial natriuretic peptide
SIA	standard intervention arm

index (BMI), regular physical activity, and an optimal profile of serum cholesterol, blood pressure, and glucose.[5] Along this line, overweight individuals have lower than expected circulating concentrations of ANP and proANP fragments,[6–12] and it has been proposed that a relative ANP deficiency could play a role in overweight-related disorders and health problems, such as hypertension and inability to lose weight.[1,6,7] Furthermore, the relationship between overweight and low circulating concentrations of ANP could be causal because weight loss is associated with an increase in circulating concentrations of proANP fragments.[13–15] The exact mechanisms behind the lower than expected circulating concentrations of ANP and proANP fragments in overweight individuals are still not clear, but overweight-induced insulin resistance with compensatory hyperinsulinemia has been suggested as a possible mechanism.[1,8,10,16–18]

So far, no data have been published on the effect of weight loss and increased physical activity on circulating concentrations of ANP and its prohormone fragments in overweight children from the general childhood population. Therefore, in this study, we tested the hypothesis that an intensive lifestyle intervention with an intended weight loss, as a result of increased physical activity and a healthy diet, would lead to increases in plasma concentrations of a proANP fragment in overweight children. We took advantage of (1) already existing data from the randomized controlled OOIS (Odense Overweight Intervention Study),[19] which showed that a 6-week day-camp intervention, with increased physical activity and a healthy diet, effectively reduced BMI and improved metabolic health of overweight children as compared with a standard control intervention, and (2) the OOIS's biobank, which enabled us to measure fasting plasma concentrations of MR-proANP (midregional proANP), which is a stable marker of ANP secretion.[20]

METHODS

The authors declare that all supporting data are available within the article.

Study Population

The OOIS population has been described in detail elsewhere.[19,21,22] In public schools in Odense, Denmark, all fifth-grade school children were screened during their annual health examination in 2011 to 2012 and 2012 to 2013 to identify candidates for the study. The study took place in Odense from mid-May to the end of June in 2012 and 2013, respectively. Overweight was defined according to the International Obesity Task Force.[23] In total, 115 overweight children (11–13 years, girls 55%) were randomized, but as described elsewhere,[19] only 106 entered the study. For this OOIS substudy, we focused on the 99 children who had at least 1 measurement of MR-proANP. The study protocol was approved by The Regional Scientific Ethical Committee for Southern Denmark (Approval number: S-20120015) and registered with the Danish Data Protection Agency and at ClinicalTrial.gov (Registration

number: NCT01574352). Written informed consent from children and their parents or legal guardians were obtained.

Intervention Arms

The overweight children were informed about the weight-loss program, which entailed a sex-stratified block randomization (1:1) to either a day-camp intervention arm (DCIA) or to a standard intervention arm (SIA). DCIA consisted of 2 parts: a 6-week day-camp intervention and a subsequent 46-week family-based intervention program. Children in DCIA stayed at the camp from 7:30 AM to 8:30 PM for 7 days per week. Here they were engaged in physical activity and sports (3 hours per day) as well as other health-promoting initiatives. At the camp, 3 meals and 3 snacks were prepared and served following the Danish dietary guidelines without caloric restriction.[19,21] After the 6-week day-camp intervention, the 46-week family-based lifestyle intervention began. This intervention consisted of 1 activity day and 4 meetings with the families promoting daily physical activity and healthy dietary behavior. SIA consisted of 1 weekly fun-based physical activity session (2 hours of duration) for 6 weeks. Further, 1 healthy lifestyle promoting session for the parents took place.

Measurements

Weight and height were measured with underwear without shoes. Pubertal development was assessed according to Tanner stages by self-evaluation. Body composition was assessed by dual energy X-ray absorptiometry.

Cardiorespiratory fitness was assessed using a progressive bicycle ergometer protocol (Monarch Ergomedic 839e) until total exhaustion with indirect calorimetry (Innovision, AMIS 2001) and a Polar RS800CX heart rate monitor.

Venous blood samples in the fasting state were obtained in the morning. MR-proANP in plasma was measured with a commercially available sandwich chemiluminescence immunoassay (Thermo-Fisher, Hennigsdorf/Berlin, Germany). This MR-proANP assay has a limit of quantitation of 6.0 pmol/L, and the intra-assay coefficient of variation is <10% and <20% for samples containing 23 to 3000 and 18.0 to 22.9 pmol/L MR-proANP, respectively. Insulin in plasma and glucose in serum were measured as described elsewhere,[19,21] and CRP (C-reactive protein), leptin, and adiponectin in serum were determined as described in detail in another publication.[22] We used the homeostasis model assessment (HOMA) method to estimate insulin resistance from the fasting glucose and insulin concentrations.[24]

Blood pressure was measured on the left upper arm with an automatic blood pressure monitor (Welch Allyn 300 series) after 5 minutes of rest in the sitting position. Measurements were taken once per minute (min. 5 measurements) until blood pressure was stable. The mean of the last 3 measurements was used.

Statistical Analysis

For statistical analyses, we used Stata version 15.1 SE (StataCorp LP, College Station, TX, USA) and Sirius version 11.0 (Pattern Recognition Systems AS, Bergen, Norway). Significance level was set at $P<0.05$. The Shapiro–Wilk test was used to test continuous data for normality,[21] and normally distributed continuous data are presented as means±SD and nonnormally distributed data are presented as medians with interquartile ranges. Categorical data are presented as frequency percentage and as numbers. The anthropometric, hemodynamic, and metabolic variables selected for analyses in this OOIS substudy (Table 1) are variables shown to associate with circulating concentrations of proANP fragments and suggested to affect ANP physiology.[1,25,26]

The primary outcome measure of this OOIS substudy was group difference in change in plasma MR-proANP concentrations after 6 weeks intervention. A secondary outcome measure was group difference in change in plasma MR-proANP concentrations 52 weeks after the start of the study. To determine the primary outcome measure consistent with the CONSORT statement for reporting parallel group randomized trials,[27] and as described in detail elsewhere,[19,21] we used linear mixed-effects modeling for repeated measures to estimate the difference in the mean change with 95% CIs in fasting MR-proANP concentrations in plasma between the 2 intervention arms after week 6. We also used mixed-effects modeling for repeated measures to determine the secondary outcome measure and to determine demographic, anthropometric, hemodynamic, and metabolic outcome measures. Furthermore, we extracted estimates from the linear mixed models to report within-group changes. In the case of nonnormally distributed data, the analyses for difference in change were performed on logarithmically transformed data, which was necessary to do to fulfill linear mixed model assumption criteria. The results were subsequently exponentially back transformed and presented as percentage change between groups.

We used partial least squares (PLS) regression analysis to identify possible candidate mediators of the hypothesized increase in plasma concentrations of MR-proANP related to the intensive lifestyle intervention. Because we already knew that the effect of the

Table 1. Baseline Characteristics of Study Population

Variables	Day-camp intervention arm (n=54)	Standard intervention arm (n=45)
Demographic		
Female sex, % (n)	54% (29)	56% (25)
Age, y	12.0 ± 0.3	12.0 ± 0.5
Pubertal development, Tanner stages 1/2/3/4/5, n	2/15/27/10/0	1/12/23/7/2
Anthropometric		
Weight, kg	61.7 ± 8.7	59.5 ± 9.0
Body mass index, kg/m^2	25.2 ± 2.9	24.5 ± 3.0
Body composition		
Total fat mass, %	39.6 ± 6.3	38.7 ± 6.3
Abdominal fat, %	48.1 ± 7.1	47.0 ± 7.6
Hemodynamic		
Systolic blood pressure, mm Hg	106.8 ± 7.6	104.1 ± 8.8
Diastolic blood pressure, mm Hg	55.9 ± 6.9	53.7 ± 5.8
Metabolic		
Glucose, mmol/L	5.0 ± 0.4	4.9 ± 0.4
Insulin, µIU/mL	9.4 (6.6–12.3)	8.2 (6.3–13.4)
Homeostatic model assessment for insulin resistance, units	2.1 (1.5–2.9)	1.7 (1.3–2.9)
C-reactive protein, mg/L	2.5 (0.5–5.4)	1.1 (0.5–3.0)
Adiponectin, µg/mL	31.2 (21.4–50.9)	31.1 (19.2–48.3)
Leptin, ng/mL	5.8 ± 2.5	5.3 ± 2.7
Natriuretic		
Midregional proatrial natriuretic peptide, pmol/L	35.5 ± 10.6	39.3 ± 11.3
Exercise		
Fitness, mL O_2/min per kg	33.1 ± 5.5	35.1 ± 5.3

Normally distributed continuous data are presented as mean±SD and nonnormally distributed data are presented as median (interquartile range). Categorical data are presented as frequency percentage and numbers.

intensive lifestyle intervention diminished over time,[21] our mediation analysis included only data from baseline and week 6. The PLS regression analysis methodology has been described in detail elsewhere.[28] PLS regression analysis determines the multivariate association pattern between a selected outcome variable (here change in plasma MR-proANP concentrations from baseline to week 6) and selected explanatory variables (here change in the variables in Table 1, except demographic variables, from baseline to week 6, without and with adjustment for treatment arms).[28] Through decomposing the explanatory variables into orthogonal linear combinations (PLS components), while simultaneously maximizing the covariance with the outcome variable, PLS regression can handle collinear variables.[28] Before PLS regression analysis, all variables were centered and standardized to unit variance.[28] The Monte Carlo resampling method with 250 repetitions was used to select the number of PLS components optimizing the predictive performance of the models by randomly keeping 50% of the children as an external validation set.[29] For each cross-validated PLS regression model, a single predictive component was calculated expressing all the predictive variance in the selected explanatory variables related to change in plasma MR-proANP concentrations in a single vector.[30,31] Selectivity ratios with 95% CIs were obtained as the ratio of this explained predictive variance to the total variance for each selected explanatory variable.[32,33] These results are shown in a selectivity ratio plot (Figure), which quantitatively displays each explanatory variables' importance for increase in plasma MR-proANP concentrations. The size of selectivity ratio for each explanatory variable describes how large a proportion of the variance it explains and thus also how large a proportion of the mediating effect related to the intensive lifestyle intervention it explains.

RESULTS

Baseline characteristics of the study population stratified by intervention arms are summarized in Table 1.

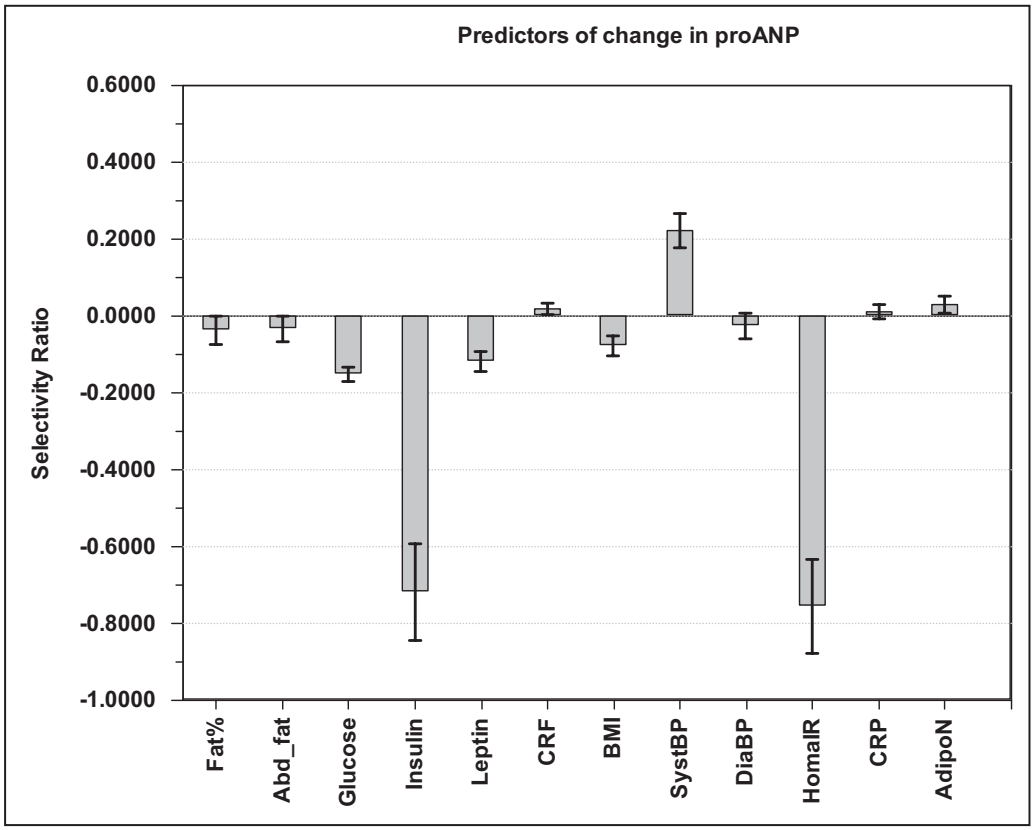

Figure. The multivariate association pattern between change in MR-proANP (midregional proatrial natriuretic peptide) concentrations and change in selected explanatory variables, from baseline to week 6, are displayed as a selectivity ratio (SR) plot.

The explanatory variables included change in total fat mass % (Fat% in figure), change in abdominal fat % (abd fat in figure), change in fasting glucose concentrations (Glucose in figure), change in fasting insulin concentration (Insulin in figure), change in fasting leptin concentrations (Leptin in figure), change in cardiorespiratory fitness (CRF in figure), change in body mass index (BMI in figure), change in systolic blood pressure (SysBP in figure) change in diastolic blood pressure (DiaBP in figure), change homeostatic model assessment for insulin resistance (HomaIR in figure), change in C-reactive protein concentration (CRP in figure), and change in fasting adiponectin concentrations (AdipoN in figure). The columns in the SR plot represent percent explained change with 95% CIs of the total explained change in MR-proANP concentrations.

Mixed Model Results

The effects of the interventions on plasma concentrations of MR-proANP, weight, BMI, total fat mass %, abdominal fat mass %, systolic blood pressure, diastolic blood pressure, plasma or serum concentrations of insulin, glucose, CRP, adiponectin, leptin, HOMA estimated insulin resistance, and fitness are summarized in Table 2. After 6 weeks intervention, children in DCIA had increased their MR-proANP (P=0.022) and their fitness (P=0.012) and they had deceased their BMI (P<0.001) as compared with children in SIA. In fact, MR-proANP did not change from baseline to week 6 in SIA. Furthermore, after 6 weeks intervention, children in DCIA, as compared with children in SIA, had decreased their total fat mass %, abdominal fat mass %, systolic blood pressure, diastolic blood pressure (P≤0.005), and their serum concentrations of glucose, CRP, and leptin (P≤0.002), whereas the differences in change in plasma insulin concentrations and in HOMA estimated insulin resistance were nonsignificant (P=0.076 and P=0.054, respectively), although the differences in change at 6 weeks were substantial between the children in SIA and DCIA (–22% (95% CI, –40.8 to 2.6%) and –24.5% (95% CI, –43.3 to 4.9%), respectively). The reason for this was that some of the children in SIA had a substantial decrease in plasma insulin concentrations between baseline and week 6 as evidenced in the lower 95% CI: –7.1% (–24.7% to 14.7%). The corresponding figures for the children in DCIA were –27.6% (–39.4% to –13.6%). In addition, no significant difference in change at 6 weeks was observed in serum adiponectin concentrations between the 2 intervention arms (P=0.16), but the 95% CI for the mean difference in change was wide (–11.6% to 115.3%).

Table 2. The Effects of the 2 Intervention Arms on Plasma Midregional Proatrial Natriuretic Peptide Concentrations, Anthropometry, Body Composition, Blood Pressure, Metabolic Factors, and Fitness

Variable	Mean±SD or Median (Interquartile) Standard Intervention Arm/Day-Camp Intervention Arm			Mean Difference in Change Day-Camp Intervention Arm vs Standard Intervention Arm at 6 weeks		Mean Difference in Change Day-Camp Intervention Arm vs Standard Intervention Arm at 52 weeks	
	Baseline	6 weeks	52 weeks	Mean (95% CI)	*P* Value	Mean (95% CI)	*P* Value
Midregional proatrial natriuretic peptide, pmol/L	39.3 ± 11.3/35.5 ± 10.6	39.8 ± 11.5/41.6 ± 11.2	39.8 ± 11.6/34.1 ± 11.3	5.40 (0.79 to 10.02)	0.022	−0.82 (−5.65 to 4.00)	0.74
Weight, kg	59.6 ± 9.0/61.7 ± 8.7	59.3 ± 9.2/56.6 ± 8.2	64.2 ± 11.1/62.5 ± 9.4	−5.19 (−6.45 to −3.94)	<0.001	−2.18 (−4.08 to −0.27)	0.025
Body mass index, kg/m^2	24.5 ± 3.0/25.2 ± 2.9	24.1 ± 3.1/22.7 ± 2.6	24.6 ± 3.7/23.8 ± 3.0	−2.12 (−2.59 to −1.65)	<0.001	−0.94 (−1.63 to −0.25)	0.008
Total fat mass, %	38.7 ± 6.3/39.6 ± 6.3	37.5 ± 7.2/33.0 ± 7.4	36.5 ± 8.2/34.8 ± 7.0	−4.95 (−6.29 to −3.61)	<0.001	−1.21 (−3.08 to 0.66)	0.20
Abdominal fat, %	46.9 ± 7.6/48.1 ± 7.1	44.9 ± 8.2/39.0 ± 9.9	43.4 ± 10.1/41.9 ± 9.2	−6.87 (−8.75 to −4.99)	<0.001	−1.49 (−3.97 to 0.99)	0.24
Systolic BP, mm Hg	104.1 ± 8.8/106.8 ± 7.6	102.3 ± 7.7/100.3 ± 6.8	103.9 ± 6.3/104.9 ± 9.0	−4.41 (−6.85 to −1.98)	<0.001	−3.00 (−6.11 to 0.10)	0.058
Diastolic BP, mm Hg	53.7 ± 5.8/55.9 ± 6.9	52.6 ± 6.5/51.2 ± 6.2	52.9 ± 7.2/54.6 ± 5.6	−3.35 (−5.66 to −1.04)	0.005	−0.22 (−2.64 to 2.20)	0.86
Glucose, mmol/L	4.9 ± 0.4/5.0 ± 0.4	5.0 ± 0.3/4.9 ± 0.3	5.0 ± 0.3/4.9 ± 0.3	−0.26 (−0.41 to −0.10)	0.002	−0.15 (−0.3 to 0.01)	0.073
Insulin, µIU/mL*	8.2 (6.3–13.4)/9.4 (6.6 –12.3)	8.7 (5.9–13.4)/6.5 (4.7–10.8)	9.0 (5.8–14.7)/8.8 (6.8–11.2)	−22.0% (−40.8 to 2.6%)	0.076	2.7% (−22.1 to 35.4%)	0.85
Homeostatic model assessment for insulin resistance, units*	1.7 (1.3–2.9)/2.1 (1.5–2.9)	1.9 (1.4–3.1)/1.4 (1.0–2.3)	1.9 (1.3–3.4)/2.0 (1.4–2.5)	−24.5% (−43.3 to 4.9%)	0.054	0.57% (−25.3 to 32.4%)	0.97
C-reactive protein, mg/L*	1.1 (0.5–3.0)/1.9 (0.5–5.1)	1.1 (0.7–2.3)/0.5 (0.2–1.1)	0.6 (0.3–1.9)/0.5 (0.2–1.8)	−55.2% (−73.8% to −23.5%)	0.003	−13.3% (−49.6% to 49.0%)	0.61
Adiponectin, µg/mL*	31.1 (19.2–48.3)/31.2 (21.4–50.9)	28.8 (17.5–42.5)/31.6 (22.4–50.3)	28.0 (19.7–36.2)/29.4 (20.9–42.3)	38.0% (−11.6% to 115.3%)	0.16	0.1% (−40.0% to 67.1%)	0.99
Leptin, ng/mL	5.35 ± 2.72/5.77 ± 2.49	4.63 ± 2.56/2.53 ± 1.68	4.29 ± 2.61/4.59 ± 2.17	−2.56 (−3.43 to −1.69)	<0.001	0.09 (−0.79 to 0.97)	0.84
Fitness, mL O_2/min per kg	35.1 ± 5.3/33.1 ± 5.5	36.4 ± 6.1/37.7 ± 6.1	36.0 ± 5.6/35.2 ± 5.8	2.33 (0.52 to 4.14)	0.012	0.55 (−1.30 to 2.41)	0.56

BP indicates blood pressure.

*Data summarized with median and interquartile range due to nonnormal distributed data. The analyses for difference in change are performed on logarithmic transformed data. The results have been exponentially back transformed and are presented in percentage change between groups.

At week 52, 46 weeks after the end of the interventions, plasma MR-proANP concentrations in the children in the DCIA had basically returned to baseline concentrations, and the only significant differences in mean change at 52 weeks were observed in weight and BMI.

Finally, we systematically looked for sex-based differences in the responses to the 2 interventions performing interaction analysis. We found only 1 significant interaction between sex and intervention arms ($P<0.001$), which showed that in DCIA, serum concentrations of CRP decreased significantly only among the boys.

Mediation Analysis Results

The results of our mediation analysis are shown in Figure. As mentioned previously, as explanatory variables with change in plasma concentrations of MR-proANP from baseline to week 6 as outcome variable, we had selected changes in BMI, total fat mass %, abdominal fat mass %, systolic and diastolic blood pressure, plasma or serum concentrations of glucose, insulin, CRP, adiponectin, leptin, HOMA estimated insulin resistance, and fitness from baseline to week 6. These 12 variables explained 18.9% of the increase in plasma concentrations of MR-proANP. Changes in circulating concentrations of glucose, insulin, and leptin, and changes in HOMA estimated insulin resistance and BMI were significantly negatively associated with change in plasma concentrations of MR-proANP, whereas change in systolic blood pressure was significantly positively associated with change in plasma concentrations of MR-proANP. None of the other explanatory variables were significantly associated with change in plasma MR-proANP. Further, as is seen in Figure, the decreases in fasting insulin and in estimated insulin resistance were the 2 factors that explained most of the observed increase in plasma MR-proANP concentrations. Finally, including the 2 treatment arms in the mediation analysis did not materially change the relationship between fasting insulin, estimated insulin resistance, and MR-proANP (data not shown).

DISCUSSION

Main Results

The major new finding of this study in overweight children was that a highly intensive multicomponent health intervention led to a significantly increase in plasma MR-proANP concentrations as compared with a standard low intensity control intervention. Furthermore, it was a major finding of this study that the decreases in plasma insulin concentrations and in estimated insulin resistance were the factors that explained most of the observed increase in plasma MR-proANP concentrations among the children randomized to the highly intensive multicomponent health intervention.

Results from Other Intervention Studies

There are apparently no other published trials of the effect of a multicomponent intervention, like the OOIS intervention, on circulating concentrations of ANP or proANP fragments. However, in a subgroup of the Action for Health in Diabetes trial, the intervention arm, which led to a greater improvement in cardiorespiratory fitness (estimated from treadmill tests) and to a greater weight loss,[34] was associated with an increase in fasting concentrations of N-terminal B-type natriuretic peptide,[35] a stable marker of BNP (B-type natriuretic peptide) secretion.[20] In addition, in a cohort study of patients with coronary heart disease and individuals at high risk of coronary heart disease, a 3-month comprehensive lifestyle intervention, which led to an improvement in functional capacity (assessed as metabolic equivalent) and to weight loss, increased fasting concentrations of BNP (18.0 [11.0–35.0], pg/mL to 28.0 [14.0–52.3], pg/mL, $P<0.001$).[36] Nevertheless, several weight reduction studies, although not all,[37] using hypocaloric diets or gastric bypass,[13–15,37] have shown that weight loss is accompanied by increases in fasting concentrations of proANP fragments.[13–15] However, 1 additional study should be mentioned that reported only a marginal increase in fasting concentrations of proANP fragments despite a substantially drop in BMI and protocol recommendation of increased physical activity.[38] This study induced weight loss by serving very low energy diets and did not report changes in fitness.[38]

Insulin, ANP Secretion, ANP Clearance, and ANP Action

We found that changes in plasma insulin concentrations and in estimated insulin resistance were candidate mediators of the observed change in plasma MR-proANP concentrations. In humans, proANP fragments are believed not to activate the natriuretic peptide receptor A,[37] which mediates the actions of ANP.[1] However, recombinant proANP has recently been shown to have biological effects in canines and in human embryonic kidney cells,[39] so we cannot totally rule out possible biological effects of MR-proANP in our study. Further, in humans, proANP fragments are believed not to be degraded by the natriuretic peptide clearance receptor or by neprilysin,[37,40,41] the 2 main degrading pathways of ANP.[1] Therefore, the changes in MR-proANP concentrations observed in this study are probably only a solid marker of changes in bioactive ANP secretion and not ANP clearance, although insulin appears to play a

role in the degradation of bioactive ANP.[1] Thus, studies have shown that hyperinsulinemia or estimates of insulin resistance are associated with increased natriuretic peptide clearance receptor expression in adipose tissue[16,42] and increased neprilysin activity.[43]

Several studies have found inverse associations between fasting concentrations of proANP fragments and fasting concentrations of insulin or estimates of insulin resistance.[8,10,37,38,44] In addition, other weight reduction invention studies have found inverse associations between changes in fasting concentrations of proANP fragments and changes in fasting concentrations of insulin or estimates of insulin resistance, even though successful weight loss by itself did not lead to higher fasting concentrations proANP fragments.[37,38] Nevertheless, the mechanistic links between insulin, insulin resistance, and ANP secretion await clarification.

Although improved insulin sensitivity could lead to increased ANP secretion, increased ANP secretion, caused by increased physical activity,[45] could have led to the improved insulin sensitivity in our study. Thus, the Malmo Diet and Cancer Study showed that plasma concentrations of MR-proANP high within the normal range at baseline were associated with a lower risk of insulin resistance after a median follow-up time of 16.5 years.[46] Further, treatment of obese hypertension patients with a combination drug that includes the neprilysin inhibitor sacubitril, which leads to increased ANP concentrations,[40] improved insulin sensitivity.[47] Finally, although we did not find a significant inverse relationship between an increase in fasting MR-proANP concentrations and decreases in total fat mass % and abdominal fat mass %, it is relevant to mention that increased ANP secretion during increased physical activity may have contributed to the observed weight loss and reduction in fat mass through increased lipolysis and fat oxidation during exercise.[1]

Glucose and Blood Pressure

In this study, we also found a significant inverse association between change in plasma glucose concentrations and change in plasma MR-proANP concentrations, although the strength of this association was weaker than the association found between changes in plasma insulin concentrations and in estimated insulin resistance and change in plasma MR-proANP concentrations. In cross-sectional studies, we have also found significant inverse associations between circulating glucose concentrations and circulating MR-proANP concentrations, although again the strength of these associations was weaker than the associations found between circulating insulin concentrations and estimated insulin resistance and circulating plasma MR-proANP concentrations in these studies.[10,44] Further, in another weight loss study, which did not include measurement of insulin, a decrease in plasma glucose concentrations was found to be associated with an increase in plasma MR-proANP concentrations, and this relationship was independent of weight loss and decrease in fat mass.[15] Finally, Arora and colleagues have provided evidence that glucose can downregulate ANP mRNA levels leading to a decrease in ANP secretion,[18] so glucose could in fact be directly involved in the overweight-related ANP deficiency.

In this study, we found a positive association between change in systolic blood pressure and change in plasma MR-proANP concentrations. In fact, based on the medical literature,[1,4,9] we had expected that an increase in plasma MR-proANP concentrations would have been associated with a lower systolic blood pressure. We are not sure how to explain this systolic blood pressure result, but the healthy diet in DCIA may have affected the response relationship between systolic blood pressure and MR-proANP.

Leptin, Adiponectin, and CRP

We have previously reported that, as compared with SIA, DCIA resulted in reductions in serum concentrations CRP and leptin but adiponectin,[22] and that the effect of the DCIA intervention on leptin levels was explained by changes in body fat mass.[22] In this study, we found that the reduction in serum leptin concentration also explained a part of the observed increase in plasma MR-proANP concentrations, whereas the reduction in CRP did not. We consulted the medical literature on possible mechanistic links between leptin and ANP secretion in humans and received no clear results. However, studies in animals found that intraperitoneal injection of leptin had no effect on plasma concentrations of ANP.[48]

Strengths and Limitations

We believe that our study population of overweight children from the general childhood population is unique and a great strength of our study. Thus, we could study physiological relationships in a population free of cardiovascular comorbidities. Further, it is a major strength of this study that OOIS is a randomized controlled study.

The greatest limitation of our study is lack of power. In this context, it should be noted that we primarily compared the effect of 2 interventions on MR-proANP, a high-intensive intervention versus a low-intensive intervention. We speculate that we may have got more straightforward results if the high-intensive arm had been compared with a nonintervention control arm or our sample size had been bigger. It is also a major

limitation that we could not measure intact ANP and only measured MR-proANP in the fasting state. We speculate that if we had measured MR-proANP several times during the day, including periods with high physical activity, we would have detected much greater difference in MR-proANP concentrations, which may have enabled us to detect additional significant relationships. Further, it is a limitation that we have no assessment of salt intake in OOIS. We speculate that the healthy diet in DCIA contained less salt compared with the participants' usual diet, which may have blunted the response of MR-proANP to DCIA and relationship between MR-proANP and systolic blood pressure. We also must acknowledge that it is a limitation that our study is a substudy with post hoc defined outcomes and accordingly can be regarded as only a hypothesis-generating study. Finally, it is a limitation that normal MR-proANP reference values for children in our age group are still not well defined,[49,50] so we do not know whether the OOIS participants in fact had low plasma MR-proANP concentrations compared with age-matched children of normal weight.

CONCLUSIONS

Higher concentrations of ANP and its prohormone fragments are associated with a poor prognosis among patients with cardiovascular disease,[2,3] and this also holds true in pediatric patients with heart failure .[49] Nevertheless, there are now several studies published that indicate that proANP fragment concentrations high in the normal range are markers of a healthy lifestyle, metabolic health, and a favorable cardiovascular disease prognosis.[1,4] The results of this OOIS substudy suggest that a healthier lifestyle increase proANP fragments, but further studies are needed. Finally, our findings support the view that close links exist between abnormalities in insulin and glucose metabolism and the endocrine heart.[1]

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Disclosures

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