

PAPER

Decrease of plasma apolipoprotein A-IV during weight reduction in obese adolescents on a low fat diet

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OBJECTIVE: Apolipoprotein (apo) A-IV is an antiatherogenic apolipoprotein, which may be involved in the regulation of food intake. Plasma apoA-IV is elevated in human obesity and apoA-IV polymorphisms have been associated with the extent of obesity. Our aim was to determine the effects of weight loss on plasma apoA-IV in obese adolescents and to examine the relation of apoA-IV with the degree of obesity.

DESIGN: Longitudinal intervention study of a low fat hypocaloric diet conducted in a dietary camp.

SUBJECTS: Two groups of obese adolescents ($n=47$ and $n=29$), age: 12.7 ± 1.7 and 11.7 ± 2.6 y, relative body mass index (RBMI): 168 ± 24 and $175\pm 34\%$, respectively.

MEASUREMENTS: Plasma total apoA-IV, apoA-I, apoB, plasma distribution of apoA-IV, leptin, lipids, and lipoproteins before and after 3 weeks of weight reduction.

RESULTS: Plasma apoA-IV decreased from 11.5 ± 4.1 mg/dl before to 6.7 ± 2.2 mg/dl after weight reduction in the first group ($P<0.001$) and to a similar extent in the second group. The relative amount of lipid-free apoA-IV and apoA-IV associated with apoA-I increased slightly, whereas apoA-IV associated with lipoproteins devoid of apoA-I decreased. ApoA-IV levels before and after weight reduction and the changes in plasma apoA-IV did not independently correlate with RBMI, weight loss, or plasma leptin.

CONCLUSION: Plasma apoA-IV decreases markedly in overweight adolescents undergoing short-term weight reduction. The decrease is not directly related to the degree of weight loss and the mechanisms underlying this reduction remain to be clarified.

International Journal of Obesity (2004) 28, 1509–1513. doi:10.1038/sj.ijo.0802789

Published online 31 August 2004

Keywords: apolipoprotein A-IV; leptin; obesity lipoproteins; weight reduction; adolescents

Introduction

Apolipoprotein (apo) A-IV is a protein component of triglyceride (TG)-rich lipoproteins and HDL or is unassociated with lipoproteins.¹ Studies in transgenic animals^{2,3} as well as in humans^{4–6} provided evidence for a strong protective effect of apoA-IV against atherosclerosis independent of plasma HDL levels. The antiatherogenic properties of apoA-IV may be explained by its role in reverse cholesterol transport and its antioxidative properties.^{7,8} In humans, apoA-IV is almost exclusively expressed in the intestine.⁹ ApoA-IV may facilitate

enterocyte lipid transport.¹⁰ Several studies in rodents point to a role for apoA-IV as a satiety factor acting via the central nervous system to suppress food intake.¹¹

Despite the potential role of apoA-IV as a satiety factor few data are available on apoA-IV in obesity. In human obesity, apoA-IV protein polymorphisms were reported to be associated with differences in body mass index and percentage of body fat and with the response of HDL levels to weight reduction.^{12–14} In leptin receptor-deficient obese rats and in leptin-deficient obese mice, the expression of the apoA-IV gene is enhanced and its dietary regulation is altered.^{15,16} Recently, Verges *et al*¹⁷ reported that in obese patients fasting plasma apoA-IV is significantly elevated and postprandial plasma apoA-IV is markedly increased. There is little information, however, on the relation of plasma apoA-IV concentrations with the degree of obesity and it is unknown whether weight reduction

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Received 29 October 2003; revised 3 May 2004; accepted 27 June 2004; published online 31 August 2004

alters plasma apoA-IV levels. To address these questions, we measured plasma apoA-IV concentrations in obese adolescents before and after short-term weight reduction in a dietary camp.

Patients and methods

We studied two independent groups of obese children participating in a 3-week dietary camp on an inpatient basis: group I comprised 23 boys and 24 girls with a mean age of 12.7 ± 1.7 y and a mean relative body mass index (RBMI) of $168 \pm 24\%$ (mean \pm s.d.). The RBMI was calculated using the reference values of Kromeyer-Hauschild *et al.*¹⁸ The patients received a low fat hypocaloric mixed diet. The mean daily energy intake during the 3-week period was 4.40 ± 0.47 MJ (1052 ± 112 kcal) with $49 \pm 8\%$ of total energy as carbohydrate, $25 \pm 3\%$ as protein, and $26 \pm 4\%$ as fat. No electrolyte or vitamin supplementation was provided. During the dietary camp, the participants regularly attended a program of moderate physical activity lasting 30 min/day. The mean weight loss was 5.1 ± 1.3 kg.

EDTA plasma samples were collected after an overnight fast on the day before starting the diet and on the last day the participants received the diet. Plasma cholesterol (C) and TG were measured by enzymatic methods. HDL-C was determined using polyanion precipitation. LDL-C was calculated using the Friedewald formula. Plasma apoA-I and apoB were quantitated by immunoturbidimetry (Roche Diagnostics GmbH, Mannheim, Germany). Plasma total apoA-IV concentrations were determined in frozen samples by an enzyme-linked immunosorbent assay that uses affinity-purified rabbit anti-human apoA-IV polyclonal antiserum as the capture antibody and the same antibody coupled to horseradish peroxidase as detection antibody. The intra-assay coefficient of variation of this assay is 4.5%.¹⁹ Lipid-free apoA-IV, apoA-IV associated with apoA-I (LpA-IV:A-I), and apoA-IV associated with lipoproteins devoid of apoA-I (LpA-IV) were measured in a randomly selected subgroup of patients by a combination of precipitation of all lipoproteins with 40% phosphotungstic acid and 4 M MgCl₂ and immunoprecipitation of all apoA-I containing lipoproteins as described recently.²⁰ Plasma leptin was measured using a commercially available enzyme-linked immunosorbent assay (Alexis Corporation, CH-4415 Lausen, Switzerland).

To confirm the observed changes in plasma apoA-IV during weight reduction, we studied a second group of obese children in whom no other lipid or lipoprotein parameters were measured. Group II comprised 11 boys and 18 girls with a mean age of 11.7 ± 2.6 y, a mean body weight of 80.5 ± 22.3 kg, and a mean RBMI of $175 \pm 34\%$ (mean \pm s.d.). Daily energy intake was 4.52 ± 0.45 MJ (1082 ± 109 kcal) with $47 \pm 7\%$ of total energy as carbohydrate, $25 \pm 5\%$ as protein, and $28 \pm 2\%$ as fat. The mean weight loss in group II was 4.9 ± 1.4 kg. The study was approved by the Ethics Review Board of the Medical Faculty, University of Vienna (Protocol #304/2002).

Means of continuous variables before and after weight reduction were compared by paired Student's *t*-tests. Multivariate stepwise regression analyses using the SAS program package (SAS for Windows, Ver. 6.11) aimed to identify independent variables associated with apoA-IV levels after weight reduction and with the changes of plasma apoA-IV. Independent variables with a *P*-value < 0.05 were retained in the models.

Results

Effect of weight reduction on plasma apoA-IV

During weight reduction, the mean plasma apoA-IV levels decreased from 11.5 ± 4.1 to 6.7 ± 2.2 mg/dl ($P < 0.001$, Table 1). The decrease of apoA-IV was more pronounced than the concomitant decreases of apoA-I, apoB, total-C, and HDL-C. The mean plasma apoA-IV levels before weight reduction did not differ between boys and girls (11.9 ± 4.8 mg/dl in boys vs 11.0 ± 3.3 mg/dl in girls, mean \pm s.d., NS). To confirm the marked reduction of total apoA-IV during weight loss, we studied a second group of 29 adolescents with a similar degree of obesity attending the dietary camp at a later time (group II). Again total apoA-IV declined significantly from 9.7 ± 2.5 to 5.6 ± 1.3 mg/dl (mean \pm s.d., $P < 0.001$).

To determine whether the decrease of apoA-IV during weight loss was mainly due to a decrease of one of the lipoprotein-bound fractions and/or of lipid-free apoA-IV, we measured lipid-free apoA-IV, LpA-I:A-IV, and LpA-IV in 14 of the participants (eight boys and six girls). We found that the relative amount of lipid-free apoA-IV and LpA-IV:A-I increased slightly, whereas LpA-IV decreased (Table 2).

Univariate correlations of apoA-IV with the degree of obesity and of weight reduction

Plasma apoA-IV measured before weight reduction was not significantly related to RBMI (Table 3). ApoA-IV levels measured after weight reduction were inversely related to

Table 1 Effect of weight reduction on plasma lipids, apolipoprotein, leptin, and RBMI (mean \pm s.d., group I, $n = 47$)

	Before weight reduction	After weight reduction	P-value*
Body weight	77.5 ± 15	72.4 ± 14	< 0.001
RBMI (%)	168 ± 24	154 ± 31	< 0.001
Leptin (ng/ml)	38.4 ± 18.0	14.2 ± 10.7	< 0.001
Total C (mmol/l)	4.55 ± 0.62	3.43 ± 0.57	< 0.001
Total TG (mmol/l)	1.01 ± 0.40	0.88 ± 0.35	NS
LDL-C (mmol/l)	2.88 ± 0.55	1.92 ± 0.49	< 0.001
HDL-C (mmol/l)	1.19 ± 0.21	1.09 ± 0.21	< 0.001
ApoA-IV (mg/dl)	11.5 ± 4.1	6.7 ± 2.2	< 0.001
ApoA-I (mg/dl)	155 ± 18	134 ± 23	< 0.001
ApoB (mg/dl)	75 ± 17	55 ± 16	< 0.001

NS = not significant. *Paired Student's *t*-tests.

Table 2 Total apoA-IV plasma concentrations and relative distribution of apoA-IV plasma fractions in 14 obese adolescents before and after weight reduction

	Before weight reduction	After weight reduction	P-value*
Total apoA-IV (mg/dl)	9.7 ± 3.8	5.2 ± 1.8	<0.001
Lipid-free apoA-IV (%)	9.2 ± 3.2	11.5 ± 3.5	0.002
LpA-IV:A-I (%) ^a	20.6 ± 8.7	26.1 ± 6.0	NS
LpA-IV (%) ^b	70.2 ± 7.8	62.4 ± 7.1	0.022

NS = not significant. *Paired Student's *t*-test. ^aLpA-IV:A-I, apoA-IV associated with apoA-I. ^bLpA-IV, apoA-IV associated with lipoproteins devoid of apoA-I.

Table 3 Univariate correlations of plasma apoA-IV with RBMI and plasma leptin (group I, *n* = 47, 23 boys, 24 girls)

	ApoA-IV before weight reduction		ApoA-IV after weight reduction		ApoA-IV difference	
	Boys	Girls	Boys	Girls	Boys	Girls
RBMI before	-0.29	0.05	-0.46*	-0.03	-0.07	0.09
RBMI after	-0.32	0.22	-0.47*	0.03	-0.09	0.24
RBMI difference	0.20	-0.17	0.02	-0.08	0.25	-0.15
Leptin before	-0.29	0.09	-0.34	0.09	-0.15	0.05
Leptin after	-0.28	0.15	-0.32	0.17	-0.14	0.06
Leptin difference	0.26	0.01	0.29	0.06	0.14	-0.03

**P* < 0.05.

RBMI before and after weight reduction in boys only. These correlations, however, did not prove to be independent in multiple correlation analysis (see below). The reduction of plasma apoA-IV did not correlate with the changes of RBMI.

Neither plasma apoA-IV measured before or after weight loss nor the change of plasma apoA-IV was significantly related to leptin levels. Plasma leptin levels before and after weight reduction were closely related to RBMI (*r* = 0.59 in boys and *r* = 0.67 in girls before weight reduction, *r* = 0.64 in boys and *r* = 0.45 in girls after weight reduction, *P* < 0.05). Weight reduction was associated with a decrease of plasma leptin levels by about 60% (Table 1), which did not correlate significantly with the changes in RBMI (*r* = -0.04 in boys, *r* = 0.03 in girls).

Univariate correlations of apoA-IV with plasma lipids and lipoproteins

Plasma apoA-IV and its change during weight reduction did not correlate significantly with total plasma C, TG or HDL-C, or their changes (data not shown). In girls, apoA-IV was positively correlated with apoA-I after weight reduction and negatively related to apoB and LDL-C before and after weight loss (Table 4). Only the correlation of apoA-IV with apoA-I after weight reduction proved to be independent in multiple correlation analysis (see below). The decrease of apoA-IV was negatively correlated with apoB measured in girls after

Table 4 Univariate correlations of plasma apoA-IV with LDL-C, apoA-I, and apoB before and after weight reduction (*n* = 47, group I, 23 boys, 24 girls)

	ApoA-IV before weight reduction		ApoA-IV after weight reduction		ApoA-IV difference	
	Boys	Girls	Boys	Girls	Boys	Girls
LDL-C before	0.10	-0.41*	-0.12	-0.28	0.20	-0.29
LDL-C after	0.02	-0.41*	-0.10	-0.22	0.10	-0.34
LDL-C difference	0.10		-0.04	-0.12	0.15	0.02
		-0.05				
ApoA-I before	0.20	0.37	0.06	0.34	0.23	0.20
ApoA-I after	0.19	0.10	0.24	0.46*	0.09	-0.20
ApoA-I difference	0.06	-0.21	0.36	0.19	-0.17	-0.39
ApoB before	-0.04	-0.42*	-0.04	-0.19	0.08	-0.37
ApoB after	-0.09	-0.45*	-0.01	-0.14	-0.11	-0.44*
ApoB difference	0.18	0.01	-0.03	-0.07	0.25	0.06

**P* < 0.05.

weight reduction. This correlation persisted after multivariate adjustment (see below). Plasma apoA-IV levels measured before weight reduction were closely correlated with apoA-IV levels after weight reduction (*r* = 0.62, *P* < 0.05) and with the change in plasma apoA-IV (*r* = 0.64, *P* < 0.05).

Multiple correlation analysis

Two stepwise multiple correlation analyses with (1) apoA-IV after weight reduction or (2) the change of apoA-IV during weight loss as the dependent variables were performed. Sex, apoA-I after weight reduction, and the levels of apoB, LDL-C, and RBMI determined before and after weight reduction were offered to the models. Only plasma apoA-I after weight reduction emerged as an independent predictor of apoA-IV after weight reduction (multiple *R*: 0.43, *P* = 0.011; partial correlation: 0.39, *P* = 0.006, *n* = 47). ApoB after weight loss proved to be independently correlated with the change of apoA-IV during weight loss (multiple *R*: 0.38, *P* = 0.028; partial correlation: -0.37, *P* = 0.0128, *n* = 47). In contrast, none of the parameters determined before the intervention nor their changes during weight reduction could independently predict the changes of plasma apoA-IV or the apoA-IV plasma levels after weight loss.

Discussion

The main findings of our study are that (1) the mean plasma apoA-IV levels decrease to almost half of baseline levels during weight reduction on a low fat hypocaloric diet and (2) plasma apoA-IV is not correlated with the degree of obesity or with the extent of weight reduction in obese adolescents.

The marked decrease of apoA-IV during weight reduction was observed in two independent groups of obese patients. The reduction of apoA-IV exceeded the concomitant decreases of apoA-I, apoB, total-C, and HDL-C. A disproportionate decrease of plasma apoA-IV has also been observed in patients receiving total parenteral nutrition.²¹ The physiological significance of the decrease of apoA-IV during

weight loss remains to be elucidated. Fasting apoA-IV is a good marker for the TG response after an oral fat load.¹⁷ Thus, the decrease of apoA-IV during weight reduction may indicate an improvement in postprandial lipid handling. It remains unclear as to whether the reduction of apoA-IV would persist during a longer period of weight reduction or whether apoA-IV would increase to pre-diet levels as described for apoA-I.²²

Weight reduction altered the relative plasma distribution of apoA-IV slightly with an increase in lipid-free apoA-IV and LpA-I:A-IV. Clearly, these small relative changes cannot explain the major absolute changes of total apoA-IV. One might speculate that these two apoA-IV fractions may have the most pronounced antiatherogenic properties. Contrary to this hypothesis, we found no major differences in the three plasma fractions between patients with coronary artery disease and controls.²⁰ Interestingly, we observed a higher proportion of lipid-free apoA-IV as well as LpA-I:A-IV in adolescents when compared to an adult population.²⁰ Whether this can be explained by age-specific differences in reverse cholesterol transport, by the obesity of our patients, or by nutritional factors remains to be clarified in future studies.

In our patients, plasma apoA-IV did not correlate significantly with the degree of obesity as assessed by RBMI and plasma leptin levels. This is consistent with the results of Verges *et al*,¹⁷ who studied a group of adults with android obesity. A weak positive correlation of plasma apoA-IV with BMI ($r=0.17$, $P<0.05$) was observed in a large group of middle-aged women.²³

In the present study, the marked reduction of plasma apoA-IV occurring during weight reduction did not correlate with the changes of RBMI or plasma leptin levels. This suggests that the decrease of plasma apoA-IV was not a direct effect of the reduction of body fat mass. A somewhat smaller fall of plasma apoA-IV, caused by a decrease in apoA-IV synthesis, was observed in individuals consuming a Step II National Cholesterol Education Program diet containing 26% of calories as fat and designed to maintain body weight.²⁴ Plasma apoA-IV levels are very sensitive to acute changes in dietary fat content and are significantly correlated with the percent of total calories consumed as dietary TG.²⁵ The hypocaloric diets used in this study had a mean fat content of 26 and 28 energy percent, respectively, compared to an average of 34% consumed by Austrians of this age group.²⁶ It is therefore conceivable that the low fat diet may have contributed to the decrease of apoA-IV in our patients. In addition to the hypocaloric diet, the patients participated in a moderate exercise program. The effects of exercise on plasma apoA-IV have not been studied in detail, but it appears that moderate exercise has no pronounced influence on apoA-IV levels.²⁷ Thus, the change of physical activity during the dietary camp is unlikely to explain the dramatic drop of plasma apoA-IV. The reasons for the marked decrease of apoA-IV during weight reduction therefore remain to be elucidated.

The pronounced fall of plasma leptin levels to less than half of the initial values clearly exceeded the decrease expected from the weight reduction of about 6% over a period of 3 weeks. This indicates that our patients were still in the dynamic phase of weight loss on the last day of the dietary camp. Plasma leptin has been reported to be markedly lower during active weight loss than during maintenance of reduced body weight.^{28,29} During negative energy balance, leptin levels may more closely reflect a reduced secretion of leptin due to decreased adipocyte glucose uptake/metabolism and increased lipolysis than the reduction in body fat mass.³⁰

Studies in rats showed that leptin is a suppressor of intestinal apoA-IV synthesis.³¹ In the present study, plasma apoA-IV fell considerably in the presence of a profound drop in plasma leptin. This finding could suggest that in humans a hypocaloric low fat diet is more powerful in regulating intestinal apoA-IV synthesis than plasma leptin levels. It is unknown, however, whether the suppression of intestinal apoA-IV production by leptin found in rodents also occurs in humans. Moreover, the adolescents studied here were obese and thus may have been resistant to the action of leptin.³²

Like in other studies, we found little correlation of plasma apoA-IV with other lipid and lipoprotein variables. Plasma apoA-I measured after weight loss explained part of the variation in plasma apoA-IV at that point of the study and apoB after weight reduction was correlated to the change of plasma apoA-IV. A weak independent correlation of plasma apoA-IV with apoA-I has been observed previously and may reflect the association of the two apo's on LpA-I:A-IV particles.²³ A significant correlation between apoA-IV and fasting plasma TG has been reported, but was not confirmed in this and in other studies.^{4,17,33,34} The reasons for these conflicting results remain unclear.

In summary, our data show that plasma apoA-IV decreases significantly during short-term weight reduction in obese patients. Our results, however, do not provide evidence for a close relationship of plasma apoA-IV levels with the degree of obesity or with fat mobilization in humans.

Acknowledgements

This study was supported by the Jubiläumsfonds der Österreichischen Nationalbank (Project #8830 to W Strobl and Project #9331 to F Kronenberg) and in part by the Austrian Science Fund (Project #13649 BIO to W Strobl).

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