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ORIGINAL CONTRIBUTION

Moderate effects of apple juice consumption on obesity-related markers in obese men: impact of diet–gene interaction on body fat content

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Abstract
Purpose The effect of polyphenol-rich cloudy apple juice (CloA) consumption on plasma parameters related to the obesity phenotype and potential effects of interactions between CloA and allelic variants in obesity candidate genes were assessed in obese men.
Methods In this controlled, randomized, and parallel study, $n = 68$, non-smoking, non-diabetic men with a BMI $\geq$27 kg/m^2 received 750 mL/day CloA (802.5 mg polyphenols) or 750 mL/day control beverage (CB, isocaloric equivalent to CloA) for 4 weeks. Further, study participants were genotyped for single-nucleotide polymorphisms in PPARγ (rs1801282), UCP3 (rs1800849), IL-6 (rs1800795), FABP2 (rs1799883), INSIG2 (rs7566605), and PGC1 (rs8192678) genes. At the beginning and at the end of intervention plasma lipids, distinct adipokines and cytokines as well as anthropometric parameters were determined.
Results CloA compared to CB had no significant effect on plasma lipids, plasma adipokine and cytokine levels, BMI, and waist circumference. However, CloA consumption significantly reduced percent body fat compared to CB (Δ % body fat: CloA: -1.0 ± 1.3 vs. CB: -0.2 ± 0.9, $p < 0.05$). The IL-6-174 G/C polymorphism showed an interaction with body fat reduction induced by CloA. Solely in C/C, but not in G/C or G/G variants, a significant reduction in body fat after 4 weeks of CloA intervention was detectable.
Conclusion The observed diet–gene interaction might be a first indication for the impact of individual genetic background on CloA-mediated bioactivity on obesity-associated comorbidities.

Keywords Polyphenols · Human intervention study · Inflammation · Adipokines · Cytokines

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Introduction

The prevalence of obesity is rising in European countries as a consequence of changes that have occurred in modern societies, which are characterized by high intake of caloric-rich food and low physical activity [1]. The increase in body fat, especially the intra-abdominal adipose tissue is a major contributor to the development of hyperlipidemia, insulin resistance, and hypertension and is associated with chronic diseases such as type 2 diabetes, coronary heart disease, and increased incidence of certain forms of cancer [2]. The current management of obesity is directed primarily to reduce energy intake and increase energy expenditure. An increased fruit and vegetable consumption is supportive to achieve an initial weight reduction and the following weight stabilization [3]. Beyond its impact on energy balance, fruit and vegetables provide phytochemicals, such as polyphenols, which might affect lipid metabolism and the development of obesity [3–7]. In many Western countries, apples are important contributors of polyphenols [8, 9].

Animal studies have suggested a significant bioactivity of apples on obesity-associated changes in metabolism.

In obese Zucker rats, a 20% lyophilized apple supplementation lowered plasma cholesterol, LDL cholesterol, and the triglyceride accumulation in the heart and liver [10]. Additionally, apple polyphenols significantly reduced plasma cholesterol in cholesterol fed rats [11] and prevented diet-induced increase in white adipose tissue [12, 13]. In humans, apple polyphenols also reduced plasma cholesterol and visceral fat area [14], while consumption of whole apples lowered energy density of food intake and resulted in body weight reduction in middle-aged women [15]. Such an effect of apples on body weight may contribute to the observed risk reduction for type 2 diabetes in women eating daily one or more apples compared to no apple consumption [16]. Whether apple polyphenols mediate the mechanisms behind these findings is not clear so far.

Most research into polyphenols has focused on their properties as antioxidants, and many of their beneficial effects have been attributed to this ability [17]. In addition, polyphenols also affect cellular function by a direct interaction with enzymes, transcription factors, and/or intracellular signaling pathways, which leads to the modulation of protein function and/or the modification of gene expression [18]. Derived from animal experiments, interactions of polyphenols with genes involved in fat synthesis and transport might lead to individual responses to intervention with polyphenols [11, 12, 18–20].

Obesity is known to be a complex trait, influenced by multiple genetic and environmental factors. To date, several common obesity loci have been identified through genome-wide association studies and associations have been confirmed for several candidate genes [21, 22]. However, it is unclear whether DNA sequence variation in these genes might affect the outcome of intervention studies with polyphenol-rich food.

Correspondingly, the present randomized controlled intervention study has been carried out to investigate whether or not the consumption of polyphenol-rich cloudy apple juice (CloA) compared to an isocaloric control beverage (CB) modifies obesity-associated metabolic and endocrine parameters in a well-characterized study population of obese males. Additionally, we investigated whether a diet–gene interaction exists regarding possible effects of CloA on the obesity phenotype.

Materials and methods

Study population

On the basis of physical examination and medical history, $n = 68$ obese (BMI >27), non-smoking, non-diabetic male study participants, 23–69 years of age (mean age 49), were recruited. The study population comprised only Germans with Caucasian background from Karlsruhe and surroundings. All study participants underwent a standard 75 g oral glucose tolerance test (Dextro® O.G.-T, Roche, Germany), and only individuals classified as non-diabetic according to the German Diabetic Association criteria 2004 were included [$n = 60$ were normoglycemic, and $n = 8$ had impaired glucose tolerance (IGT)]. Individuals with hemoglobin A1c (HbA1c) levels above 6.2% or taking hypolipemic drugs were not included. Three subjects were on medication to treat high blood pressure. The study was approved by the Medical Ethics Committee of the Landesärztekammer Baden-Württemberg (number 030–05f), and all study participants provided their written consent.

Study design

A randomized, parallel, controlled, and blinded study design was used. A 2-week run in period was followed by a 4-week intervention period with daily consumption of either 750 mL CloA ($n = 38$) or control beverage (CB; $n = 30$), equilibrated for sugar, mineral, acid, and vitamin C composition of apple juice (Table 1). The beverages were produced, analyzed, and stored as described recently [23]. The polyphenolic constituents of the CloA have been published recently [18]. The study participants were advised to consume the beverages together with their main meals. Further, the participants were instructed to maintain their normal life style but not to consume apple products and dietary supplements during the entire study period. Also, tea, coffee, and alcohol consumption were limited to one serving per day.

Clinical parameters

All clinical parameters were determined before and after the intervention period following a 12-h overnight fast. Further the study participants were advised not to perform exercise sport, visit the sauna, or to drink alcohol the day before the examinations. The body mass index (BMI) was calculated as the weight (kg) divided by square height (m), and the body composition was measured by bioelectric impedance analysis (BIA, body composition analyzer, biodynamics model 310e, Biodynamics, Seattle, USA). Waist circumference (cm) measurements were performed according to WHO guidelines (http://www.who.int/chp/steps/Part3_Section3.pdf).

Fasting venous blood samples were collected in serum and EDTA monovettes (Sarstedt, Germany). EDTA monovettes were placed directly on ice in the dark, and plasma was collected after centrifugation at 1,500*g* for 10 min at 4 °C. For serum collection, blood was allowed to clot at room temperature for 30 min and then centrifuged at

Table 1 Sugar, mineral, and fruit acids content in control beverage (CB) and cloudy apple juice (CloA)

	CB	CloA
Glucose (g/L)	21.4	21.2
Fructose (g/L)	63.8	67.0
Saccharose (g/L)	32.4	36.2
L-malic acid (g/L)	5.9	9.8
Citric acid (g/L)	2.6[a]	0.1
Ascorbic acid (mg/L)	253	311
Potassium (mg/L)	1,023	1,217
Calcium (mg/L)	57	68
Magnesium (mg/L)	61	57
Total phenols (mg/L)[b]	0	1,070

[a] The higher citric acid concentration in the control beverage is due to minerals added to the mix as citrate derivatives

[b] Total polyphenol content has been determined by the Folin-Ciocalteu method [56]

1,500*g* for 10 min. Until analysis, serum and plasma were stored at −80 °C. Serum resistin and adiponectin (Mediagnost, Reutlingen, Germany), leptin (DRG Diagnostics, Marburg/Lahn, Germany), adipocyte fatty acid-binding protein (a-FABP, BioVendor, Heidelberg, Germany), retinol-binding protein 4 (RBP4, Immundiagnostik, Bensheim, Germany), C-reactive protein (CRP, Diagnostic Systems Laboratories, Sinsheim, Germany) as well as tumor-necrosis factor-alpha, interleukin-6, soluble vascular, and intercellular adhesion molecule 1 (TNF-α, IL-6, sVCAM-1, sICAM-1, Immunotech, Marseille, France) were analyzed by ELISA kits. Non-esterified fatty acids (NEFA C kit, Wako, Neuss, Germany), low-density lipoprotein cholesterol (LDL-C), and high-density lipoprotein cholesterol (HDL-C) (LDL und HDL kits, WAKO, Neuss, Germany) were determined with the respective assay kits. The analysis of biochemical parameters such as triglycerides, cholesterol, glucose, and HbA1c was performed by an accredited medical diagnostic laboratory (MZV, Karlsruhe, Germany).

DNA isolation and genotyping

For genotyping, genomic DNA was extracted from blood lymphocytes by the total RNA and DNA Purification kit (NucleoSpin® RNA/DNA buffer set) in combination with RNA/Protein Kit (NucleoSpin® RNA/Protein Kit, Macherey–Nagel, Düren, Germany). Lymphocytes were isolated from blood drawn in EDTA monovettes by density gradient centrifugation using Lymphoprep (Life Sciences, Eggenstein-Leopoldshafen, Germany). All $n = 68$ study participants were genotyped for a total of six SNPs (Table 2). SNPs were selected based on a known or expected association with obesity and the resulting metabolic complications. Genotyping was performed by the Amplifluor® SNPs Genotyping System (Millipore, Schwalbach/Ts, Germany), which combines allele-specific polymerase chain reaction [24, 25] with "universal" energy-transfer (ET)-labeled primers [26]. The 10 µL PCR reaction contained 2 µL of 2.5 ng/µL of genomic DNA, 25 nM each of the two-tailed allele-specific primers, 375 nM of reverse primer, 250 nM each of green, and red universal ET-labeled Amplifluor primers, 0.2 mM dNTPs (each), 1 µL 10 × reaction buffer S, 0.5 unit of Taq Polymerase (Titanium® Taq DNA Polymerase, Takara/Clontech, Saint-Germain-en-Laye, France), and 4,6 µL nuclease-free water. Allele-specific primers were designed with the assay architect software (https://apps.serologicals.com/AAA/). For primer sequence information, see Table 2. The PCR was performed in a thermocycler (PTC 200, MJ Research Inc, Waltham, Ma), and cycling conditions were preheated block 96 °C, and after 4 min at 96 °C, the first amplification step was carried out for 20 cycles of 10–15 s at 96 °C, 5 s at 55–60 °C, 10 s at 72 °C. This first amplification step was followed by 22–25 cycles of 10–15 s at 96 °C, 20 s at 55 °C, 40 s at 72 °C, followed by incubation at 72 °C for 3 min. PCR conditions were optimized for each SNP within the indicated range. Fluorescence intensity measurements were routinely performed in a micro plate reader (PHERAstar, BMG-Labtech, Offenburg, Germany) using excitation and emission filters for fluorescein (485/520 nm) and sulforhodamine (575/620 nm). The Assay Auditor provided by Chemicon was used to identify and delineate respective genotypes from scatter plots. The measurement was considered significant when the signal was at least five standard deviations above the background for fluorescein and sulforhodamine emission of water controls. DNA probes of known genotype were used in all experiments as positive controls.

Statistical evaluation

Possible differences at baseline among treatment groups were assessed by unpaired *t* test or nonparametric test when normality was not given. Treatment effects between groups were calculated as the difference between post- and pre-intervention data by unpaired *t* test or nonparametric test when normality was not given.

Table 2 Obesity candidate gene polymorphisms

Polymorphism/ NCBI db rs number[a]	Function	Effect of polymorphism	Allele-specific forward primers[b]	Common reverse primer	Genotype frequencies[c]	χ^2-value HWE[d]
FABP2 Ala54Thr G/A (rs1799883)	Lipid transport	Increased transport of long-chain fatty acids and secretion of triglycerides in human intestinal cell line [3]	(tail 1) GAAGGAAATAAATTCACAGTCAAAGAATCAAGC*A* (tail 2) GGAAATAAATTCACAGTCAAAGAATCAAGC*G*	GGTGACACCAAGTTCAAAAACAA	GG 34 GA 30 AA 4	0.62
IL-6-174 G/C (rs1800795)	Production of IL-6	C allele showed lower transcriptional activity [10]	(tail 1) TTTTCCCCCTAGTTGTGTCTTGC*G* (tail 2) TTTTCCCCCTAGTTGTGTCTTGC*C*	GGGGCTGATTGGAAACCTTATT	GG 21 GC 35 CC 12	0.22
INSIG2-10 kb G/C (rs7566605)	Control of lipid synthesis	Hypercholesterolemia [57]	(tail 1) TGTACAGACCTAAAGGACCAC*G* (tail 2) GTACAGACCTAAAGGACCAC*C*A	TCTCTCCTACCTCCCTCCAAT	GG 30 GC 30 CC 8	0.01
PGC1 Gly482Ser G/A (rs8192678)	Regulation of adipocyte differentiation and function	Activity may [7] or may not [29] be impaired	(tail 1) GACGAAGCAGACAAGACC*G* (tail 2) CGACGAAGCAGACAAGACC*A*	CTGAAATCACTGTCCCTCAGTT	GG 29 GA 31 AA 8	0.00
PPARγ Pro12Ala C/G (rs1801282)	Regulation of adipocyte differentiation and function	Decreased binding affinity to the cognate promoter element and reduced ability to transactivate responsive promoters [8]	(tail 1) AGTGAAGGAATCGCTTTCTG*C* (tail 2) AGTGAAGGAATCGCTTTCTG*G*	TGGGTGAAACTCTGGGAGATT	CC 53 GC 13 GG 1	0.04
UCP3-55 C/T (rs1800849)	Efficiency of energy expenditure	Might affect transcriptional activity	(tail 1) GGCACTGGTCTTATACACAC*G* (tail 2) CTTGGCACTGGTCTTATACACAC*A* (reverse strand)	GGCTGTCAACCAACTTCTCT	CC 43 TC 23 TT 2	0.27

[a] Fatty acid-binding protein 2, intestinal (FABP2), interleukin 6 (IL-6), Insulin-induced gene 2 (INSIG2), peroxisome proliferator-activated receptor-gamma; coactivator-1 (PGC1), peroxisome proliferative activated receptor γ (PPAR γ), uncoupling protein 3 (UCP3)

[b] (tail 1) = GAAGGTGACCAAGTTCATGCT; (tail 2) = GAAGGTCGGAGTCAACGGATT

[c] Of the $n = 408$ samples analyzed, $n = 1$ (0.3%) could not be successfully genotyped (PPARγ)

[d] *HWE* Hardy–Weinberg equilibrium

Table 3 Effect of cloudy apple juice (CloA) compared to control beverage (CB) consumption on anthropometric parameters and plasma lipids in obese, non-diabetic male study participants

Parameter	CB		CloA	
	Before intervention	After intervention	Before intervention	After intervention
Body weight (kg)	97.6 ± 13.0	97.8 ± 12.9	99.0 ± 14.2	99.3 ± 14.3
BMI (kg/m^2)	30.5 ± 3.0	30.6 ± 3.2	31.1 ± 3.6	31.1 ± 3.6
Body fat (%)	29.0 ± 2.7	28.8 ± 2.7	29.3 ± 3.6	28.3 ± 3.9
Lean body mass (kg)	69.1 ± 7.6	69.5 ± 7.4	69.4 ± 7.9	70.8 ± 7.9
Waist circumference (cm)	106.9 ± 7.9	107.0 ± 7.9	107.3 ± 9.9	107.4 ± 9.7
HDL-C (mg/dL)	45.5 ± 6.6	43.7 ± 6.8	47.1 ± 5.9	45.4 ± 6.0
LDL-C (mg/dL)	142.1 ± 21.7	146.8 ± 22.6	152.1 ± 32.0	150.7 ± 31.1
Cholesterol (mg/dL)	206.3 ± 29.6	209.8 ± 33.8	211.5 ± 43.0	216.9 ± 45.0
Triglycerides (mg/dL)	138.2 ± 58.4	165.6 ± 79.4*	141.6 ± 80.5	189.2 ± 110.8*
NEFA (mmol/L)	0.41 ± 0.13	0.37 ± 0.11	0.38 ± 0.13	0.41 ± 0.17

CB $n = 30$; CloA: $n = 38$

* $p < 0.05$, significant difference within group

Agreement with Hardy–Weinberg expectations was tested by Chi-square (χ^2) analysis. We tested the codominant model for the IL-6 and the PGC1 polymorphisms. For the UCP3, the PPARγ, the INSIG2, and the FABP2 SNP, we tested only the dominant model, because of the limited number of subjects carrying the minor allele of the corresponding SNP. Possible differences at baseline within the genotypes between CloA and CB group for each SNP were calculated by ANOVA for the codominant model and by unpaired *t* test for the dominant model. The analysis of the intervention–genotype interaction on anthropometric and biochemical parameters was calculated using the difference between post- and pre-intervention data for ANOVA with "CloA/CB" and "genotype" as independent factors. For post hoc comparison of means, Tukey's honest significant difference test was used. All statistical calculations were performed with the StatView program (1998; SAS Institute Inc., Cary, NC, USA), and significance level was set at $p < 0.05$. Results are given as means with their standard deviations (SD) if not otherwise stated.

Results

All study participants complied with the study protocol and completed the intervention without reporting any illness. Those drinking 750 mL/day CloA consumed 802.5 mg polyphenols with juice. Anthropometric and biochemical characteristics of the study subjects at the time of the entry into the study and after intervention are shown in Tables 3 and 4. At baseline, no differences have been observed between CloA and CB group regarding these anthropometric and biochemical parameters. Analyses showed that plasma triglyceride levels significantly increased independent of the intervention in both the CloA and the CB group (Table 3) Further, the consumption of CloA significantly reduced the percentage of total body fat (Fig. 1; CloA vs. CB: $p = 0.001$; power with alpha = 0.05: 0.81) while increasing the lean body mass (Fig. 1; CloA vs. CB: $p = 0.019$; power with alpha = 0.05: 0.52) as compared to the CB. Consumption of CloA did not show additional significant changes on any other parameter as compared to CB (Table 3). In serum, neither adipokines (leptin, adiponectin, resistin, a-FABP, RBP4) nor markers of systemic (CRP, IL-6, TNF-α) or vascular inflammation (sICAM-1, sVCAM-1) were affected by intervention (Table 4).

The change in total body fat prompted us to investigate whether functionally relevant single-nucleotide polymorphisms (SNPs) in candidate obesity genes might have been involved. Study participants were genotyped for six obesity-related SNPs, and genotype allelic frequencies are shown in Table 2. They were not different from the Hardy–Weinberg prediction (Table 2).

Carriers of the IL-6-174 C/C variant showed a significant loss of body fat mass by CloA, whereas body fat mass of G-allele carriers (G/C, G/G) was not affected by the CloA intervention (Fig. 2; p for interaction (intervention × genotype) = 0.011; power with alpha = 0.05 for interaction (intervention × genotype) = 0.68). No additional intervention–genotype interaction effect has been observed on any of the analyzed biochemical or anthropometric parameters and any other SNP (data not shown).

Discussion

The present study was initiated in order to evaluate the effect of a 4-week polyphenol-rich CloA consumption on

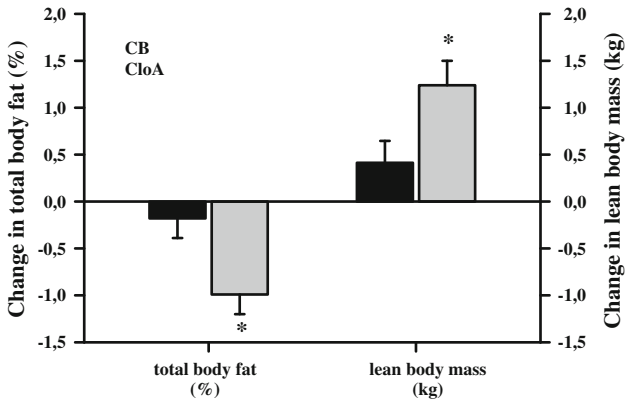

Fig. 1 Changes (differences between post- and pre-intervention) in total body fat and lean body mass in obese men (BMI >27) after consumption of 750 mL cloudy apple juice (*CloA*) or control beverage (*CB*) for 4 weeks. *Bars* represent means ± SEM. Changes in total body fat and lean body mass were significantly different (*$p < 0.05$) after apple juice consumption compared to control (CB: $n = 30$; CloA: $n = 38$)

plasma parameters associated with the obesity phenotype. Cholesterol, triglyceride, HDL, LDL, and NEFA plasma levels did not change by 4 weeks intervention with CloA compared to CB. Most interestingly, the percentage body fat has been slightly but significantly reduced by CloA.

These data are in line with other in vivo studies that apple polyphenols in fact exert beneficial effects on body fat. Supplementation of 1% apple polyphenols for 9 weeks prevented the increase in white adipose tissue in rats on a high fat diet [12, 13]. Supplementation of 5% apple polyphenols for 3 weeks reduced white adipose tissue in rats under normal feeding condition [27]. Further, a human intervention trial performed over 12 weeks with daily supplementation of 600 mg apple polyphenol capsules reduced the visceral fat area measured by computer tomography in humans with a mean BMI of 26 kg/m^2 [14]. These data have recently been confirmed by the same group using an apple polyphenol-containing beverage instead of an encapsulated polyphenolic powder [28]. In both intervention trials, the analyses of body weight changes over time showed a gradually and progressive decrease after 4 weeks and even stronger after 12 weeks of apple polyphenols intake. At the end of intervention, the body weight and visceral fat tissue mass finally differed significantly between the polyphenol and the placebo group [14, 28]. Therefore, the small magnitude of the effect on percent body fat observed in our study; although the polyphenol intake by CloA was comparable (800 mg/day by CloA vs. 600 mg by capsules or beverage), the shorter intervention period of four compared to 12 weeks [14, 28] might explain that the reduction in body fat mass in our study was not accompanied by changes in body weight or BMI.

Several association studies have described a diet–genotype interaction between energy restriction or macronutrient dietary intake with different single-nucleotide polymorphisms in genes that generally associate with the obesity phenotype [29]. However, it is unclear whether DNA sequence variation in these same genes affects the outcome of intervention studies.

Although the present study has originally not been designed to focus on genotype–diet interactions, the observed intervention effects on body fat content has prompted us to also analyze such SNPs that are associated with, e.g., lipid/energy homeostasis, overweight/obesity, or inflammation. Among the different analyzed SNPs, the IL-6-174 G/C SNP located within the IL-6 promoter region has been shown to be relevant. Our data show that the body fat lowering effect by apple juice observed among the entire study collective was mainly due to a significant decrease in IL-6-174 C/C gene variants but not in G-allele carriers (G/C, G/G). The IL-6-174 SNP has originally been associated with overweight in male and female individuals [30] and more recently with fat mass in elderly men [31]. Further, it has been reported that the C allele as a factor not only increases the risk of developing obesity comorbidities such as hypertension and cardiovascular disease [32] but is also accompanied with a higher risk of developing insulin resistance in obese individuals [33, 34]. Very recently, the IL-6-174 G/C SNP in combination with a flavonol-rich diet has been associated with a lower risk of adenoma recurrence [35]. Further, the IL-6-174 G/C genotype might be relevant for elderly individuals more likely to benefit from nutraceutical anti-inflammatory interventions [36]. Taken together, these published and our data on the C/C variant not only resemble a supposed high-risk group for obesity-related disorders but also indicates those individuals as responders to apple-associated constituents. Intervention–genotype interaction effects related to other investigated SNPs (PGC1, UCP3, PPARγ, INSIG2, FABP2) were not detected. So far, dietary intervention studies investigating gene–nutrient interactions are scarce. The impact of UCP SNPs on body composition has been investigated in an overfeeding study in male twins [37] and a low-calorie diet study in females [38]. UCP3 genotype had no effect on change in fat mass due to overfeeding [37], while a UCP2-3 gene cluster is significantly associated with low-calorie diet-induced loss in fat mass [38]. Comparable intervention studies with polyphenol-rich diets are not available so far.

The body composition was assessed by bioelectrical impedance analysis (BIA), which is a widely used, non-invasive, valid, and reliable method. The consistency and accuracy in BIA measurements are predicated on the use of standardized procedures for testing, as the results could be affected by body position during measurement, recent exercise, dietary and fluid intake, hydration status, and skin

Table 4 Effect of cloudy apple juice (CloA) compared to control beverage (CB) consumption on plasma adipokines and inflammation markers obese, non-diabetic male study participants

Parameter	CB		CloA	
	Before intervention	After intervention	Before intervention	After intervention
Adiponectin (μg/mL)	4.7 ± 2.8	5.1 ± 2.9	5.3 ± 2.8	5.5 ± 2.8
Leptin (ng/mL)	11.4 ± 9.8	13.6 ± 9.5	12.7 ± 9.5	14.5 ± 10.0
Resistin (ng/mL)	3.0 ± 0.7	3.1 ± 0.7	3.2 ± 0.8	3.3 ± 0.8
a-FABP (ng/mL)	29.2 ± 7.1	28.3 ± 8.3	29.4 ± 9.7	29.4 ± 9.0
RBP4 (mg/L)	44.7 ± 7.9	47.5 ± 8.2	48.0 ± 11.2	49.9 ± 11.1
CRP (μg/mL)	5.2 ± 4.6	6.0 ± 5.5	9.9 ± 22	7.2 ± 6.4
IL-6 (pg/mL)	8.9 ± 5.9	8.3 ± 5.6	9.5 ± 6.8	8.1 ± 5.5
TNF-a (pg/mL)	8.9 ± 4.4	7.7 ± 2.9	10.2 ± 4.7	10.1 ± 5.1
sICAM-1 (ng/mL)	562 ± 152	586 ± 151	581 ± 126	596 ± 133
sVCAM-1 (ng/mL)	951 ± 346	952 ± 327	911 ± 263	927 ± 225

CB: $n = 30$; CloA: $n = 38$. *a-FABP* adipocyte fatty acid-binding protein, *RBP4* retinol-binding protein 4, *CRP* C-reactive protein, *IL-6* interleukin-6, *TNF-α* tumor-necrosis factor-alpha, *sICAM-1* s inter-cellular adhesion molecule 1, *sVCAM-1* s vascular-cell adhesion molecule 1

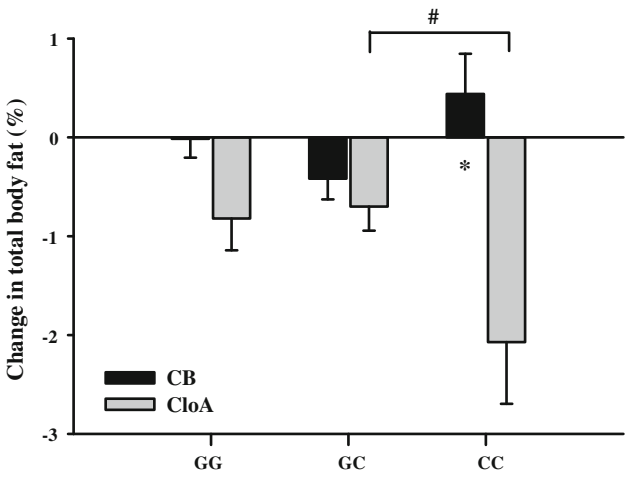

Fig. 2 Impact of IL-6-174 G/C single-nucleotide polymorphism on changes (%) (difference between post- and pre-intervention) in total body fat in obese men (BMI >27 kg/m^2) after consumption of 750 mL apple juice (*CloA*) or control beverage (*CB*) for 4 weeks. *Bars* represent means ± SEM (CloA group: $n = 14$ G/G, $n = 17$ G/C, $n = 7$ C/C; CB group: $n = 7$ G/G, $n = 18$ G/C, $n = 5$ C/C, $p < 0.05$). A significant interaction between intervention (CloA/CB) and IL-6-174 G/C SNP could be detected (ANOVA: $p = 0.011$). Significant differences among genotype groups within each intervention (CloA, CB) are indicated by $^{\#}p < 0.05$. Significant differences for CloA versus CB within a genotype are indicated by $^{*}p < 0.05$

temperature (reviewed by [39]). Although BIA is highly correlated with fat-free mass or body fat mass, caution is advised when parameter changes are discrete but statistically significant. According to theses methodological obligations, we have measured body composition under controlled and standardized conditions with study participants randomly allocated to the CloA and CB groups. Therefore, it is unlikely that the observed changes in percent body fat in the CloA but not in the CB group is ascribed to measurement artifacts.

The mechanisms how apple polyphenols modulate body fat might include regulation of lipid metabolism, reduction in energy intake, and/or decrease in fat absorption as indicated by experimental data. The reduction in a postprandial increase in plasma triglycerides by simultaneous consumption of dietary fat and apple polyphenols in mice and humans [38] has been linked to an inhibition of pancreatic lipase activity, according to the in vitro inhibition of pancreatic lipase by apple polyphenols [40–42]. Vogels et al. [43] suggested that procyanidins prevent weight gain or help losing weight by reducing energy intake, since an intake of 300 mg grape seed extract (>90% procyanidins) decreased 24-h energy intake in normal to overweight subjects with a mean energy requirement of ≥7.5 MJ/day. Furthermore, a direct modulation of cellular processes in adipocytes such as lipolysis with fatty acids release, lipid synthesis, and adipogenesis might be additional mechanisms functionally underlying the effect of apple polyphenols on pathologies associated with obesity [42, 44–46].

The polyphenol-rich CloA did not alter leptin levels compared to controls. Leptin plays a major role in the control of body fat storage through the regulation of food intake and total body energy expenditure and circulating levels correlate closely with both the BMI and the total amount of body fat (reviewed by [47]). Apple polyphenols have been shown to reduce leptin levels in animals on a high fat diet [13]; yet, it could not be distinguished if the effect on leptin levels was due to the reduction in body fat by polyphenols or due to a direct effect of polyphenols on adipocytes' leptin synthesizing/secreting capacities. Experiments in 3T3-L1 adipocytes showed that some phenolic acids and flavonoids inhibited the expression of

leptin at the protein level [45], indicating also a direct action of polyphenols on adipokine expression. However, in line with our results, recently published human intervention studies could not show changes in leptin plasma levels regardless of a lower visceral adipose tissue mass after consumption of apple polyphenols for 12 weeks [14, 28]. Besides unaffected leptin plasma concentrations, also other adipocyte-derived factors such as adiponectin, resistin, RBP4, and a-FABP were unaffected by the CloA intervention. To date, no study has been conducted to analyze the effect of CloA or apple polyphenols consumption in overweight individuals on resistin, RBP4, and a-FABP, which have been considered to contribute to insulin resistance in obesity [48–50].

Obesity is associated with inflammation [51, 52], and in this functional context, adipocytes play a major role being the origin of inflammatory mediators [48]. In our study, obesity-related markers of inflammation were not affected by CloA compared to CB. Since intervention-related changes in total body fat was quite moderate and BMI as well as waist circumference did not change, we may speculated that the intervention period was too short to result in more substantial effects on body fat homoeostasis and subsequent changes in plasma inflammation markers.

Although an inhibiting biofunctionality of apple polyphenols on pancreatic lipase activity has been suggested [41, 42], an increase in fasting triglyceride levels not only in CB but also in CloA group has been observed. This can be explained by the relevant fructose intake by both beverages as already reported in frame of an intervention study showing an increase in fasting triglycerides by 32% due to fructose intake of 80 g/day compared to those consuming glucose [53]. In the present study, daily fructose intake supplied by the beverages reached 50.3 g/day in the CloA and 47.9 g/day in the CB group. Mechanistically, either a stimulation of hepatic triglyceride synthesis and/or a decreased triglyceride clearance might have contributed to the increased triglycerides after fructose consumption [54]. Whether this increase in triglycerides is of clinical relevance [55] cannot be judged from our data.

Taken together, this is the first intervention study that addressed the question whether a polyphenol-rich CloA shows bioactivity on obesity-associated biomarkers also including the potential role of genetic polymorphisms regarding a potential diet–gene interaction. Although CloA compared to CB failed to modulate plasma parameters related to the obesity phenotype, CloA induced a significant reduction in the percent body fat in obese men. Therefore, the CloA showed biofunctional characteristics being already suggested for apples [10] and apple polyphenols [12–14, 27]. However, on the basis of this pilot study, the role of SNPs in obesity-associated candidate genes regarding the reduction in total body fat has to be confirmed in frame of larger cohort studies.

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Conflict of interest The authors declare no conflict of interest.

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