RESEARCH ARTICLE

Highly bioavailable micellar curcuminoids accumulate in blood, are safe and do not reduce blood lipids and inflammation markers in moderately hyperlipidemic individuals

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Scope: Curcuminoids are poorly bioavailable, but potentially lipid- and inflammation-lowering phytochemicals. We hypothesized that curcuminoids, when administered as a micellar formulation with hundredfold enhanced bioavailability, decrease blood lipids and inflammation in subjects with moderately elevated cholesterol and C-reactive protein concentrations.

Methods and results: We carried out a randomized, double-blind, crossover study (4-wk washout phase) with 42 subjects consuming 294 mg curcuminoids per day (as micelles) or placebo for 6 wk. At the beginning, after 3 wk and at the end (6 wk) of each intervention, we collected fasting blood samples to determine curcuminoids, blood lipids, and markers of inflammation, glucose and iron homeostasis, and liver toxicity. Daily ingestion of 98 mg micellar curcuminoids with each principal meal for as little as 3 wk resulted in fasting curcuminoid plasma concentrations of 49 nmol/L. Neither blood lipids, nor markers of inflammation, glucose and iron homeostasis, or liver enzymes differed between curcuminoid and placebo interventions.

Conclusion: Consumption of 98 mg of highly bioavailable curcuminoids with each principal meal sufficed to achieve curcuminoid accumulation in the blood, was safe, and did not alter blood lipids, inflammation, glucose, or iron homeostasis in healthy subjects with slightly elevated blood cholesterol and C-reactive protein.

Keywords:
Blood lipids / Cholesterol / Curcumin micelles / Curcuminoids / Inflammation / Safety

1 Introduction

Cardiovascular diseases are one of the leading causes of mortality in the Western world [1]. Atherosclerosis is the immediate cause of cardiovascular disease and its development is associated with hypertension, smoking, hyperlipidaemia, and chronic inflammation [2]. At present, the most common therapy used to prevent cardiovascular events is the prescription of lipid-lowering drugs, such as statins, which inhibit cholesterol biosynthesis and enhance the clearance of circulating low-density lipoprotein cholesterol (LDL-C) from blood [3]. Furthermore, statins reduce C-reactive protein (CRP), an acute phase reactant and risk factor for cardiovascular diseases [4]. However, statins have several side effects, including rhabdomyolysis and myopathy [5]. For this reason, there is increasing interest in finding alternatives or complements to statins for lowering blood lipids. Curcuminoids are among these proposed alternative lipid-lowering agents.

Curcuminoids are lipophilic phytochemicals from the plant *Curcuma longa Linn*. Curcumin makes up 75–85% of the curcuminoids with demethoxycurcumin (DMC) and bis-demethoxycurcumin (BDMC) making up 15–20 and 2–8%, respectively [6]. Curcumin is present in spices containing turmeric and used as food colorant (E100) by the food industry. The mean daily intake of curcumin was estimated to be 0.4–1.5 mg/kg bodyweight in India [7] and 0.48 mg/kg bodyweight in France [8]. Besides its use as food

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Abbreviations: **ALT**, alanine transaminase; **AST**, aspartate transaminase; **BDMC**, bis-demethoxycurcumin; **CRP**, C-reactive protein; **DMC**, demethoxycurcumin; **HOMA-IR**, insulin resistance index; **TAG**, triacylglycerol

colorant (E100), curcumin possesses a wide range of potential biological activities, which have been suggested to be useful in the treatment of chronic diseases including cancer, diabetes mellitus, neurodegenerative or cardiovascular diseases [9,10]. So far, the lipid- and inflammation-lowering effects of curcumin have been demonstrated in different animal models and human studies [11–13]. The results from human studies, however, are inconclusive [12, 14, 15].

Native curcuminoids have poor oral bioavailability [16–18], which may explain why previous human studies testing native curcuminoids have led to variable results. We hypothesize that curcuminoids with improved bioavailability will lower plasma lipids and inflammation in subjects at risk for the metabolic syndrome. We investigated the effects of curcuminoids on plasma lipids and markers of inflammation, administered in form of a novel oral delivery system (micelle) with hundredfold enhanced bioavailability [17, 18], in a randomized, double-blind, crossover study with a duration of 6 wk per intervention in subjects with moderately elevated cholesterol and CRP. Secondary outcomes were the effects of curcuminoids on glucose and iron metabolism based on earlier studies suggesting that curcuminoids may affect these parameters [19–21].

2 Subjects and methods

2.1 Formulations

The curcuminoid micelles were produced with native curcumin powder (Jupiter Leys, Cochin, Kerala State, India), which contained 82% curcumin, 16% demethoxycurcumin (DMC), and 2% bis-demethoxycurcumin (BDMC). Curcuminoid micelles comprised 7% curcumin powder (equal to 6% curcumin) and 93% Tween-80 (Kolb, Hedingen, Switzerland) and were produced by and a kind gift of AQUANOVA AG (Darmstadt, Germany). The placebo capsules contained only Tween-80. Tween-80 (placebo) and the liquid micelles were filled into Licaps (Capsugel, Colmar, France). Each curcuminoid capsule contained 20.1 mg curcumin, 3.9 mg DMC, and 0.5 mg BDMC.

2.2 Subjects

Seventeen men (50 ± 20 years) and twenty-five women (52 ± 16 years) took part in the study. Inclusion criteria to participate in the study were total cholesterol concentrations >200 mg/dL, and/or LDL-C >135 mg/dL and/or triacylglycerol concentrations >200 mg/dL and/or CRP concentrations >2 mg/L. Exclusion criteria were daily intake of medications (with the exception of contraceptives), pregnancy or lactation, chronic diseases, drug or alcohol abuse, smoking, intake of dietary supplements, extreme physical exercise, and hypersensitivity to curcuminoids. Each subject gave informed written consent to participate in the study. The study protocol was reviewed and approved (F-2013-048) by the Ethics committee of the State Medical Society of Baden-Württemberg, Germany. The trial is registered at ClinicalTrials.gov (NCT01925547).

2.3 Study design

This study was designed as a randomized, double-blind, crossover trial in which each subject received 241.2 mg curcumin, 47.1 mg DMC, and 5.9 mg BDMC (294.2 mg curcuminoids) per day. Curcuminoid or placebo capsules were taken three times per day, four capsules (equivalent to 80.4 mg curcumin, 15.6 mg DMC, and 2.0 mg BDMC) with each principal meal, for six wk and then crossed over to the alternate regimen. The treatment periods were separated by a 4-week wash-out phase. Fasting blood samples (≥10 h) were collected at the beginning, after 3 and 6 wk of each intervention. At each time point, height and weight were determined and BMI calculated. During the study, subjects were advised not to change their lifestyle. In order to determine the compliance, the remaining capsules were collected from each subject and counted.

2.4 Sample preparation and measurement of curcuminoids

Sample preparation and measurement of plasma curcuminoid concentrations were previously reported in detail [17,18]. Briefly, 1 mL plasma was acidified with 10 μL 6 M hydrochloric acid and incubated with 100 μL beta-glucuronidase type H1 from Helix Pomatia (1 mg/100 μL in 0.1 M sodium acetate buffer, Sigma-Aldrich Chemie GmbH, Schnelldorf, Germany) for 45 min at 37 °C. After triplicate extraction with 95% ethyl acetate and 5% methanol (v/v), supernatants were evaporated to dryness and resuspended in 150 μL methanol, vortexed for 20 s, stored in the dark for 10 min, vortexed for 20 s, and transferred into HPLC vials. Twenty microliters of each sample were injected into the HPLC system.

Curcuminoids were quantified on a Jasco HPLC system (Jasco GmbH, Gross-Umstadt, Germany) with a fluorescence detector (excitation wavelength 426 nm, emission wavelength 536 nm) and separated on a Reprosil-Pur C18-AQ column (150 mm × 4 mm, 3 μm particle size; Dr. Maisch GmbH, Ammerbuch, Germany) maintained at 40 °C. The mobile phase comprised 52% de-ionized water (adjusted to pH 3 with perchloric acid), 34% acetonitrile, and 14% methanol. Curcuminoids were quantified against external standard curves (curcumin, purity ≥ 97.2%, CAS # 458-37-7; DMC, purity ≥ 98.3%, CAS # 22608-11-13; BDMC, purity ≥ 99.4%, CAS # 24949-16-; Chromadex, Irvine, USA).

2.5 Sample preparation and measurement of serum safety parameters, lipid profiles, and C-reactive protein

Serum samples for the determination of lipid profiles (triacylglycerols, total cholesterol, HDL-C and LDL-C, LDL-C/HDL-C), glucose metabolism (fasting glucose, insulin, and HOMA-IR), iron status (iron, ferritin, transferrin, and transferrin saturation) and safety parameters (aspartate transaminase (AST), alanine transaminase (ALT)) were collected; stored at room temperature for 30 min and centrifuged (1008 × *g*, 15 min, 20 °C). EDTA-plasma for the determination of inflammation markers (CRP, IL-6) and curcumin, DMC, and BDMC was immediately centrifuged after collection (1008 × *g*, 10 min, 4 °C), aliquoted and stored at −80 °C.

Triacylglycerols, total cholesterol, high density lipoprotein (HDL-C) and LDL-C, LDL-C/HDL-C, fasting glucose, insulin, iron, ferritin, transferrin, AST and ALT were analyzed by the clinical laboratory Laborärzte Sindelfingen (Sindelfingen, Germany) using standard clinically validated ELISA-based techniques. Insulin resistance index (HOMA-IR) was calculated according to the following formula: [fasted insulin (mU/L) × fasted glucose (mg/dL)]/405.

2.6 Quantification of IL-6

Duplicates of plasma samples (100 μL) were analyzed using the Human Ready-Set-Go! ELISA kit for IL-6 (#88-7066; IL-6, eBioscience, Frankfurt, Germany) according to the manufacturer's instructions.

2.7 Statistical analyses

Values were expressed as means with their standard deviation (SD) or standard error of the mean (SEM), as indicated. The standardized residuals were tested for normality with the Shapiro–Wilk test and skewed data were log transformed. Two-factor repeated-measures ANOVA (factors were treatment and time) was used to test for significant effects of treatment or time on changes from baseline values as well as treatment × time interactions. Where an overall treatment or time effect or treatment × time interaction was observed, Bonferroni post-hoc tests were carried out to test which groups were different. Data analyses were performed using SPSS (IBM Corp., IBM SPSS Statistics for Windows, Version 22.0. Armonk, NY, USA) and GraphPad Prism version 6.01 (GraphPad Software, San Diego, CA, USA).

3 Results

3.1 Subjects, compliance, and side effects

Seventeen men and twenty-five women completed the study (Table 1). One woman dropped out because of diarrhea during the intake of curcuminoid micelles, and one woman and one man for personal reasons. Compliance, based on capsule returns, was 93.5% and did not differ between treatments. Side effects observed during both interventions were similar and unspecific, with the exception of yellow feces, which was only observed following the ingestion of curcuminoids (Table 2). During both intervention periods, subjects gained weight (intervention, 0.7 kg; placebo, 0.3 kg) and consequently underwent increases in their body mass index, but this effect was only significant for curcumin ($p<0.01$ for time; week 0 versus week 6, $p<0.009$; Table 3). However, no significant differences in body weight were observed between curcuminoid and placebo treatment at the end of the study.

Table 1. Baseline characteristics (mean ± SD) of the 42 subjects who completed the trial[a)]

Variable	Women ($n = 25$)	Men ($n = 17$)	$p<$
Age (y)	52 ± 16	50 ± 20	n.s.
Body height (m)	1.6 ± 0.1	1.8 ± 0.1	0.0001
Body weight (kg)	73.6 ± 15.3	85.0 ± 11.6	0.05
Body mass index (kg/m^2)	27.0 ± 5.2	26.0 ± 3.3	n.s.
Total cholesterol (mg/dL)	240.2 ± 56.4	219.6 ± 36.7	n.s.
LDL-C (mg/dL)	157.5 ± 44.6	156.3 ± 30.5	n.s.
HDL-C (mg/dL)	62.8 ± 10.5	44.9 ± 9.1	0.0001
Triacylglycerols (mg/dL)	114.2 ± 46.5	120.1 ± 47.0	n.s.
C-reactive protein (mg/L)	4.9 ± 3.6	3.1 ± 1.8	n.s.

[a)] Differences between women and men were analyzed by an unpaired Student's *t*-test or Mann–Whitney U test, as appropriate.

Table 2. Number of subjects, out of the 17 men and 25 women who completed the study, reporting side effects during 6 wk of daily supplementation with 294 mg micellar curcuminoids (equivalent to 241 mg pure curcumin) or placebo capsules

Side effects	Curcumin Women	Placebo Men	Women	Men
Softer feces			1	
Diarrhea	2	3	1	1
Yellow feces	1	1		
Bloating	1		2	
Pyrosis	1		2	
Stomach ache		1		1
Headache	2	1		1
Hot flushes			1	

3.2 Plasma curcuminoids

Fasting concentrations of plasma curcuminoids were not detectable in any subject at baseline, nor during or after placebo intervention. Treatment (curcumin, $p<0.001$; DMC, $p<0.03$; BDMC, $p<0.000$), time (curcumin, $p<0.001$; DMC, $p<0.047$;

Table 3. Mean concentrations (± SD) of serum lipids, and biomarkers of inflammation, glucose, and iron metabolism, and mean values of anthropometric measurements, and liver enzyme activities in 42 volunteers (25 women, 17 men) before and after 3 respectively 6 wk of supplementation with 294 mg micellar curcuminoids (equivalent to 241 mg pure curcumin) or placebo capusles[§]

Week	Curcumin			Placebo			P for treatment	P for time	P for treatment x time
	0	3	6	0	3	6			
Serum lipids									
Triacylglycerols (mg/dL)	120.6 ± 56.5	111.8 ± 49.1	111.1 ± 47.2	115.2 ± 47.5	119.3 ± 54.2	121.4 ± 53.2	n.s.	n.s.	n.s.
Total cholesterol (mg/dL)	234.5 ± 45.1	235.3 ± 47.2	237.2 ± 45.9	232.5 ± 51.9	238.5 ± 47.2	239.3 ± 50.1	n.s.	n.s.	n.s.
LDL-C (mg/dL)	162.0 ± 37.8	159.7 ± 36.4	160.8 ± 36.4	157.8 ± 39.5	161.0 ± 35.8	162.5 ± 38.0	n.s.	n.s.	n.s.
HDL-C (mg/dL)	55.5 ± 13.0	56.3 ± 14.8	55.6 ± 14.9	55.0 ± 13.3	55.2 ± 13.9	55.3 ± 15.1	n.s.	n.s.	n.s.
LDL/HDL-C (mg/dL)	3.1 ± 1.0	3.0 ± 0.9	3.1 ± 1.0	3.0 ± 0.9	3.1 ± 0.8	3.1 ± 0.9	n.s.	n.s.	n.s.
Inflammation markers									
CRP (mg/L)	3.4 ± 2.4	3.5 ± 2.3	3.4 ± 1.8	3.6 ± 2.2	3.2 ± 2.0	3.4 ± 2.0	n.s.	n.s.	n.s.
IL-6 (pg/mL)	9.9 ± 16.5	8.2 ± 11.3	7.2 ± 7.9	8.1 ± 12.5	7.8 ± 11.1	9.6 ± 15.7	n.s.	n.s.	n.s.
Glucose metabolism									
Fasting glucose (mg/dL)	89.5 ± 9.1	88.7 ± 10.9	91.9 ± 10.0	89.2 ± 9.4[a]	89.8 ± 8.4[a]	92.5 ± 9.5[b]	n.s.	0.001	n.s.
Insulin (μU/mL)	9.0 ± 4.7	8.4 ± 4.7	8.9 ± 4.5	7.8 ± 4.1	8.9 ± 5.4	8.5 ± 3.1	n.s.	n.s.	n.s.
HOMA-IR	2.0 ± 1.01	1.9 ± 1.3	2.0 ± 1.2	1.7 ± 1.0	1.9 ± 1.3	1.9 ± 0.8	n.s.	n.s.	n.s.
Iron metabolism									
Iron (μg/dL)	91.5 ± 31.4	82.3 ± 31.7	86.3 ± 35.5	89.1 ± 27.2	85.9 ± 26.0	88.4 ± 27.7	n.s.	n.s.	n.s.
Ferritin (μg/L)	150 ± 161	144 ± 164	142 ± 160	145 ± 152[a]	141 ± 145[a,b]	138 ± 151[b]	n.s.	0.001	n.s.
Transferrin (g/L)	2.9 ± 0.5	2.8 ± 0.5	2.8 ± 0.6	2.9 ± 0.6	2.8 ± 0.5	2.9 ± 0.6	n.s.	n.s.	n.s.
Transferrin saturation (%)	23.3 ± 8.7	21.3 ± 9.0	22.6 ± 9.9	22.5 ± 8.3	22.1 ± 8.0	22.7 ± 7.6	n.s.	n.s.	n.s.
Body weight and BMI									
Body weight (kg)	78.4 ± 15.1[a]	78.8 ± 15.3[a,b]	79.1 ± 15.2[b]	78.7 ± 15.1	78.7 ± 15.3	79.0 ± 15.5	n.s.	0.010	n.s.
BMI (kg/m^2)	26.7 ± 4.6[a]	26.8 ± 4.7[a,b]	26.9 ± 4.7[b]	26.8 ± 4.6	26.8 ± 4.6	26.9 ± 4.8	n.s.	0.011	n.s.
Liver enzymes									
AST (U/L)	24.7 ± 6.5	24.7 ± 6.5	26.5 ± 11.2	24.6 ± 5.7	24.8 ± 7.0	24.5 ± 6.0	n.s.	n.s.	n.s.
ALT (U/L)	25.6 ± 15.9	23.0 ± 13.0	24.8 ± 15.2	24.6 ± 13.6	26.3 ± 23.9	23.7 ± 13.0	n.s.	n.s.	n.s.

[§]Different superscript letters within a row indicate significant differences between time points within each group. Statistical significances were calculated by means of repeated measures 2-way ANOVA (treatment and time as factors) with Bonferroni post-hoc tests.

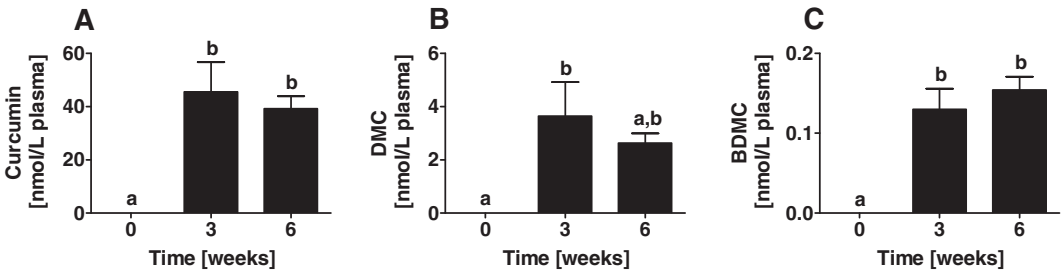

Figure 1. Mean fasting plasma total curcumin (A), demethoxycurcumin (DMC) (B), and bis-demethoxycurcumin (BDMC) (C) concentrations in 42 volunteers (25 women, 17 men) before and after 3 respectively 6 wk of supplementation with 294 mg micellar curcuminoids (equivalent to 241 mg curcumin). Curcuminoids were not detectable in the plasma samples of subjects during the placebo intervention. Error bars indicate standard error of the mean. Bars not sharing a common superscript letter are significantly different at $p< 0.05$.

BDMC, $p<0.000$) and treatment x time effects (curcumin, $p<0.001$; DMC, $p<0.047$; BDMC, $p<0.000$) were observed for all curcuminoids. Except for DMC at week 6, fasting plasma curcuminoids were significantly higher 3 (curcumin and BDMC, $p<0.000$; DMC, $p<0.049$) and 6 wk (curcumin and BDMC; $p<0.000$) after the intake of curcuminoid micelles in comparison to baseline and placebo, but did not differ between three and six wk of curcumin consumption (Fig. 1).

3.3 Serum lipids and inflammation markers

Total cholesterol, triacylglycerols (TAG), LDL-C, HDL-C, and LDL/HDL-C concentrations did neither differ between interventions nor change over time (Table 3). CRP and IL-6 concentrations were neither affected by treatment nor time (Table 3).

3.4 Glucose metabolism

A time effect ($p<0.001$), but no treatment effect or treatment × time interaction, was observed for fasting glucose concentrations, which were increased after six wk of placebo consumption compared to zero ($p<0.008$) and three wk ($p<0.001$; Table 3). Fasting insulin concentrations and HOMA-IR did not change between interventions or over time.

3.5 Iron homeostasis

Free iron, transferrin, and transferrin saturation were neither altered by treatment nor time (Table 3). A time effect ($p< 0.001$) was observed for ferritin concentrations, which were significantly lower after 6 wk of placebo consumption compared to baseline ($p<0.01$). There was no treatment effect on ferritin concentrations (Table 3).

3.6 Liver enzymes

No treatment or time effects were observed for alanine transaminase (ALT) and aspartate transaminase (AST) activities (Table 3).

4 Discussion

In this randomized, double-blind, placebo-controlled, crossover study, we investigated the lipid-lowering and anti-inflammatory effects as well as the safety of 294 mg bioavailability enhanced micellar curcuminoids (equivalent to 241 mg pure curcumin) in otherwise healthy women and men with moderately elevated blood lipids and inflammation markers. We did not detect overall sex effects in any of the measured parameters (although baseline and endpoint values for a number of parameters differed between sexes) and therefore discuss only effects in the entire study population below.

4.1 Fasting plasma curcuminoid concentrations

We observed mean fasting total plasma curcumin concentrations of ca. 45 nmol/L (curcuminoid concentrations ca. 49 nmol/L) after intervention with only 241 mg curcumin (294 mg curcuminoids) for as little as 3 wk, which is higher than maximum plasma concentrations achieved with even higher doses of native curcumin in single-dose pharmacokinetic studies [17, 18]. To the best of our knowledge, fasting plasma concentrations of curcuminoids have not been reported in any other human intervention trial with curcuminoids. This observation suggests that ingestion of as little as 98 mg curcuminoids (80 mg curcumin) with each principal meal, when administered as highly bioavailable micellar formulation [17, 18], is sufficient to achieve accumulation of curcuminoids in the body.

4.2 Blood lipids

Total triacylglycerols, total cholesterol, LDL-C, and HDL-C concentrations and the LDL-C/HDL-C ratio were neither affected by treatment, nor time (Table 3). The observed lack of effect of supplementary curcuminoids on blood lipids in moderately hyperlipidemic subjects is in agreement with previous reports using native curcuminoids [14,22,23]. In patients with

acute coronary heart syndrome, the daily intake of up to 180 mg native curcumin and of native curcuminoids (equivalent to 300 mg/day curcumin) in type 2 diabetes mellitus patients for 2 months did not alter total cholesterol, LDL-C, HDL-C, or TAG concentrations compared to placebo [22, 23].

On the other hand and as described in the following, there are a number of studies reporting moderate lipid-lowering effects of curcuminoids in humans [12, 15, 19, 20, 24–27]. In healthy middle-aged subjects (40–60 years; $n = 19$ per group), TAG, but not cholesterol, slightly decreased compared to placebo (starch) upon intake of 80 mg/day curcumin as solid lipid particles (Longvida) for 1 month; however; TAG concentrations were higher in the curcumin than placebo group already at baseline and remained higher even after the intervention [15]. The same amount of this formulation taken for 1 month by healthy elderly people (60–85 years) decreased cholesterol and LDL-C, but not TAG concentrations [24]. The extent of these inconsistent effects on different blood lipid parameters, however, was very small (<10 mg/dL) and may have been affected or even caused by within-person variations between blood draws, which, for total cholesterol, have been reported to be in the range of ~5 mg/dL [28] to ~17 mg/dL [29].

In patients with diabetes mellitus type 2, the daily intake of 1.5 g native curcuminoids for 6 months ($n = 99$) significantly decreased TAG, but not total cholesterol, LDL-C, and HDL-C, compared to placebo ($n = 100$) [20]. Reduced TAG and unchanged blood cholesterol concentrations were also observed in overweight/obese type 2 diabetics (mean BMI 27 kg/m^2) who consumed 300 mg/day curcuminoids for 3 months [19]. Furthermore, the intake of 1.89 g curcumin extract for 3 months significantly improved the lipid profile of patients with metabolic syndrome [27]. The latter studies suggest that the lipid-lowering potential of curcuminoids may require an intervention time of 3 months or longer and/or may be more pronounced in patients suffering from the metabolic syndrome.

Curcumin may lower cholesterol concentrations by inhibiting cholesterol *de novo*-synthesis [11] and/or by reducing its absorption [30–32], inter alias by competing for incorporation into mixed micelles. The latter mechanism would explain the lack of efficacy in the current study, where curcuminoids were already prepackaged into micelles and thus did not interfere with the formation of mixed micelles.

4.3 Inflammation markers

The pathogenesis of many chronic diseases, including atherosclerosis, is associated with systemic inflammation [33, 34]. Therefore, we also investigated the effects of curcuminoid treatment on serum concentrations of the acute-phase protein CRP and the cytokine IL-6, which stimulates the expression of CRP [35]. Neither inflammation marker was altered by curcuminoid intervention in the present study (Table 3).

This is in agreement with earlier reports that the daily intake of 80 mg curcumin (Longvida) for 1 month did not affect CRP concentrations in healthy middle-aged people [15] and treatment of patients with oral lichen planus with 6 g/day curcuminoids for 2 wk did neither alter CRP nor IL-6 concentrations [36]. The maximum duration of curcuminoid intervention in these trials and ours was 6 wk. For effective anti-inflammatory activity, longer interventions as well as patients with more severe inflammation may be required. However, the intake of 200 mg/day liposomal curcumin (Meriva) for 3 months reduced CRP concentrations only compared to baseline and only in a subpopulation of osteoarthritis patients ($n = 50$) with initially high CRP (168 mg/L), but not compared to control [13]. In a second trial using the same intervention (200 mg/day liposomal curcumin) in osteoarthritis patients for 8 months, a similar reduction from baseline was observed for IL-6 and IL-1β, but again, no differences to the control group were observed [37]. In a parallel-design, placebo-controlled ($n = 21$) trial investigating the effects of curcuminoids ($n = 23$) on blood lipids and inflammatory markers in type 2 diabetics, the daily intake of curcuminoids (equivalent to 600 mg curcumin) for 8 wk reduced IL-6 and tumor necrosis factor alpha compared to baseline, but not compared to control [23].

Overall, it appears that the potent anti-inflammatory effects of curcuminoids observed in cell culture and animal studies may not translate into similar effects in humans [38] or may be observable only in more severely afflicted patients suffering from different inflammatory diseases (e.g. inflammatory bowel disease) and/or require different treatment regimen.

4.4 Glucose homeostasis

We observed a significant time effect on fasting glucose concentrations (Table 3), but as this was caused by a slight increase after the consumption of placebo for 6 wk, we conclude that it is likely without biological relevance. Our observation that curcuminoids did not alter glucose homeostasis in healthy subjects, is supported by similar findings in diabetes mellitus type 2 patients treated with curcuminoids (equivalent to 600 mg/day curcumin for 2 months) [23] and in patients with the metabolic syndrome ingesting 1.89 g/day curcumin for 3 months [27].

There is, however, evidence that treatment of prediabetic subjects with 1.5 g curcuminoids per day for 9 months prevents the progression to diabetes mellitus type 2 [39]. A reduction in HOMA-IR following ingestion of 1.5 g curcuminoids per day was observed in type 2 diabetics already at 3 months and even more pronounced at 6 months of intervention [20]. As little as 300 mg curcuminoids per day for 3 months reduced fasting blood glucose and HOMA-IR in diabetes mellitus type 2 patients in another study [19].

The discrepancies between these trials and our findings are likely explained by differences in study populations

(pre-/diabetic patients versus healthy subjects) and the duration of intervention. Based on the studies described above, effects on blood glucose concentrations appear to be limited to patients with already impaired glucose metabolism and require curcuminoid interventions of 3 months or more.

4.5 Iron homeostasis

We assessed iron status by measuring serum iron, transferrin, and ferritin concentrations in our subjects, because reduced iron status was observed after long-term curcuminoid feeding in previous mouse studies [21,40]. None of these iron status parameters differed between treatments (Table 3). In mice, which were fed for 6 months with a Western type diet supplemented with 0.2% curcuminoids, iron contents in liver and spleen and liver hepcidin and ferritin expression were reduced. The authors suggested that curcumin may complex iron in the intestine and thereby inhibit its absorption [21]. As we administered curcuminoids in the form of micelles in the present study, such an iron-chelating activity, if biologically relevant at all, may have been reduced.

4.6 Safety of the bioavailability enhanced micellar curcuminoids

Enhancing the bioavailability of a substance and thereby the achieved blood and tissue concentrations may change its safety profile. In order to assess if the intake of micellar curcuminoids for a prolonged time may lead to hitherto unknown toxicity or adverse effects, we recorded any adverse reactions reported by our volunteers and measured liver enzyme activities in serum (Tables 2 and 3). No significant treatment or time effects were observed. Thus, the used micellar formulation appears to be safe and well tolerated in moderately overweight and hyperlipidemic subjects, which is in agreement with our previous observations in healthy young and aged volunteers [17,18].

4.7 Factors that may have contributed to the observed lack of effect

In addition to the points discussed in the individual chapters above, the lack of effect of micellar curcumin, despite of its enhanced bioavailability and demonstrated accumulation in the body (Fig. 1), may be explained by any of the following or a combination thereof: (i) Maximum plasma respective tissue concentrations may not have reached the minimum effective concentration required for biological activity, and/or (ii) the health beneficial effects of curcumin may be facilitated by interactions of the curcumin molecule with (a) the absorption of other nutrients or bioactive compounds in the gastro-intestinal tract, (b) direct interactions of curcumin with (anti-) inflammatory targets in/on intestinal cells, or (c) effects of unabsorbed curcumin with gastro-intestinal bacteria and changes to the composition of the gut microbiota; all of which may have been altered by the enhanced intestinal absorption of micellar curcumin and/or by the entrapment of the phytochemical in the core of the micelle.

The micellar structure itself might thus impact the biological activity of the contained active agent. It is currently assumed that micelles release their contained active substance, which is then absorbed into small intestinal cells and from thereon underlies the same metabolic and transport processes as the native (unformulated) compound. The micellar structure could, at least theoretically, be recognized by cell surface receptors, which could then trigger endocytosis of the entire micellar particle. If the micelles were indeed endocytosed and even secreted into the blood circulation as a whole, this could significantly alter the interaction of the micellized substance with molecular targets. However, the uptake mechanism of curcumin from micelles respectively of curcumin micelles into intestinal epithelial cells has not been investigated yet and thus remains a pressing topic for future research. To the best of our knowledge, there is no evidence available in the published literature suggesting that orally administered polysorbate 80 micelles may enter the systemic circulation. In support of this, we did not find free curcumin in the circulation of our volunteers (Fig. 1), but only conjugated phase II metabolites, which suggests that the curcumin contained in the micelles was released and accessible to phase II enzymes.

Another mechanism by which micellar formulation may alter curcumin disposition and thereby activity is by interaction of the surfactant (polysorbate 80) with membrane transporters that are themselves involved in the export of xenobiotics and their conjugates (e.g. glucuronides) from cells. Such an inhibition of ATP-binding cassette transporter C2 (aka multidrug resistance associated protein 2) mediated drug export was reported, even though only at very high concentrations, for polysorbate 80 [41]. The inhibition of efflux transporters by the excipient used in the formulation, however, is more likely to enhance the biological availability and activity of curcumin rather than reducing its efficacy. Accordingly, improved oral bioavailability and inhibition of P-glycoprotein (ATP-binding cassette transporter B1) has been reported for (nonpolysorbate 80) mixed micelles [42].

Further well-designed trials will be required to elucidate if the lack of effect observed in the present study was due to the dose (too low?), duration of the intervention (too short?), or any respectively a combination of the aforementioned factors. Last but not least, it is also possible that the blood-lipid lowering and anti-inflammatory activities of curcumin observed in cell culture experiments and animal models do not translate into comparable activities in humans.

4.8 Conclusion

In conclusion, daily supplementation for 6 wk with 294 mg highly bioavailable curcuminoids (98 mg with each principal

meal) was sufficient to achieve accumulation of curcuminoids in fasting plasma, was well tolerated and safe, and did not alter blood lipids, inflammatory status, glucose, or iron homeostasis in healthy subjects with slightly elevated blood lipids and inflammation markers.

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J.F. is a consultant to AQUANOVA AG, the company manufacturing micellar curcumin. All other authors have declared no conflict of interest.

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