# Micellar Curcumin: Pharmacokinetics and Effects on Inflammation Markers and PCSK-9 Concentrations in Healthy Subjects in a Double-Blind, Randomized, Active-Controlled, Crossover Trial

*Juergen Grafeneder, Ulla Derhaschnig, Farsad Eskandary, Nina Buchtele, Nadine Sus, Jan Frank, Bernd Jilma,\* and Christian Schoergenhofer*

**Scope:** Preclinical models have demonstrated the anti-inflammatory and lipid-lowering effects of curcumin. Innovative formulations have been developed to overcome the poor bioavailability of native curcumin. The study hypothesizes that the bioavailability of micellar curcumin is superior to native curcumin and investigates the potential anti-inflammatory and proprotein convertase subtilisin/kexin type 9 (PCSK9) concentration lowering effects. **Methods and results:** In this double-blind, randomized, crossover trial, 15 healthy volunteers receive micellar or native curcumin (105 mg day$^{-1}$) for 7 days with a $\geq$7 days washout period. Curcumin and metabolite concentrations are quantified by high-performance liquid chromatography with fluorescence detection (HPLC-FD), and pharmacokinetics are calculated. To analyze anti-inflammatory effects, blood samples (baseline, 2 h, 7 days) are stimulated with 50 ng mL$^{-1}$ lipopolysaccharides (LPS). Interleukin (IL)-6, tumor-necrosis factor (TNF-$\alpha$), and PCSK9 concentrations are quantified. Micellar curcumin demonstrates improved bioavailability ($\approx$39-fold higher maximum concentrations, $\approx$14-fold higher area-under-the-time-concentration curve, $p < 0.001$) but does not reduce pro-inflammatory cytokines in the chosen model. Subjects receiving micellar curcumin have significantly lower PCSK9 concentrations ($\approx$10% reduction) after 7 days compared to baseline ($p = 0.038$).
**Conclusion:** Micellar curcumin demonstrates an improved oral bioavailability but does not show anti-inflammatory effects in this model. Potential effects on PCSK9 concentrations warrant further investigation.

## 1. Introduction

Turmeric (*Curcuma longa*) is a perennial plant native to India and southeast Asia containing the bioactive substance curcumin.[1] Curcumin has been found to have beneficial effects in clinical trials investigating a range of medical conditions including rheumatoid arthritis, various cancers, and atherosclerosis.[2–5] Curcumin's antioxidant properties involve elimination of reactive oxygen species,[6,7] however its anti-inflammatory characteristics are of particular interest,[8–11] including the inhibition of activation of nuclear factor kappa-light-chain-enhancer of activated B cells (NF$\kappa$B),[12,13] a major inflammation pathway. In several studies, curcumin reduced tumor necrosis factor $\alpha$ (TNF-$\alpha$) concentration.[14,15] Furthermore, curcumin reduced the inflammatory response after stimulation with lipopolysaccharides (LPS). It reduces the upregulation of toll-like receptor 4 and myeloid differentiation protein 88.[16–19] In this context, curcumin reduced pro-inflammatory cytokines in various cell-based and animal

J. Grafeneder
Department of Emergency Medicine
Medical University of Vienna
Vienna 1090, Austria

U. Derhaschnig, B. Jilma, C. Schoergenhofer
Department of Clinical Pharmacology
Medical University of Vienna
Vienna 1090, Austria
E-mail: bernd.jilma@meduniwien.ac.at
F. Eskandary
Division of Nephrology, Department of Medicine III
Medical University of Vienna
Vienna 1090, Austria

**Table 1.** Pharmacokinetic parameters (mean ± standard deviation) of curcumin, desmethoxycurcumin (DMC), bis-desmethoxycurcumin (BDMC), and total curcuminoids (TC) in healthy humans (six women, nine men) following the intake of a single oral dose of 43 mg micellar or native curcuminoids.

| | Micellar | | | | Native | | | |
|---|---|---|---|---|---|---|---|---|
| | Curcumin | DMC | BDMC | TC | C | DMC | BDMC | TC |
| $C_{max}$ [nmol L$^{-1}$] | 433.2 ± 237.7** | 74.4 ± 39.3** | 3.1 ± 1.9* | 510.6 ± 275.8** | 10.0 ± 5.7** | 3.1 ± 2.0** | 0.83 ± 1.2* | 13.2 ± 8.0** |
| $T_{max}$ [h] | 0.7 ± 0.3** | 0.7 ± 0.3* | 0.87 ± 0.40* | 0.73 ± 0.32* | 4.57 ± 2.80** | 4.03 ± 3.21* | 2.17 ± 2.19* | 4.1 ± 2.97* |
| AUC | 652.2 ± 318.7** | 101.0 ± 72.8** | 9.7 ± 14.2 | 762.9 ± 376.1** | 39.7 ± 21.2** | 11.5 ± 8.3** | 2.5 ± 2.8 | 53.7 ± 29.9** |

* $p < 0.05$ for paired $t$-test (micellar vs native formulation). ** $p < 0.001$ for paired $t$-test (micellar vs native formulation).

models using LPS as a stimulus,[20–22] as well as in clinical trials in patients with various underlying inflammatory conditions.[23–26] Curcumin has shown anticoagulant and antiplatelet effects.[27] Its intake had positive effects on blood lipid profiles and other cardiovascular risk factors.[28,29] This effect seems to be at least partly mediated by decreased concentrations of proprotein convertase subtilisin/kexin type 9 (PCSK9) after curcumin treatment.[22]

Curcumin modulates multiple cellular targets and shows numerous biological effects.[30] These include antioxidant,[31] anti-inflammatory,[32] antiviral, antibacterial, antifungal, antiproliferative, nephroprotective, neuroprotective, hepatoprotective, immunomodulatory,[33] and anticancer activities.[34,35] However, its efficacy is limited by its low oral bioavailability and short half-life.[36,37] Micellar formulation increases the bioavailability of curcumin in healthy humans: a single oral dose consisting of six capsules containing in total 207 mg micellar curcumin results in a 57-fold increased bioavailability compared to capsules containing native curcumin.[38] In another study, 410 mg micellar curcumin (=500 mg curcuminoids) was mixed with 50 g syrup and improved bioavailability 185-fold increase compared with native powder dispersed in syrup.[39] Moreover, micellar formulation most likely increases the post-digestive stability and solubility of curcumin,[38] which might also result in a more pronounced pharmacodynamic effect. Kocher et al.[40] investigated the effects of taking 294 mg of micellar curcumins daily on inflammation markers and blood lipid concentrations in moderately hyperlipidemic patients. Although safe and tolerable, intake of 12 capsules of curcumin per day was insufficient to achieve the desired effects. The current study was designed to use a realistic and convenient dosing regimen (three capsules per day) that would be acceptable for long-term treatment of patients and ensure therapy adherence.

The focus of the trial was to investigate anti-inflammatory effects in an ex vivo model of acute inflammatory response after intake of realistic doses of micellar or non-micellar curcumin. Furthermore, we determined the pharmacokinetics, safety, tolerability, and possible effects on PCSK9 concentrations in healthy human volunteers. The use of healthy volunteers allowed the investigation of these endpoints in a homogenous human population.

## 2. Results

Twenty subjects were screened. One subject did not meet the in- and exclusion criteria and another subject withdrew consent before study initiation. Of the remaining 18 subjects, three ended the trial prematurely: one subject developed thrombophlebitis in another sub-study, one subject dropped out of the study due to unforeseen unavailability, and one subject with multiple food intolerances developed diarrhea and dropped out.

The final per protocol population consisted of nine male and six female subjects, who were 40.3 ± 9.5 years old, with a mean height of 172.5 ± 9.7 cm, a mean weight of 74.6 ± 15.2 kg, and a body mass index of 25.0 ± 4.1 kg m$^{-2}$.

### 2.1. Pharmacokinetics

Micellar curcumin was absorbed better and faster than native curcumin, with significantly higher $C_{max}$ for bis-demethoxycurcumin ($p = 0.002$), demethoxycurcumin, curcumin, and total curcuminoids (TC; all $p < 0.001$; **Table 1**, **Figure 1**, Figure S1, Supporting Information). The $C_{max}$ of total curcuminoids for micellar curcumin (510.6 nmol L$^{-1}$) was 39-fold higher than that for native curcumin (13.2 nmol L$^{-1}$). The higher bioavailability of micellar relative to native curcumin was also reflected by a 14-fold higher area under the curve (762.9 nmol L$^{-1}$ h$^{-1}$ respectively 53.7 nmol L$^{-1}$ h$^{-1}$, $p < 0.001$; Table 1). Micellar curcumin was absorbed significantly faster ($T_{max}$, 0.73 h) than native curcumin ($T_{max}$, 4.1 h; $p < 0.001$). All pharmacokinetic parameters were significantly different in favor of the micellar curcumin, apart from the bis-demethoxycurcumin area under the curve (AUC), which was only numerically, but not significantly higher in the micellar group ($p = 0.08$; Table 1). Trough concentrations of total curcuminoids, curcumin, demethoxycurcumin, and bis-demethoxycurcumin on days 3 and 7 were higher in subjects taking micellar curcumin, reflecting the augmented bioavailability with the micellar formulation (**Figure 2**).

### 2.2. Effects on TNF-α, IL-6, and PCSK9

In our model, the intake of micellar and native curcumin neither affected concentrations of TNF-α and IL-6 in unstimulated

N. Buchtele
Department of Medicine I
Medical University of Vienna
Vienna 1090, Austria

N. Sus, J. Frank
Department of Food Biofunctionality (140b)
Institute of Nutritional Sciences
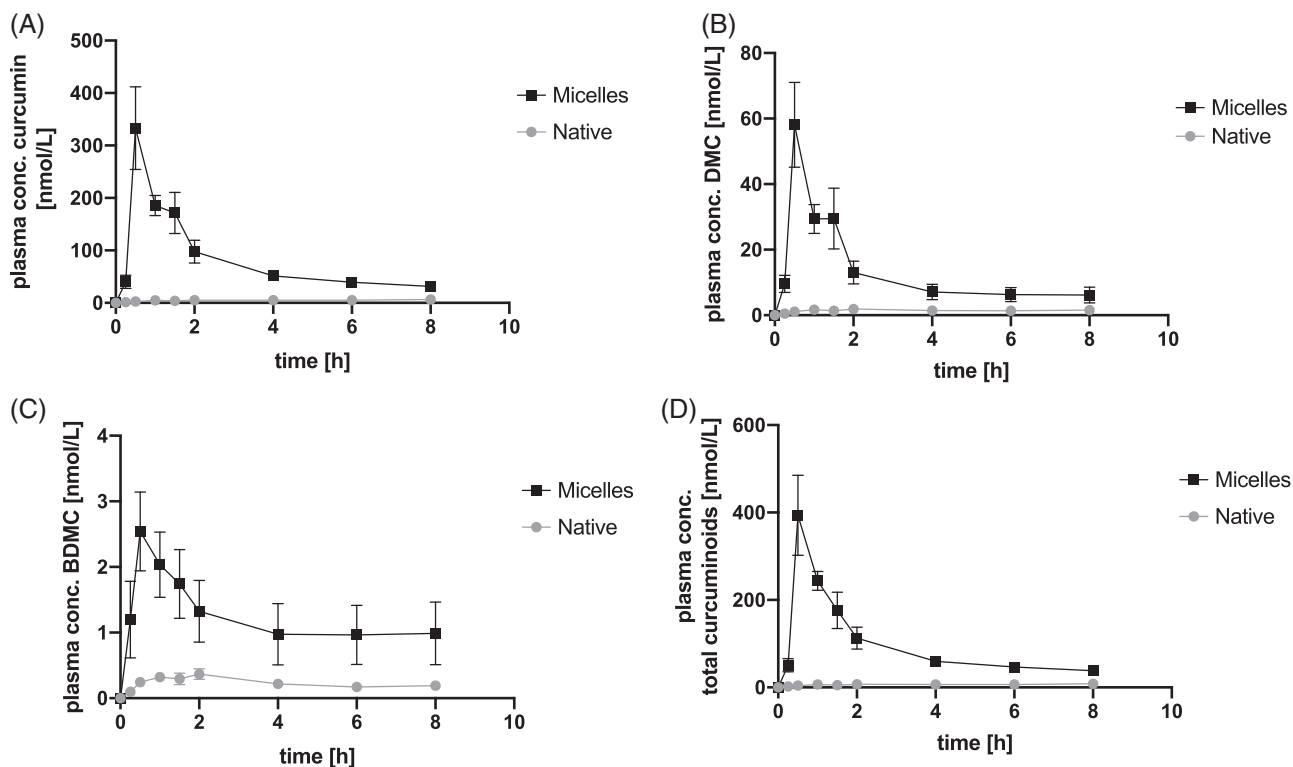University of Hohenheim
Stuttgart, Germany

**ADVANCED
SCIENCE NEWS**
www.advancedsciencenews.com

**Molecular Nutrition
Food Research**
www.mnf-journal.com

(A)



(B)

(C)

(D)

**Figure 1.** Concentration–time curves (means ± standard deviation) of A) curcumin, B) desmethoxycurcumin (DMC), C) bis-desmethoxycurcumin (BDMC), and D) total curcuminoids (all in nmol $L^{-1}$) following the intake of a single oral dose of 35 mg curcumin (in the form of capsules containing either 43 mg of micellar curcuminoids or native curcuminoids).
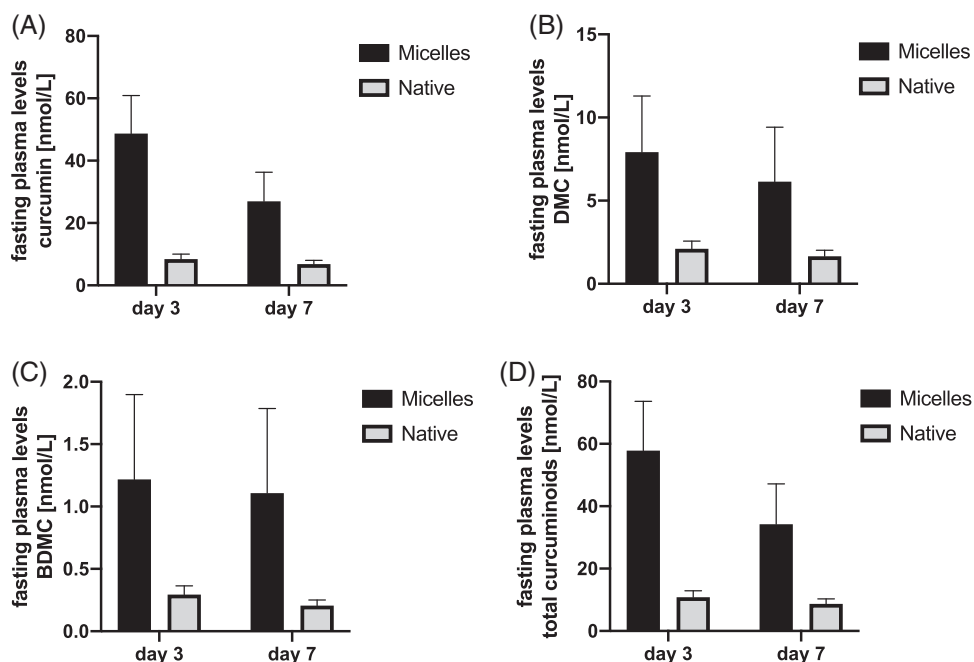
(A)



(B)

(C)

(D)

**Figure 2.** Mean trough concentrations (error bars indicate standard deviation) of curcumin A), desmethoxycurcumin (DMC) B), bis-desmethoxycurcumin (BDMC) C), and total curcuminoids D) on days 3 and 7 following the daily intake of 129 mg micellar or native curcuminoids.
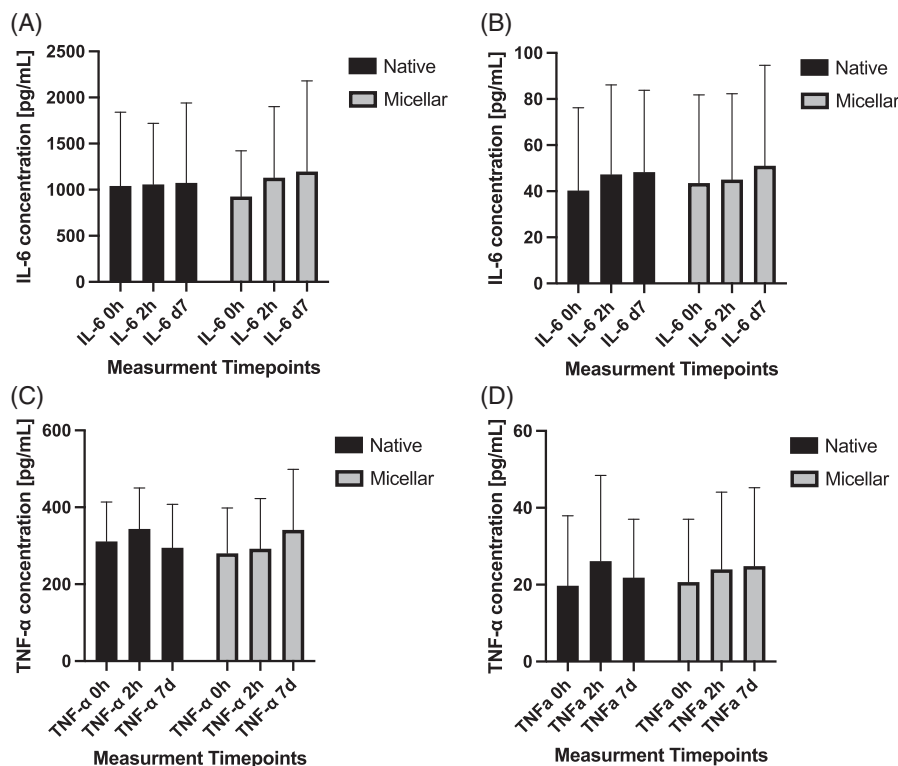
**Figure 3.** Concentrations of IL-6 and TNF-$\alpha$ in whole blood samples from healthy volunteers following an overnight fast after ingesting 129 mg day$^{-1}$ of native or micellar curcumin for 7 days; unstimulated whole blood A and C) and whole blood after ex vivo stimulation with LPS B and D).

samples, nor after ex vivo stimulation with LPS (**Figure3**). This was the case for all time points. The addition of LPS to whole blood samples increased TNF-$\alpha$ concentrations $\approx$70–130-fold and IL-6 concentrations $\approx$200–300-fold compared to unstimulated samples.

Micellar curcumin, but not native curcumin, reduced plasma PCSK9 concentrations on day 7 compared to baseline by approximately 10% ($p = 0.038$, **Figure4**). To confirm this finding, PCSK9 concentrations were quantified in a second population consisting of 42 moderately hyperlipidemic patients who received micellar curcumin (241 mg day$^{-1}$) or placebo for 6 weeks. However, curcumin had no effect on PCSK9 concentrations in this population (**Figure5**).

PCSK9 concentrations were approximately 40% higher in hyperlipidemic patients when compared to baseline values from the healthy controls in this study.

### 2.3. Blood Chemistry

Routine blood chemistry parameters investigated on days 3 and 7 did not change from baseline following the administration of either micellar or native curcumin (**Table2**, Figure S2, Supporting Information). The numerical increase in CK concentrations on day 7 in the micellar group was caused by one participant who had undertaken an unusually heavy workout the previous day. The increase in LDH concentrations on day 7 in the native group was due to one subject completing a long run the day before. Both participants reported sore muscles. The numerically
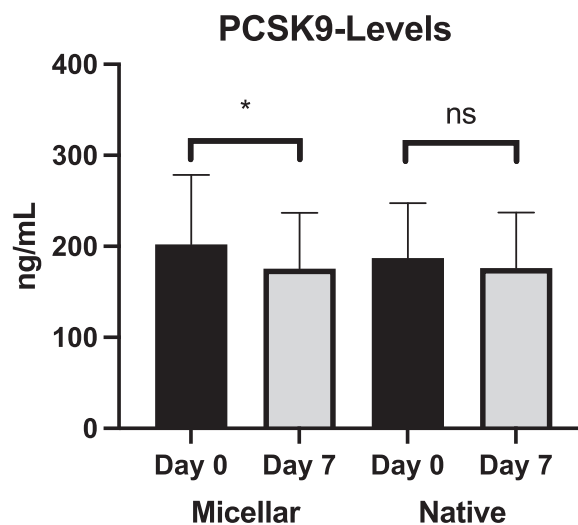


**Figure 4.** Serum concentrations of proprotein convertase subtilisin/kexin type 9 (PCSK9) in healthy humans (six women, nine men) before (day 0) and after 1 week (day 7) of daily intake of 129 mg micellar or native curcumin.

higher CRP concentrations were caused by two participants: one (CRP 0.96 mg dL$^{-1}$) had a newly diagnosed laryngitis, the other had only slightly elevated CRP (0.63 mg dL$^{-1}$), which was deemed not clinically relevant, as no other signs of infection were present. All numerically increased blood chemistry parameters had normalized by the next routine examination.

**Table 2.** Blood chemistry parameters (mean ± standard deviation) in healthy humans (six women, nine men) following the oral intake of 129 mg day$^{-1}$ micellar or native curcuminoids before (D0) and after 3 (D3) or 7 days (D7).

| Parameter [unit] (RV) | Micellar | | | Native | | |
|---|---|---|---|---|---|---|
| | D0 | D3 | D7 | D0 | D3 | D7 |
| Hb [g dL$^{-1}$] (12–16) | 13.7 ± 1.2 | 13.7 ± 1.2 | 13.8 ± 1.3 | 14 ± 1.3 | 13.9 ± 1.2 | 13.9 ± 1.1 |
| Platelets [G L$^{-1}$] (150–350) | 5.6 ± 1.4 | 5.7 ± 1.5 | 6.1 ± 2 | 5.7 ± 1.1 | 6.2 ± 2.7 | 5.7 ± 1.8 |
| Creatinine [mg dL$^{-1}$] (0.5–0.9) | 0.8 ± 0.1 | 0.8 ± 0.1 | 0.8 ± 0.1 | 0.8 ± 0.1 | 0.8 ± 0.2 | 0.9 ± 0.2 |
| Bilirubin [mg dL$^{-1}$] (0–1.2) | 0.5 ± 0.4 | 0.6 ± 0.6 | 0.6 ± 0.6 | 0.6 ± 0.5 | 0.6 ± 0.5 | 0.5 ± 0.6 |
| ASAT [U L$^{-1}$] (<35) | 22.3 ± 7.2 | 23.6 ± 8.1 | 24.4 ± 12.2 | 23.5 ± 7.5 | 22.1 ± 5.9 | 27.2 ± 14.4 |
| ALAT [U L$^{-1}$] (<35) | 22 ± 11.6 | 21.8 ± 11.3 | 23.1 ± 13.8 | 22.3 ± 8.9 | 20.3 ± 8 | 26.3 ± 26.8 |
| LDH [U L$^{-1}$] (<250) | 166.5 ± 24.2 | 171.3 ± 24.8 | 171.7 ± 23.7 | 163 ± 22.9 | 166 ± 28 | 173.1 ± 26 |
| CK [U L$^{-1}$] (<170) | 156.2 ± 139.6 | 194.4 ± 183.1 | 302.7 ± 556.5 | 151.9 ± 92.6 | 164.2 ± 120.6 | 151 ± 121.6 |
| CRP [mg dL$^{-1}$] (<0.5) | 0.1 ± 0.1 | 0.1 ± 0.2 | 0.1 ± 0.1 | 0.1 ± 0.1 | 0.1 ± 0.2 | 0.2 ± 0.3 |

ALAT, alanine transaminase; ASAT, aspartate transaminase; LDH, lactate dehydrogenase; CK, creatine kinas; CRP, C-reactive protein; D0, first day of study; D3, day 3 in study; D7, Day 7 in study; EoS, end of study; Hb, hemoglobin; RV, reference values.
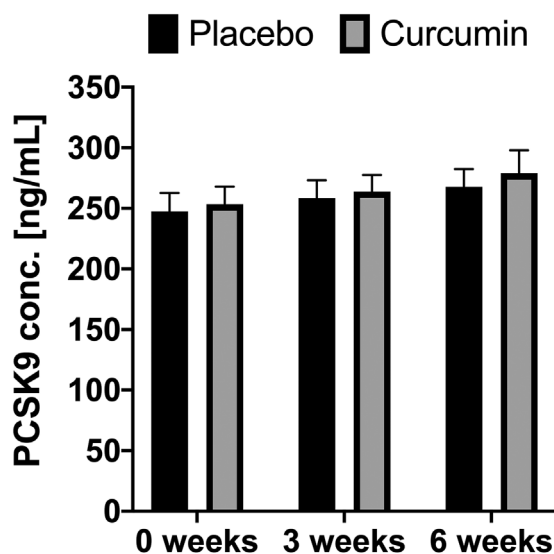


**Figure 5.** Plasma concentrations of proprotein convertase subtilisin/kexin type 9 (PCSK9) in 42 moderately hyperlipidemic patients who received a dose of micellar curcumin (294 mg per day) or placebo for 6 weeks.

### 2.4. Safety

Intake of both curcumin formulations was generally considered safe. In total, 13 adverse events occurred before or during intake of native curcumin (one episode of diarrhea, one abdominal pain, three common cold, two sore throats, one meteorism, one headache, one laryngitis, one otalgia, one xerostomia, and one inguinal pain) and 16 adverse events occurred before or after intake of micellar curcumin (one episode of nausea, one vomiting, one diarrhea, one meteorism, one abdominal pain, one common cold, four headaches, one vertigo, one sinusitis, one orthostatic reaction, one cough, one skin irritation, one blocked ear, one urolithiasis). Two serious adverse events occurred but were unrelated to the intake of curcumin. One subject broke his clavicle, required surgery and was consequently hospitalized. Another

subject suffered from urolithiasis and was hospitalized; this was, however, already the subject's second episode of urolithiasis, the first one occurring 2 years before inclusion in this trial. Both serious adverse events were judged to be unrelated to curcumin intake and both subjects recovered fully.

### 3. Discussion

This prospective, randomized, double-blind, active-controlled crossover trial investigated the pharmacokinetics and effects of micellar and native curcumin on pro-inflammatory markers and PCSK9 concentrations in healthy subjects. The results of this study show 1) the enhanced bioavailability of the micellar formulation of curcumin, which agrees with earlier findings,[38–43] 2) no effects on IL-6 or TNF-$a$ in an ex vivo model of LPS, 3) inconclusive effects of micellar curcumin on PCSK9 concentrations, and 4) a good safety profile for curcumin when administered three times a day for 1 week.

Both bioavailability and delivering curcumin to target tissues has been a problem in the use of curcumin so far. However, this has partially been solved by the introduction of micellar curcumin formulations.[38,39,41,44] Our study confirms the pharmacokinetic superiority of the micellar formulation compared to native curcumin. Based on data reported by Schiborr et al.,[39] which showed that plasma concentrations of total curcuminoids are almost back to baseline 8 h after intake, we chose a dosing regimen of three capsules per day. Trough concentrations on day 3 and day 7 were significantly higher for micellar than for native curcumin, remained stable, and showed no signs of accumulation (Figure 2), which agrees with earlier findings that fasting total curcuminoid concentrations after 3 and 6 weeks of daily intake of 294 mg micellar curcuminoids (98 mg with each principal meal) resulted in fasting plasma curcuminoid concentrations in the same range as observed here (Figure 2), and did not increase with time.[40] Together, these data suggest that steady-state concentrations of micellar curcumin may be reached within 3 days when individual doses are taken within 6–8 h.

**ADVANCED
SCIENCE NEWS**

www.advancedsciencenews.com

**Molecular Nutrition
Food Research**

www.mnf-journal.com

The main hypothesis of this study was that curcumin reduces pro-inflammatory cytokines upon stimulation with LPS by inhibition of the NF$\kappa$B pathway, which was based on numerous animal studies and in vitro experiments reporting the reduction of LPS-induced inflammation with curcumin.[45–49] It is difficult to directly compare animal studies to our experiments as both LPS doses and the mode of curcumin administration differ.[50] Nevertheless, in an adjuvant-induced rat model of arthritis, the oral administration of native curcumin (5 mg kg$^{-1}$ body weight) did not reduce edema or serum TNF-$\alpha$ and IL-6 concentrations, whereas the same dose of micellar curcumin significantly reduced all three parameters and was as effective as diclofenac (3 mg kg$^{-1}$ bodyweight).[51]

The concentrations of curcumin used in in vitro experiments,[52,53] however, are substantially higher than the maximum concentrations observed in our study (Table 1). For instance, the downregulation of microRNA-155 in LPS-stimulated macrophages was observed with curcumin concentrations of 5–15 µmol L$^{-1}$, which clearly exceeds our mean $C_{max}$ of 433 nmol L$^{-1}$ by a factor of 12–35.[54] Curcumin only decreased TNF-$\alpha$ and IL-6 concentrations in RAW.264 cells at concentrations exceeding 12.5 µmol L$^{-1}$.[55]

Another reasonable explanation may be the health status of our volunteers, as they were all generally healthy and did not have increased inflammatory markers (Table 2). In the previously mentioned human trial,[40] supplementation with 294 mg micellar curcuminoids per day in healthy subjects without signs of inflammation did not reduce the concentrations of inflammatory markers. On the other hand, a meta-analysis of clinical trials investigating their anti-inflammatory efficacy found that overall curcuminoids had an IL-6-lowering effect; this lowering effect was more pronounced in subjects with a high pro-inflammatory state at the beginning of the trial.[56]

The ex vivo-experiment chosen for this study was sufficiently sensitive to detect significant differences in proinflammatory cytokines after treatment with colistin relative to placebo.[57] Colistin, however, covalently binds LPS and neutralizes it, whereas the anti-inflammatory mechanism of curcumin is due to suppression of NF$\kappa$B activation.[58] Thus, it is possible that the concentration of LPS used in our experiment (50 ng mL$^{-1}$), which is substantially higher than the dose used in human in vivo trials (1–4 ng kg$^{-1}$ body weight), may have been too high and may have overwhelmed the NF$\kappa$B-suppressing activity of curcumin.

In summary, the findings discussed above suggest that the explanation for the lack of effect on TNF-$\alpha$ and IL-6 in the present ex vivo experiment may be 1) the comparatively low dose and resulting low blood concentrations in our in vivo study, which were far from the concentrations used in in vitro experiments,[52–55] 2) the good health status and lack of pro-inflammatory state of our volunteers, as anti-inflammatory effects of curcuminoids appear to be more pronounced in patients with elevated inflammatory cytokines,[56] and/or 3) the use of a too strong inflammatory stimulus (in the form of high-dose LPS) in the ex vivo model. Last, but not least, we also need to consider the possibility that the reported NF$\kappa$B-inhibiting activity of curcumin may not be relevant in this model.

Interestingly, in healthy volunteers, micellar curcumin, but not native curcumin, significantly decreased PCSK9 concentrations by approximately 10% (Figure 4), corroborating re-

sults from two previous studies, one cell-based[59] and one rat experiment.[22] We aimed to confirm this finding in a study conducted in moderately hyperlipidemic patients treated with micellar or native curcumin for 6 weeks. However, in this population intake of curcumin did not affect PCSK9 concentrations.[40] Of note, in this study curcumin also had no effects on other blood lipids, which contrasts with findings from other studies supplementing curcumin for longer periods, as discussed by Kocher et al.[40] For instance, a meta-analysis reports that curcumin may improve blood lipid profiles and specifically reduce low-density lipoprotein concentrations in patients with cardiovascular risk factors.[60] Thus, these results are inconclusive and should be interpreted with caution. However, to the best of our knowledge, this is the first randomized clinical trial in humans demonstrating that curcumin may reduce PCSK9 concentrations. PCSK9 plays a key role in lipoprotein homeostasis by interaction with the low-density lipoprotein receptor[61] and has become an important pharmacological target to lower LDL concentrations.[62] In two well-powered trials, the two monoclonal antibodies targeting PCSK9, alirocumab, and evolocumab, have effectively reduced the risk of further cardiovascular events in patients with existing cardiovascular disease.[63,64] Moreover, PCSK9 may play a role in the detoxification of bacterial toxins and therefore may have anti-inflammatory effects during bacterial infections.[65] Thus, micellar curcumin may be an interesting alternative to reduce PCSK9 concentrations in high-risk populations. However, given the inconclusive results in various populations, further studies are needed to define the potential role of curcumin as a possible treatment option to lower PCSK9 and consecutively blood lipid concentrations. Additionally, it may exert anti-inflammatory effects via that pathway. In summary, available data are inconclusive. However, given the potential clinical value of PCSK9 inhibition by curcumin (e.g., as an alternative or adjunct to established lipid-lowering therapy), further investigation may be worthwhile.

## 3.1. Strengths and Limitations

The strengths of this study include its randomized, double-blind, active-controlled design. However, this study also has specific limitations. First, a limited sample size precludes it from detecting smaller effects. Second, curcumin was administered for 7 days only and some of its anti-inflammatory effects may require higher doses or longer treatment durations. Third, the ex vivo model of LPS-induced inflammation has relevant limitations (e.g., LPS dosing, no endothelial cells, etc.), some of which have been discussed above. Furthermore, we only used LPS as an inflammatory stimulus. Other inflammatory stimuli (e.g., lipoteichoic acid, interferons) should be investigated in future studies. Fourth, we only quantified TNF-$\alpha$ and IL-6 as pro-inflammatory cytokines in the in vitro model after LPS stimulation, because of i) their central importance in the regulation of inflammatory responses, ii) our experience with these parameters in comparable models, and iii) the strong correlation with other cytokines.[57] As previously shown by Matzneller et al.,[57] the ex-vivo model is not sensitive in detecting effects of curcumin on IL-1ß. Therefore, we may have missed such an effect in our study. Sixth, the use of capsules is convenient, but limited the total curcumin dose per subject. We have only investigated one dose and did not perform

**ADVANCED
SCIENCE NEWS**
www.advancedsciencenews.com

**Molecular Nutrition
Food Research**
www.mnf-journal.com

a dose-finding study. Higher doses would have been achievable by taking even more capsules (e.g., 6–9 per day). However, this would most likely limit long-term therapy adherence in patients. Dose finding studies and especially higher doses, possibly using alternative drug formulations, should be conducted. Finally, the use of an active control instead of a placebo may have also reduced the strength of this study in detecting small effects.

## 4. Conclusion

In this randomized, active-controlled, crossover trial in healthy volunteers, the bioavailability of micellar curcuminoids was significantly higher than that of native curcuminoids. The superior bioavailability of micellar over native curcuminoids was confirmed by significantly higher trough concentrations as soon as 3 days after initiation of intake. The daily consumption of 129 mg micellar and native curcuminoids for 1 week was safe, but without anti-inflammatory effects in an LPS-stimulated ex vivo model. To the best of our knowledge, this study for the first time reports a reduction in plasma PCSK9 concentrations in healthy subjects following supplementation with a natural dietary bioactive compound. These data could not be confirmed in a population of moderately hyperlipidemic patients and hence, further clinical studies are warranted.

## 5. Experimental Section

The trial was conducted in accordance with the Good Clinical Practice guidelines and the principles set forth in the Declaration of Helsinki. The independent ethics committee of the Medical University of Vienna approved the trial before its initiation (study number: 1580/2018). The study took place at the Department of Clinical Pharmacology at the Medical University of Vienna between October 2018 and August 2019. Written and oral informed consent was obtained from all healthy volunteers before any trial-related activity was performed.

Furthermore, confirmatory analyses were performed in samples from another clinical trial, which was completed at the Institute of Nutritional Sciences, University of Hohenheim. The detailed study design and main results were published elsewhere.[40] In short, 42 moderately hyperlipidemic subjects received micellar curcumin (total dose 241 mg day$^{-1}$; total curcuminoids, 294 mg day$^{-1}$) or placebo in a crossover study with a 4-week-washout period.

*Intervention Drug*: The study drugs were an in-kind contribution of Aquanova AG (Darmstadt, Germany). Native curcumin powder (Jupiter Leys, Cochin, Kerala State, India), which was used for all formulations, consisted of 82% curcumin, 16% demethoxycurcumin (DMC), and 2% bis-demethoxycurcumin (BDMC). Each capsule contained 43 mg curcumin powder (accordingly corresponding to 35.3 mg curcumin, 6.9 mg DMC, and 0.9 mg BDMC). Curcumin micelles furthermore consisted of polysorbate-80 (Kolb, Hedingen, Switzerland). The study drugs were taken three times a day, approximately 8 h apart, for 7 days.

*Trial Population*: Healthy volunteers aged 18–60 years were included in the trial. The key exclusion criteria were treatment with drugs which would interfere with the trial's endpoints, acute illness, or systemic inflammatory reactions, as well as known allergies to the study drugs. A complete list of exclusion criteria was available in the appendix.

*Study Design, Randomization, and Blinding*: This was a prospective, double-blind, randomized, active-controlled, crossover trial which consisted of three sub-studies. One of these sub-studies focused on micellar and native (non-micellar) curcumin and was reported here.

Block randomization was performed using an open access randomization generator (https://www.randomizer.org/). For each subject, three sets of sealed codes/labels were prepared. These featured a randomization number containing information about the treatment allocation (periods A and B). Participants, investigators, and laboratory staff were blinded. Randomization/treatment allocation was performed by a third person who was only in charge of treatment preparation and not in contact with the trial subjects. The code for a particular subject could be broken in a medical emergency, if knowing the identity of the treatment allocation would influence the subject's treatment.

Active controls (*micellar* vs *native*) were chosen for this trial, meaning that trial participants were unable to differentiate between treatment and placebo based on the taste and smell of the trial substances.

Sixteen healthy volunteers were randomized to receive micellar or native curcumin and after a washout period of ≥1 week, the respective other treatment. The washout period was deemed sufficient based on the following assumptions: i) a terminal elimination half-life of micellar curcumin of 4–6 h from earlier studies was estimated,[38,39] ii) the study hypothesized that like other NF$\kappa$B inhibitors, any anti-inflammatory effects of curcumin were concentration-dependent, and iii) the study previously showed that PCSK9 was quickly regulated, and concentrations could adapt within a few hours to clinical conditions.[66,67]

On the morning of the first study day, participants arrived at the Department of Clinical Pharmacology following an overnight fast. After baseline blood sampling, participants received a single dose of 35 mg curcumin (in the form of capsules containing either 43 mg of micellar curcuminoids or native curcuminoids). Pharmacokinetic measurements and measurement of vital signs were performed 15, 30, 60, 90, 120, 240, 360, and 480 min after dosing. After 8 h, participants were discharged.

Subjects were instructed to take three curcumin capsules (cumulative dose of 129 mg curcuminoids, 105 mg curcumin) per day, approximately 8 h apart, until day 7. The time of intake was recorded by the participants themselves on a log sheet. Subjects returned to the department on the morning of days 3 and 7 for follow-up and trough level measurements after an overnight fast. After a washout period of ≥7 days, participants received the other curcumin formulation.

*Endpoints*: As primary endpoint, TNF-$\alpha$ concentrations after stimulation with LPS were compared between the two curcumin formulations. LPS was chosen as it had been shown to upregulate TNF-$\alpha$.[57]

Secondary endpoints included interleukin-6 (IL-6) concentrations after stimulation with LPS and in the unstimulated state, and TNF-$\alpha$ concentrations in the unstimulated condition after intake of *micellar* or *native* curcumin formulations. Additionally, pharmacokinetics (including maximum concentration ($C_{max}$), time of maximum concentration ($T_{max}$), and area under the concentrations–time curve (AUC)) were compared between the two formulations. Moreover, the study investigated whether a 7-day intake of either native or micellar curcumin influenced PCSK9 concentrations.

*Measurements*: An ex vivo LPS model was chosen to investigate the anti-inflammatory effects of curcumin. IL-6 and TNF-$\alpha$ concentrations were measured; these were upregulated after stimulation with LPS.[57]

At the scheduled time points, venous EDTA anti-coagulated blood samples were taken. In a further preparation step, the whole blood samples collected at baseline, 2 h and after 7 days were spiked with LPS to produce a final concentration of 50 ng mL$^{-1}$. Positive (blood + LPS only) and negative (blood + 0.9% saline solution only, also called "unstimulated condition") controls were included. Following incubation for 2 h at 37 °C, blood was centrifuged and the resulting plasma was stored at −80 °C until analysis. Eventually, IL-6, TNF-$\alpha$, and PCSK9 concentrations were determined using a commercially available cytokine enzyme-linked immunosorbent assay (ELISA) method[68,69] (IL-6 and TNF-$\alpha$: Quantikine ELISA kit, R&D Systems, Abingdon, UK; PCSK9: Circulex, Human PCSK9, MBL International Woburn, MA, USA). Differential blood counts, blood chemistry, and global coagulation assays were performed at the central laboratory of the General Hospital of Vienna, Austria.

Plasma samples were analyzed according to Schiborr et al.[39] with slight modifications. For total curcuminoid extraction, 500 μL plasma was mixed with 5 μL 6 M hydrochloric acid, 1 mL 0.1 M acetate buffer, and 100 μL $\beta$-glucuronidase type H-1 from *Helix pomatia* (1 mg $\beta$-glucuronidase in 100 μL 0.1 mol L$^{-1}$ sodium acetate buffer). Enzyme treatment was carried out for 45 min at 37 °C and 150 rpm.

Curcuminoids were extracted by adding 3 mL extraction solvent (95% ethyl acetate, 5% methanol), mixing for 30 s, and then centrifuging (1690 × g, 4 °C, 5 min). Two mL of the organic layer was transferred into a fresh tube. Extraction was performed on ice and repeated three times, in total 8 mL of the 9 mL supernatant was collected and evaporated to dryness in an RVC 2–25 CDplus centrifugal evaporator (Martin Christ Gefriertrocknungsanlagen GmbH, Osterode am Harz, Germany). The dried samples were resuspended in 500 µL methanol and evaporated again. Finally, the samples were reconstituted in 75 µL methanol and transferred to HPLC vials. Quantification was performed using a Shimadzu-HPLC system (autosampler SIL-20 AC HT, controller SIL-20A, degaser DGV-20A, mixer FCV-20 AC, pump LC-10 AT; Shimadzu Corporation, Nakagyo ku, Japan) equipped with a Reprosil-Pur C18 AQ column (150 mm × 4 mm, 3 µm particle size; Dr. Maisch GmbH, Ammerbuch) and a fluorescence detector (excitation/emission 426 nm/536 nm, Fluorescence detector RF 20A). The mobile phase, consisting of 55% deionized water adjusted to pH 3 with perchloric acid and 45% acetonitrile, was delivered at a flow rate of 1.4 mL min$^{-1}$ for a runtime of 12 min. Twenty microliters were injected and quantified by an external standard curve (curcumin purity ≥97.2%; CAS#458-37-7; demethoxycurcumin purity ≥98.3%; CAS#22608-11; and bis-demethoxycurcumin purity ≥99.4%, CAS#24949-16; Chromadex, Irvine, CA, USA). All peaks were recorded and integrated with LabSolution Software (Shimadzu, Groß-Umstadt, Germany).

*Statistical Analysis and Sample Size*: The sample size calculation was based on the results of a prior trial using an LPS model and anti-inflammatory agents.[57] After stimulation with LPS, TNF-$\alpha$ concentrations increased to $65 \pm 50$ pg mL$^{-1}$, whereas in subjects pretreated with colistin, TNF-$\alpha$ concentrations only increased to $8 \pm 9$ pg mL$^{-1}$. Based on these results, a sample size of 13 subjects should suffice to show a statistically significant difference between both groups with a power of 80% and an alpha error of 5%. The power was increased to 90% and thus 16 healthy subjects were included in the trial.

Demographics and baseline data, as well as pharmacokinetics, were presented with descriptive statistics (mean ± standard deviations (SD)). First, normal distribution was assessed using histograms and the Shapiro-Wilk test. A paired $t$-test was used for normally distributed data and a non-parametric Wilcoxon signed ranks test was used for skewed data. Hence, concentrations of pro-inflammatory cytokines (TNF-$\alpha$ and IL-6, with or without LPS stimulation) and pharmacokinetic parameters were compared using a non-parametric Wilcoxon signed ranks test, whereas the impact of both formulations on PCSK9 concentrations was analyzed with a paired $t$-test.

## Supporting Information

Supporting Information is available from the Wiley Online Library or from the author.

## Conflict of Interest

The authors declare no conflict of interest.

## Author Contributions

C.S. and B.J. designed the trial. J.G., U.D., and F.E. performed the research. C.S. and J.G. performed the statistical analysis. C.S., J.G., and J.F. interpreted the data. J.G., J.F., and C.S. drafted the manuscript. All authors critically revised the manuscript and approved the final version for publication.

[1] V. Soleimani, A. Sahebkar, H. Hosseinzadeh, *Phytother. Res.* **2018**, *32*, 985.

[2] S. C. Gupta, S. Patchva, B. B. Aggarwal, *AAPS J.* **2013**, *15*, 195.

[3] F. PourhabibiãZarandi, S. ShojaeiãZarghani, M. Rafraf, *Int. J. Clin. Pract.* **2021**, *75*, e14280.

[4] J. W. Daily, M. Yang, S. Park, *J. Med. Food* **2016**, *19*, 717.

[5] S. Mohammadian Haftcheshmeh, A. Khosrojerdi, A. Aliabadi, S. Lotfi, A. Mohammadi, A. A. Momtazi-Borojeni, *Rev. Physiol. Biochem. Pharmacol.* **2021**, *179*, https://doi.org/10.1007/112_2020_54.

[6] Y. He, Y. Yue, Xi Zheng, K. Zhang, S. Chen, Z. Du, *Molecules* **2015**, *20*, 9183.

[7] R. De A. Goulart, S. M. Barbalho, V.-C. M. Lima, G. A. De Souza, J. N. Matias, A. C. Araãjo, C. U. J. Rubira, R. R. L. Buchaim, D. V. Buchaim, A. C. A. De Carvalho, Ã. L. L. Guiguer, *J. Med. Food* **2021**, *24*, 675.

[8] B. Kocaadam, N. Anlier, *Crit. Rev. Food Sci. Nutr.* **2017**, *57*, 2889.

[9] D. Fernandez-Lazaro, J. Mielgo-Ayuso, J. Seco Calvo, A. Cordova Martinez, et al., *Nutrients* **2020**, *12*, 501.

[10] Y. Chen, C. Li, S. Duan, X. Yuan, J. Liang, S. Hou, *Biomed. Pharmacother.* **2019**, *118*, 109195.

[11] S. J. Uddin, Md. F. Hasan, M. Afroz, D. K. Sarker, R. Rouf, M. T. Islam, J. A. Shilpi, M. S. Mubarak, *Curr. Drug Targets* **2021**, *22*, 656.

[12] L. Li, B. B. Aggarwal, S. Shishodia, J. Abbruzzese, R. Kurzrock, *Cancer* **2004**, *101*, 2351.

[13] P. Liczbinski, J. Michalowicz, B. Bukowska, *Phytother. Res.* **2020**, *34*, 1992.

[14] S. C. Gupta, A. K. Tyagi, P. Deshmukh-Taskar, M. Hinojosa, S. Prasad, B. B. Aggarwal, *Arch. Biochem. Biophys.* **2014**, *559*, 91.

[15] C. A. Mohammad, *Biomed. Res. Int.* **2020**, *2020*, https://doi.org/10.1155/2020/8850924.

[16] N. N. Kuzmich, K. V. Sivak, V. N. Chubarev, Y. B. Porozov, et al., Vaccines (Basel) **2017**, *5*, 34.

[17] J. Zhang, Y. Zheng, Y. Luo, Yu Du, X. Zhang, J. Fu, *Mol. Immunol.* **2019**, *116*, 29.

[18] Z. Wang, Z. Chen, B. Li, Bo Zhang, Y. Du, Y. Liu, Y. He, X. Chen, *Pharm. Biol.* **2020**, *58*, 828.

[19] Y. Han, R. Chen, Q. Lin, Yu Liu, W. Ge, H. Cao, J. Li, *J. Cell. Mol. Med.* **2021**, *25*, 8947.

[20] B. N. Nayak, G. Kaur, H. S. Buttar, *J. Complement Integr. Med.* **2016**, *13*, https://doi.org/10.1515/jcim-2015-0024.

[21] C. T. Ford, S. N. Richardson, F. Mcardle, S. B. Lotito, A. Crozier, A. Mcardle, M. J. Jackson, *Br. J. Nutr.* **2016**, *115*, 1699.

[22] Yu Cai, Di Lu, Y. Zou, C. Zhou, H. Liu, C. Tu, F. Li, L. Liu, S. Zhang, *J. Food Sci.* **2017**, *82*, 772.

[23] N. Dhillon, B. B. Aggarwal, R. A. Newman, R. A. Wolff, A. B. Kunnumakkara, J. L. Abbruzzese, C. S. Ng, V. Badmaev, R. Kurzrock, *Clin. Cancer Res.* **2008**, *14*, 4491.

[24] F. Di Mario, L. G. Cavallaro, A. Nouvenne, N. Stefani, G. M. Cavestro, V. Iori, M. Maino, G. Comparato, L. Fanigliulo, E. Morana, A. Pilotto,

**ADVANCED
SCIENCE NEWS**

www.advancedsciencenews.com

**Molecular Nutrition
Food Research**

www.mnf-journal.com

L. Martelli, M. Martelli, G. Leandro, A. Franzè, *Helicobacter* **2007**, *12*, 238.

[25] P. R. Holt, S. Katz, R. Kirshoff, *Dig. Dis. Sci.* **2005**, *50*, 2191.

[26] P. Usharani, A. A. Mateen, M. U. R. Naidu, Y. S. N. Raju, N. Chandra, *Drugs R. D.* **2008**, *9*, 243.

[27] F. Keihanian, A. Saeidinia, R. K. Bagheri, T. P. Johnston, A. Sahebkar, *J. Cell. Physiol.* **2018**, *233*, 4497.

[28] Yi-S. Yang, Y.-F. Su, H.-W. Yang, Yu-H. Lee, J. I. Chou, K.-C. Ueng, *Phytother. Res.* **2014**, *28*, 1770.

[29] S. Saraf-Bank, A. Ahmadi, Z. Paknahad, M. Maracy, M. Nourian, *Sao Paulo Med. J.* **2019**, *137*, 414.

[30] R. Jamwal, *J. Integr. Med.* **2018**, *16*, 367.

[31] F. Piaz, A. Braca, M. Belisario, N. De Tommasi, *Curr. Med. Chem.* **2010**, *17*, 479.

[32] J. S. Jurenka, *Altern. Med. Rev.* **2009**, *14*, 141.

[33] M. Karlstetter, E. Lippe, Y. Walczak, C. Moehle, A. Aslanidis, M. Mirza, T. Langmann, *J. Neuroinflammation* **2011**, *8*, 125.

[34] M. Lopez-Lazaro, *Mol. Nutr. Food Res.* **2008**, *52*, S103.

[35] R. A. Sharma, A. J. Gescher, W. P. Steward, *Eur. J. Cancer* **2005**, *41*, 1955.

[36] Y.-J. Wang, M.-H. Pan, A.-L. Cheng, L.-In Lin, Y.-S. Ho, C.-Y. Hsieh, J.-K. Lin, *J. Pharm. Biomed. Anal.* **1997**, *15*, 1867.

[37] M. Metzler, E. Pfeiffer, S. I. Schulz, J. S. Dempe, *BioFactors* **2013**, *39*, 14.

[38] S. Flory, N. Sus, K. Haas, S. Jehle, E. Kienhöfer, R. Waehler, Günther Adler, S. Venturelli, J. Frank, *Mol. Nutr. Food Res.* **2021**, *65*, 2100613.

[39] C. Schiborr, A. Kocher, D. Behnam, J. Jandasek, S. Toelstede, J. Frank, *Mol. Nutr. Food Res.* **2014**, *58*, 516.

[40] A. Kocher, L. Bohnert, C. Schiborr, J. Frank, *Mol. Nutr. Food Res.* **2016**, *60*, 1555.

[41] A. Kocher, C. Schiborr, D. Behnam, J. Frank, *J. Funct. Foods* **2015**, *14*, 183.

[42] J. Frank, C. Schiborr, A. Kocher, J. Meins, D. Behnam, M. Schubert-Zsilavecz, M. Abdel-Tawab, *Plant Foods Hum. Nutr.* **2017**, *72*, 48.

[43] L. Beltzig, A. Frumkina, C. Schwarzenbach, B. Kaina, *Nutrients* **2021**, *13*, 2385.

[44] S. D. Tzmann, C. Schiborr, A. Kocher, U. Pilatus, E. Hattingen, J. Weissenberger, F. Gealer, J. Quick-Weller, K. Franz, V. Seifert, J. Frank, C. Senft, *Nutr. Cancer* **2016**, *68*, 943.

[45] S. Ahmadabady, F. Beheshti, F. Shahidpour, E. Khordad, M. Hosseini, *Biochem. Biophys. Rep.* **2021**, *25*, 100908.

[46] A. Kumari, N. Tyagi, D. Dash, R. Singh, *Inflammation* **2015**, *38*, 1103.

[47] Q. F. Xie, J. J. Cheng, J. F. Chen, Y. C. Feng, et al., *Evid. Based Complement Alternat. Med.* **2020**, *2020*, 8856135.

[48] Y. Hu, Y. Sheng, X. Ji, P. Liu, L. Tang, G. Chen, G. Chen, *Pulm. Pharmacol. Ther.* **2020**, *63*, 101939.

[49] Q. Wang, C. Ye, S. Sun, R. Li, X. Shi, S. Wang, X. Zeng, N. Kuang, Y. Liu, Q. Shi, R. Liu, *Int. Immunopharmacol.* **2019**, *72*, 292.

[50] A. Kumari, D. Dash, R. Singh, *Inflammopharmacology* **2017**, *25*, 329.

[51] M. T. Khayyal, R. M. El-Hazek, W. A. El-Sabbagh, J. Frank, D. Behnam, M. Abdel-Tawab, *Nutrition* **2018**, *54*, 189.

[52] J. Wang, S. S. Ghosh, S. Ghosh, *Am. J. Physiol. Cell Physiol.* **2017**, *312*, C438.

[53] Z. Gong, J. Zhou, H. Li, Y. Gao, C. Xu, S. Zhao, Y. Chen, W. Cai, J. Wu, *Mol. Nutr. Food Res.* **2015**, *59*, 2132.

[54] F. Ma, F. Liu, L. Ding, M. You, H. Yue, Y. Zhou, Y. Hou, *Pharm. Biol.* **2017**, *55*, 1263.

[55] F. Zhao, Y. Gong, Y. Hu, M. Lu, J. Wang, J. Dong, D. Chen, L. Chen, F. Fu, F. Qiu, *Mol. Med. Rep.* **2015**, *11*, 3087.

[56] G. Derosa, P. Maffioli, L. E. Simental-Mendā-A, S. Bo, A. Sahebkar, *Pharmacol. Res.* **2016**, *111*, 394.

[57] P. Matzneller, S. Strommer, C. Drucker, K. Petroczi, C. Schörgenhofer, E. Lackner, B. Jilma, M. Zeitlinger, *Clin. Pharmacol. Ther.* **2017**, *101*, 773.

[58] P. Liczbinski, J. Michalowicz, B. Bukowska, *Phytother. Res.* **2020**.

[59] Mi-H. Tai, Po-K. Chen, P.-Yi Chen, M.-J. Wu, C.-T. Ho, J.-H. Yen, *Mol. Nutr. Food Res.* **2014**, *58*, 2133.

[60] Si. Qin, L. Huang, J. Gong, S. Shen, J. Huang, H. Ren, H. Hu, *Nutr. J.* **2017**, *16*, 68.

[61] N. G. Seidah, Z. Awan, M. Chrétien, M. Mbikay, *Circ. Res.* **2014**, *114*, 1022.

[62] A. N. Tsouka, C. C. Tellis, A. D. Tselepis, *Curr. Pharm. Des.* **2018**, *24*, 3622.

[63] G. G. Schwartz, P. G. Steg, M. Szarek, D. L. Bhatt, V. A. Bittner, R. Diaz, J. M. Edelberg, S. G. Goodman, C. Hanotin, R. A. Harrington, J. W. Jukema, G. Lecorps, K. W. Mahaffey, A. Moryusef, R. Pordy, K. Quintero, M. T. Roe, W. J. Sasiela, J.-F. Tamby, P. Tricoci, H. D. White, A. M. Zeiher, *N. Engl. J. Med.* **2018**, *379*, 2097.

[64] M. S. Sabatine, R. P. Giugliano, A. C. Keech, N. Honarpour, S. D. Wiviott, S. A. Murphy, J. F. Kuder, H. Wang, T. Liu, S. M. Wasserman, P. S. Sever, T. R. Pedersen, *N. Engl. J. Med.* **2017**, *376*, 1713.

[65] K. R. Walley, K. R. Thain, J. A. Russell, M. P. Reilly, N. J. Meyer, J. F. Ferguson, J. D. Christie, T.-A. Nakada, C. D. Fjell, S. A. Thair, M. S. Cirstea, J. H. Boyd, *Sci. Transl. Med.* **2014**, *6*, 258ra143.

[66] A. Merrelaar, N. Buchtele, C. Schriefl, C. Clodi, M. Poppe, F. Ettl, H. Herkner, B. Jilma, M. Schwameis, C. Schoergenhofer, *J. Clin. Med.* **2020**, *9*, 2606.

[67] C. Schoergenhofer, P. Matzneller, J. Mühlbacher, L. Hell, M. Zeitlinger, B. Jilma, *J. Intern. Med.* **2020**, *287*, 333.

[68] J. Leitner, C. Firbas, F. Mayr, R. Reiter, B. Steinlechner, B. Jilma, *Clin. Pharmacol. Ther.* **2006**, *79*, 23.

[69] U. Derhaschnig, D. Bergmair, C. Marsik, I. Schlifke, J. Wijdenes, B. Jilma, *Crit. Care Med.* **2004**, *32*, 1136.