Journal of Functional Foods 29 (2017) 154–160

Contents lists available at ScienceDirect

Journal of Functional Foods

journal homepage: www.elsevier.com/locate/jff

Responsiveness to curcumin intervention is associated with reduced aortic stiffness in young, obese men with higher initial stiffness

Marilyn S. Campbell [a], Adam J. Berrones [a], I.M. Krishnakumar [c], Richard J. Charnigo [b], Philip M. Westgate [b], Bradley S. Fleenor [a,*]

[a] Department of Kinesiology and Health Promotion, University of Kentucky, Lexington, KY 40506, USA
[b] Department of Biostatistics, University of Kentucky, Lexington, KY 40536, USA
[c] Akay Flavours & Aromatics Pvt. Ltd., R&D Centre, Ambunadu, Malayidamthuruthu P.O., Cochin 683561, India

ARTICLE INFO

Article history:
Received 19 September 2016
Received in revised form 5 October 2016
Accepted 8 December 2016

Keywords:
Turmeric
Vascular function
Body composition
Obesity
Inflammation
Bioavailability

ABSTRACT

Obesity results in greater aortic stiffness assessed by carotid-femoral Pulse Wave Velocity (cfPWV), which is an independent predictor of cardiovascular (CV) events. We hypothesized that a novel curcumin formulation with enhanced bioavailability, CurQfen®, would reduce cfPWV and inflammation in young, obese men. In the present placebo-controlled pilot study, 22 obese subjects (BMI $\geqslant$ 30.0 kg/m^2) were randomized into placebo (n = 11, BMI = 33.18 ± 3.38 kg/m^2) and curcumin (n = 11, BMI = 33.29 ± 3.69 kg/m^2) supplemented groups. When CurQfen® was supplemented at 500 mg/day for 12 weeks, it was found that individuals who did respond to the treatment (n = 6) entered the study with higher baseline cfPWV versus those who did not respond (n = 5) (6.81 ± 0.83 m/s v. 5.84 ± 0.41 m/s, p = 0.045, group by time interaction). The curcumin responders also had increased plasma IL-13 concentrations (p = 0.018, 12 weeks v. baseline). These findings suggest CurQfen curcumin has potential to de-stiffen arteries in young, obese men with greater aortic stiffness.

1. Introduction

The prevalence of obesity in the United States remains high, with approximately 35% of the adult population considered obese (Ogden, Carroll, Kit, & Flegal, 2014). Of great concern, obesity has been linked to cardiovascular disease (CVD) risk and predisposes individuals to a 50–100% greater risk of death (Pérez Pérez, Ybarra Muñoz, Blay Cortés, & de Pablos Velasco, 2007). Cardiovascular diseases are the leading cause of death in the United States (Murphy, Xu, & Kochanek, 2013), and large artery stiffness, specifically aortic stiffening, is an indicator of cardiovascular (CV) health. Greater aortic stiffness serves as a predictor adjusted for traditional risk factors of CV events, CV mortality, and all-cause mortality (Vlachopoulos, Aznaouridis, & Stefanadis, 2010) and is associated with obesity at all ages across the lifespan (Scuteri, Najjar, Morrell, & Lakatta, 2005; Wildman, Mackey, Bostom, Thompson, & Sutton-Tyrrell, 2003). Thus, strategies to reduce aortic stiffening have potential to reduce cardiovascular risk due to the predictive nature of aortic stiffness on CV events (Cecelja & Chowienczyk, 2012).

Curcumin [1,7-bis-(4-hydroxy-3-methoxy-phenyl)-1,6-hepta diene-3,5-dione], the active component in the spice turmeric, has shown beneficial effects to many disease conditions (Gupta, Patchva, & Aggarwal, 2013). While the chemopreventive effects of curcumin have been well-studied (Duvoix et al., 2005), clinical studies with curcumin have shown significant improvements in many pathologies including inflammatory-related conditions, arthritis, chronic anterior uveitis, gastric and peptic ulcers, diabetes, acute coronary syndrome, and atherosclerosis (Gupta et al., 2013). Additional study, however, needs to be done in order to understand how curcumin can impact chronic disease, especially as it relates to CVD and large artery stiffness. Initial evidence in pre-clinical models has shown that curcumin reduces aortic stiffness (Fleenor et al., 2013; Nakmareong et al., 2012) and improves arterial compliance, an estimation of arterial stiffness, in postmenopausal women (Akazawa et al., 2013). It is not yet known,

Abbreviations: BMI, body mass index; BP, blood pressure; CCTS, Center for Clinical and Translational Science; cfPWV, carotid-femoral Pulse Wave Velocity; CGM, CurQfen; CV events, cardiovascular events; CVD, cardiovascular disease; DBP, diastolic blood pressure; EKG, electrocardiogram; IFN, Interferon; IL, Interleukin; NFκB, Nuclear Factor Kappa Beta; PP, pulse pressure; SBP, systolic blood pressure; SD, standard deviation; TNF-α, Tumor Necrosis Factor Alpha.

* Corresponding author at: University of Kentucky, 100 Seaton Building, Lexington, KY 40506, USA.

E-mail addresses: mca243@uky.edu (M.S. Campbell), adam.berrones@gmail.com (A.J. Berrones), krishnakumar.im@akay-group.com (I.M. Krishnakumar), rjcharn2@aol.com (R.J. Charnigo), philip.westgate@uky.edu (P.M. Westgate), bradley.fleenor@uky.edu (B.S. Fleenor).

http://dx.doi.org/10.1016/j.jff.2016.12.013
1756-4646/

however, whether curcumin de-stiffens arteries in obese individuals. Additionally, the poor bioavailability of curcumin in its natural state in humans is well-established, which is attributed to poor absorption, rapid metabolism, and rapid elimination (Aggarwal & Harikumar, 2009). Therefore, various formulations have been created to enhance the absorption and bioavailability of curcumin. Of note, a novel food-grade formulation of curcumin containing curcuminoids (curcumin/diferuloylmethane, demethoxycurcumin, and bisdemethoxycurcumin), CurQfen® (*hereinafter referred to as* '*CGM*'), is combined with the galactomannan soluble dietary fiber from fenugreek seeds (*Trigonella Foenum gracum*). This novel formulation has been shown to improve the oral bioavailability of curcumin in humans when compared to unformulated curcumin (Krishnakumar, Ravi, Kumar, Kuttan, & Maliakel, 2012). CGM improves the hydrophobic-hydrophilic balance of curcumin, creating a prolonged release of colloidal curcumin within the digestive tract by preventing enzymatic breakdown in the upper gastrointestinal tract (Krishnakumar et al., 2012). Though CGM suggests potential benefits due to its enhanced absorption and pharmacokinetics, the de-stiffening effects of this compound have not yet been studied in an obese population (Scuteri et al., 2005; Wildman et al., 2003).

Acute and chronic inflammatory processes are implicated in the development of aortic stiffness through activation of the Nuclear Factor Kappa Beta (NFκB) pathway, which induces transcription of inflammatory cytokines such as IL-1β, IL-6, IFN-γ, and TNF-α (McEniery & Wilkinson, 2005; Vlachopoulos et al., 2005). Inflammatory cytokine production and secretion are increased among obese individuals (Bastard et al., 2006; Greenberg & Obin, 2006), which may, in turn, influence aortic stiffness in this population. Since curcumin possesses anti-inflammatory properties (Wellen & Hotamisligil, 2003), obesity-related aortic stiffness may be attenuated by reducing inflammatory cytokines. While reductions in acute inflammation have shown a decrease in aortic stiffness in older adults (Jablonski et al., 2015), it is currently unknown whether reductions in chronic inflammation will improve aortic stiffness in a young, obese population (Fleenor, 2013). Thus, the present pilot study investigated the effect of the anti-inflammatory functional food, curcumin, to reduce obesity-related aortic stiffness. The ability to ameliorate aortic stiffness could suggest a role for curcumin as an anti-inflammatory functional food in obese men to confer a benefit above and beyond its nutritional effects as a protective and preventative agent against CVD.

We tested the hypothesis that a novel curcumin compound, CGM, with enhanced bioavailability, would ameliorate aortic stiffness in young, obese men following 12 weeks of supplementation. Furthermore, we tested the hypothesis that the CGM curcumin compound would attenuate inflammation in obese men that, in turn, would lead to reductions in aortic stiffness.

2. Material and methods

This study was conducted at the University of Kentucky in the Exercise Physiology Laboratory and the Center for Clinical and Translational Science (CCTS) with blood analyses conducted by the CCTS Biochemical Analysis Laboratory. The Institutional Review Board at the University of Kentucky approved all procedures before study commencement, and written consent from all subjects was obtained prior to participation.

2.1. Subjects

Twenty-two subjects were recruited for this study via local advertisement, including flyers and web announcements from the CCTS and ResearchMatch through the University of Kentucky. Healthy males aged 18–35 years with BMI $\geqslant$ 30 kg/m^2 were recruited for this study. Volunteers were pre-screened via e-mail to determine if age, BMI, sex, and current medication status were appropriate based on inclusion and exclusion criteria (Table S1). Additional screening occurred during their first visit to the Exercise Physiology Laboratory at the University of Kentucky, including a self-reported health history questionnaire, a preliminary blood pressure (BP) reading, and a resting 12-lead EKG.

2.2. Study design and materials

Subjects in this double-blind, placebo-controlled study were matched based on BMI and randomized by a random numbers table into the curcumin or placebo group. The intervention period lasted 12 weeks for both groups. Curcumin (CGM) and placebo (fenugreek dietary fiber) supplement capsules were obtained from Akay Flavours & Aromatics Pvt. Ltd. (Kochi, India). The intervention consisted of a two-piece hard shell gelatin capsule of (500 mg × 1) formulated curcumin (CGM) consisting of 38.6% curcuminoids infused into 60% (w/w) of soluble dietary fiber isolated from fenugreek seeds. The CGM formulated curcumin is a 100% natural food-grade formulation that is composed of 157.8 mg curcumin (diferuloylmethane), 29.6 mg demethoxycurcumin, and 5.5 mg bisdemethoxycurcumin, with a total curcuminoids content of 193.0 mg per 500 mg. The placebo pills were formulated with 500 mg of fenugreek fiber and were identical in size and shape to the intervention pills. Subjects were instructed to consume one pill per day, with pill consumption recorded on written logs. Subjects were encouraged to consume pills at the same time each day. Participants were also instructed to not change any lifestyle habits throughout the study, including maintaining habitual diet and level of physical activity.

All testing was completed after a minimum of a 4-h fast from all food, beverages other than water, and caffeine. The experimental design included four total visits to the laboratory, occurring at 0, 4, 8, and 12 weeks. On the first visit to the laboratory, subjects were screened to ensure that they met all inclusion and exclusion criteria, and baseline measures were attained for anthropometrics, body composition, BP, aortic stiffness, and plasma cytokine concentrations. Furthermore, subjects were assigned and given pills based on randomization into the intervention or placebo group. At all subsequent visits, anthropometric, body composition, BP, and aortic stiffness measures were taken. Plasma cytokine concentrations were measured again at the final visit.

2.3. Anthropometric and body composition measurements

Height and weight measures were taken with the subjects wearing light-weight clothing and no shoes. Height was measured to the nearest 0.1 cm using a wall-mounted stadiometer (PORTROD; Health-o-meter, Alsip, IL), and weight was measured to the nearest 0.01 kg using a calibrated electronic scale (DI-10; DIGU, Rice Lake, WI). Hip and waist measures were taken to the nearest 0.1 cm in triplicate and averaged, alternating between the sites with a spring-loaded fiberglass anthropometric tape (Gulick Deluxe; Baseline Evaluation Instruments, White Plains, NY). The waist circumference was measured at the level of the umbilicus, and the hip circumference was measured at the largest circumference around the gluteal muscles.

Body composition measures were taken in the supine position on a non-conducting surface with a tetrapolar bioelectrical impedance analyzer (Multi-frequency Quadscan 4000 Bioelectrical Impedance Analyzer; Bodystat Ltd., Douglas, United Kingdom). A proprietary calculation from the manufacturer was used to obtain body fat percentage.

2.4. Blood pressure and aortic stiffness measurements

Brachial BP measurements were taken after the subjects rested in the supine position for a minimum of 10 min in a quiet, thermoneutral room. Measurements were taken manually for all subjects with a sphygmomanometer by the same trained investigator in duplicate and recorded. Brachial pulse pressure (PP) was determined by subtracting diastolic BP from systolic BP.

Following BP measurements, aortic stiffness was determined by carotid-femoral Pulse Wave Velocity (cfPWV) using the Sphygmocor system (Sphygmocor; AtCor Medical, Sydney, Australia). Distance between the carotid and femoral sites was measured with a flexible fiberglass measuring tape to the nearest millimeter. Measurements were taken in triplicate by the same trained investigator and averaged, and cfPWV was determined by the Sphygmocor system as pulse wave distance traveled, divided by transit time of the pulse wave.

2.5. Blood analyses

Blood samples were collected at baseline (0 weeks) and at the completion of the study (12 weeks) by trained staff at the University of Kentucky's CCTS. After whole blood was centrifuged, plasma and serum aliquots were frozen until all blood samples were collected for analysis. Plasma samples were analyzed by a lab technician in the CCTS using the V-PLEX Proinflammatory Panel 1 (human) Kit (Meso Scale Discovery, Rockville, MD), which determined cytokine concentrations for: IFN-γ, IL-2, IL-4, IL-6, IL-8, IL-10, IL-12 p70, IL-13, and TNF-α.

2.6. Statistical analysis

All data were analyzed using SAS version 9.4 (SAS System for Windows; SAS Institute Inc., Cary, NC). Descriptive characteristics are presented as means and standard deviations, and baseline data were analyzed with paired samples *t*-tests to compare means between the intervention and placebo groups. If data were not normally distributed, logarithmic transformation of the data was employed to handle comparisons between the groups; if log transformation did not correct normality, the signed rank test was employed. Baseline data comparing the responders and non-responders (subsets of the intervention group, determined post hoc) were analyzed using an independent samples *t*-test. As a post hoc sensitivity analysis, we re-ran our analyses on IL-2, IL-4, and IL-12 p70 with log transformed versions of these quantities, since the original versions were not normally distributed. In analyzing curcumin versus placebo subjects on treatment effect throughout the study, a multilevel linear regression model was used, with random effects to account for BMI-matched pairs and an unstructured covariance matrix to account for repeated measurements. In analyzing responders versus non-responders on treatment effect throughout the study, random effects were not employed due to the absence of matching within the intervention group. The Kenward and Roger degrees of freedom method was used for inference (Kenward & Roger, 1997). The Pearson product-moment correlation was used to assess correlations between variables. Statistical significance was defined by $p < 0.05$.

3. Results

3.1. Subject characteristics

All 22 subjects completed the 12-week protocol. Clinical baseline anthropometric, body composition, and BP characteristics did not differ significantly between the curcumin and placebo groups (Table 1). Conclusions regarding statistical significance were unaltered in the sensitivity analysis using log-transformed versions of the previously indicated quantities. Overall supplement compliance was 96.74% with no differences observed between groups (98.02% placebo v. 95.43% curcumin; $p = 0.520$). No adverse side effects were reported in the curcumin or placebo groups.

3.2. Effect of curcumin on aortic stiffness, plasma cytokines, and subject characteristics

A significant reduction in brachial PP, a surrogate marker for aortic stiffness, was observed in the curcumin group following 12 weeks of curcumin treatment when compared to the placebo treatment ($p = 0.040$; Table 2). While no further significant differences were observed between curcumin and placebo groups, including body composition, BP measures, and inflammatory cytokines (Table 2), a notable trend for an increase was observed in the anti-inflammatory cytokine IL-10 with curcumin intervention ($p = 0.071$, group by time interaction; Table 2).

Interestingly, following 12 weeks of supplementation, a divergent response among the curcumin group was observed in cfPWV. Responders were individuals with reductions in cfPWV following the 12-week curcumin intervention ($n = 6$, Table 3), whereas the non-responders demonstrated no reductions in cfPWV from baseline values ($n = 5$, Table 3). Mean baseline cfPWV values were greater among the responders when compared to the non-responders (6.81 ± 0.83 m/s v. 5.84 ± 0.41 m/s, $p = 0.045$, Table 3). Additionally, the overall change in cfPWV among the curcumin responders displays a greater curcumin-induced de-stiffening effect for those with increased baseline cfPWV ($r = -0.936$, $p = 0.006$). Responders and non-responders were defined in terms of cfPWV change over time; thus, a p-value is not reported for group by time interaction with respect to the outcome of cfPWV. In sum, individuals with higher baseline cfPWV had greater reductions in aortic stiffness.

The effects of curcumin on anthropometric, body composition and BP characteristics, and inflammatory cytokine markers in the responders and non-responders for the 12-week study are given in Table 4. Of note, the decrease in cfPWV values was accompanied by an increase in anti-inflammatory IL-13 in the responders

Table 1
Baseline subject characteristics.

	Curcumin (n = 11)	Placebo (n = 11)
Age (years)	25.91 ± 4.46	26.64 ± 4.06
Body Mass Index (kg/m^2)	33.29 ± 3.69	33.18 ± 3.38
Hip circumference (cm)	114.47 ± 8.41	112.80 ± 5.87
Waist circumference (cm)	108.93 ± 11.99	106.27 ± 10.37
Body fat (%)	27.22 ± 4.90	27.18 ± 5.46
Brachial SBP (mmHg)	123.64 ± 8.48	124.91 ± 8.96
Brachial DBP (mmHg)	77.09 ± 9.01	81.82 ± 5.40
Brachial PP (mmHg)	46.55 ± 10.20	43.09 ± 6.41
cfPWV (m/s)	6.36 ± 0.82	6.31 ± 0.99
IFN-γ (pg/mL)	2.94 ± 0.94[b]	4.51 ± 2.66[a]
IL-2 (pg/mL)	0.17 ± 0.13[b]	0.45 ± 0.42[a,†]
IL-4 (pg/mL)	0.07 ± 0.05[b]	0.12 ± 0.10[a]
IL-6 (pg/mL)	0.77 ± 0.33[b]	0.62 ± 0.27[a]
IL-8 (pg/mL)	3.71 ± 0.72[b]	3.48 ± 0.72[a]
IL-10 (pg/mL)	0.29 ± 0.11[b]	0.45 ± 0.32[a]
IL-12 p70 (pg/mL)	0.32 ± 0.22[b]	0.50 ± 0.38[a]
IL-13 (pg/mL)	3.92 ± 3.50[b]	9.00 ± 7.36[a]
TNF-α (pg/mL)	1.76 ± 0.49[b]	1.71 ± 0.45[a]

Values are means ± SD. SBP = Systolic Blood Pressure; DBP = Diastolic Blood Pressure; PP = Pulse Pressure; cfPWV = carotid-femoral Pulse Wave Velocity; IFN = Interferon; IL = Interleukin; TNF = Tumor Necrosis Factor.
† $p = 0.052$, between groups.
[a] $n = 9$.
[b] $n = 10$.

Table 2
Subject characteristics with curcumin and placebo intervention.

	Curcumin (n = 11)		Placebo (n = 11)	
	Before	After	Before	After
Age (years)	25.91 ± 4.46	26.18 ± 4.38	26.64 ± 4.06	27.00 ± 3.90
Body Mass Index (kg/m^2)	33.29 ± 3.69	33.99 ± 3.42	33.18 ± 3.38	33.34 ± 3.79
Hip circumference (cm)	114.47 ± 8.41	115.36 ± 6.97	112.80 ± 5.87	112.75 ± 5.42
Waist circumference (cm)	108.93 ± 11.99	109.32 ± 11.98	106.27 ± 10.37	106.41 ± 12.32
Body fat (%)	27.22 ± 4.90	28.52 ± 4.42	27.18 ± 5.46	27.69 ± 5.57
Brachial SBP (mmHg)	123.64 ± 8.48	118.73 ± 8.64	124.91 ± 8.96	123.82 ± 7.51
Brachial DBP (mmHg)	77.09 ± 9.01	78.73 ± 7.60	81.82 ± 5.40	80.00 ± 5.37
Brachial PP (mmHg)	46.55 ± 10.20	40.00 ± 9.42	43.09 ± 6.41	43.82 ± 6.29*
cfPWV (m/s)	6.36 ± 0.82	6.30 ± 0.68	6.31 ± 0.99	6.51 ± 1.03
IFN-γ (pg/mL)	2.94 ± 0.94[b]	2.76 ± 0.69[b]	4.51 ± 2.66[a]	3.82 ± 1.89[a]
IL-2 (pg/mL)	0.17 ± 0.13[b]	0.17 ± 0.14[b]	0.45 ± 0.42[a]	0.44 ± 0.48[a]
IL-4 (pg/mL)	0.07 ± 0.05[b]	0.07 ± 0.06[b]	0.12 ± 0.10[a]	0.11 ± 0.13[a]
IL-6 (pg/mL)	0.77 ± 0.33[b]	0.70 ± 0.38[b]	0.62 ± 0.27[a]	0.80 ± 0.62[a]
IL-8 (pg/mL)	3.71 ± 0.72[b]	3.56 ± 1.17[b]	3.48 ± 0.72[a]	3.51 ± 1.19[a]
IL-10 (pg/mL)	0.29 ± 0.11[b]	0.34 ± 0.13[b]	0.45 ± 0.32[a]	0.41 ± 0.31[a,‡]
IL-12 p70 (pg/mL)	0.32 ± 0.22[b]	0.29 ± 0.23[b]	0.50 ± 0.38[a]	0.44 ± 0.48[a]
IL-13 (pg/mL)	3.92 ± 3.50[b]	3.77 ± 3.61[b]	9.00 ± 7.36[a]	9.53 ± 9.02[a]
TNF-α (pg/mL)	1.76 ± 0.49[b]	1.89 ± 0.63[b]	1.71 ± 0.45[a]	1.72 ± 0.51[a]

Values are means ± SD. SBP = Systolic Blood Pressure; DBP = Diastolic Blood Pressure; PP = Pulse Pressure; cfPWV = carotid-femoral Pulse Wave Velocity; IFN = Interferon; IL = Interleukin; TNF = Tumor Necrosis Factor.
* $p < 0.05$, group by time interaction.
‡ $p = 0.071$, group by time interaction.
[a] n = 9.
[b] n = 10.

Table 3
Baseline subject characteristics of curcumin responders and non-responders.

	Responders (n = 6)	Non-responders (n = 5)
Age (years)	25.17 ± 4.17	26.80 ± 5.12
Body Mass Index (kg/m^2)	31.87 ± 1.67	35.00 ± 4.88
Hip circumference (cm)	111.89 ± 6.53	117.57 ± 10.07
Waist circumference (cm)	103.63 ± 5.32	115.28 ± 15.21
Body fat (%)	25.60 ± 3.41	29.16 ± 6.07
Brachial SBP (mmHg)	121.00 ± 10.41	126.80 ± 4.60
Brachial DBP (mmHg)	76.33 ± 8.14	78.00 ± 10.86
Brachial PP (mmHg)	44.67 ± 11.57	48.80 ± 9.01
cfPWV (m/s)	6.81 ± 0.83	5.84 ± 0.41*
IFN-γ (pg/mL)	3.00 ± 1.18[c]	2.89 ± 0.76
IL-2 (pg/mL)	0.22 ± 0.17[c]	0.11 ± 0.05
IL-4 (pg/mL)	0.08 ± 0.07[c]	0.07 ± 0.02
IL-6 (pg/mL)	0.60 ± 0.21[c]	0.94 ± 0.35
IL-8 (pg/mL)	3.69 ± 0.69[c]	3.72 ± 0.83
IL-10 (pg/mL)	0.31 ± 0.13[c]	0.27 ± 0.09
IL-12 p70 (pg/mL)	0.38 ± 0.31[c]	0.26 ± 0.03
IL-13 (pg/mL)	4.52 ± 4.68[c]	3.31 ± 2.17
TNF-α (pg/mL)	1.54 ± 0.18[c]	1.97 ± 0.62

Values are means ± SD. SBP = Systolic Blood Pressure; DBP = Diastolic Blood Pressure; PP = Pulse Pressure; cfPWV = carotid-femoral Pulse Wave Velocity; IFN = Interferon; IL = Interleukin; TNF = Tumor Necrosis Factor.
* $p < 0.05$, between groups.
[c] n = 5.

(12 weeks v. baseline, $p = 0.018$), and the time change of IL-13 in responders was nearly significantly different ($p = 0.052$) from that of non-responders. No additional significant group differences were noted in anthropometric, body composition and BP characteristics, and inflammatory cytokine markers (Table 4).

4. Discussion

The primary finding of the present study demonstrates responsiveness to curcumin treatment in young, obese men that is dependent on increased baseline aortic stiffness measures. As such, a reduction in cfPWV was apparent among the curcumin-supplemented sub group (responders) when compared to the non-responders, indicating the importance of curcumin among people with higher baseline values of cfPWV (arterial stiffness). Previous studies have found that curcumin treatment improves aortic stiffness in aged rodents (Fleenor et al., 2013; Nakmareong et al., 2012), but young, obese individuals have yet to be studied. Importantly, these initial findings may provide support for curcumin to be used as a functional food to reduce obesity-related aortic stiffness and potentially prevent CVD, but further study is warranted. Furthermore, a mechanistic role is suggested with anti-inflammatory cytokine IL-13 in the reduction of obesity-associated aortic stiffness. Based on these findings, the ability to alter inflammation, and specifically anti-inflammatory cytokine IL-13, could determine the ability to affect obesity-associated aortic stiffness.

The present study has demonstrated an overall group by time interaction in brachial PP, a surrogate marker for aortic stiffness. Previous work has shown BP-related reductions with curcumin (Akazawa et al., 2013; Sugawara et al., 2012), though the present study is the first to demonstrate a reduction in brachial PP. There was a 13.6% average reduction in brachial PP in the curcumin group as compared to a 2.4% average increase in the placebo group. The change in brachial PP among the curcumin group is likely due to functional rather than structural changes occurring in the artery, as structural differences would have been expected to cause an overall change in cfPWV. Functional changes that occur in the arterial wall result in alterations of the vasodilatory properties of the artery without directly affecting the structural components. Conversely, structural changes result in physical modification of the arterial wall, which often impacts the two major proteins that alter the rigidity of the wall: collagen and elastin. Therefore, these data suggest different mechanisms may be contributing to the overall reduction in brachial PP in the curcumin group versus the mechanisms leading to improvements in cfPWV among the subset of responders.

Inflammation is a potential explanation of this functional change within the arteries. Curcumin has been shown to inhibit NFκB signaling and the downstream inflammatory pathways that it regulates, which can affect the production of inflammatory cytokines (Gonzales & Orlando, 2008; Thangapazham, Sharma, & Maheshwari, 2006). A trending increase of the anti-inflammatory

Table 4
Subject characteristics in curcumin responders and non-responders.

	Responders (n = 6)		Non-responders (n = 5)	
	Before	After	Before	After
Age (years)	25.17 ± 4.17	25.50 ± 4.04	26.80 ± 5.12	27.00 ± 5.10
Body Mass Index (kg/m^2)	31.87 ± 1.67	32.60 ± 2.11	35.00 ± 4.88	35.65 ± 4.16
Hip circumference (cm)	111.89 ± 6.53	112.58 ± 5.95	117.57 ± 10.07	118.69 ± 7.19
Waist circumference (cm)	103.63 ± 5.32	103.33 ± 3.85	115.28 ± 15.21	116.51 ± 14.89
Body fat (%)	25.60 ± 3.41	26.53 ± 3.03	29.16 ± 6.07	31.12 ± 4.67
Brachial SBP (mmHg)	121.00 ± 10.41	118.33 ± 10.61	126.80 ± 4.60	119.20 ± 6.72
Brachial DBP (mmHg)	76.33 ± 8.14	78.33 ± 8.89	78.00 ± 10.86	79.20 ± 6.72
Brachial PP (mmHg)	44.67 ± 11.57	40.00 ± 9.88	48.80 ± 9.01	40.00 ± 10.00
cfPWV (m/s)	6.81 ± 0.83	5.92 ± 0.36	5.84 ± 0.41	6.75 ± 0.72
IFN-γ (pg/mL)	3.00 ± 1.18[c]	2.46 ± 0.55[c]	2.89 ± 0.76	3.06 ± 0.74
IL-2 (pg/mL)	0.22 ± 0.17[c]	0.22 ± 0.17[c]	0.11 ± 0.05	0.11 ± 0.07
IL-4 (pg/mL)	0.08 ± 0.07[c]	0.10 ± 0.07[c]	0.07 ± 0.02	0.05 ± 0.02
IL-6 (pg/mL)	0.60 ± 0.21[c]	0.56 ± 0.15[c]	0.94 ± 0.35	0.84 ± 0.50
IL-8 (pg/mL)	3.69 ± 0.69[c]	4.30 ± 0.71[c]	3.72 ± 0.83	2.81 ± 1.09
IL-10 (pg/mL)	0.31 ± 0.13[c]	0.38 ± 0.12[c]	0.27 ± 0.09	0.30 ± 0.13
IL-12 p70 (pg/mL)	0.38 ± 0.31[c]	0.39 ± 0.30[c]	0.26 ± 0.03	0.20 ± 0.06
IL-13 (pg/mL)	4.52 ± 4.68[c]	4.83 ± 4.71[c]	3.31 ± 2.17	2.70 ± 2.08[†]
TNF-α (pg/mL)	1.54 ± 0.18[c]	1.74 ± 0.15[c]	1.97 ± 0.62	2.05 ± 0.90

Values are means ± SD. SBP = Systolic Blood Pressure; DBP = Diastolic Blood Pressure; PP = Pulse Pressure; cfPWV = carotid-femoral Pulse Wave Velocity; IFN = Interferon; IL = Interleukin; TNF = Tumor Necrosis Factor.
[*] $p < 0.05$, group by time interaction
[†] $p = 0.052$, group by time interaction.
[c] n = 5.

cytokine IL-10 occurred in conjunction with the decreases in brachial PP among the curcumin group when compared to the placebo group ($p = 0.071$, group by time interaction). IL-10 is a potent anti-inflammatory cytokine (Fiorentino, Zlotnik, Mosmann, Howard, & O'garra, 1991), and inflammation can affect both functional and structural properties of the artery. These changes that occur in the arterial wall can occur through direct action of inflammation on the structural components of the arterial wall or indirectly by increased oxidative stress (Napoli, de Nigris, & Palinski, 2001; Ross, 1999). Furthermore, curcumin may directly affect the vascular smooth muscles (Dewar, Clark, Singer, & Frame, 2011), which alter the active tone of the arterial wall by vasodilation and vasoconstriction (Gaballa et al., 1998), without affecting inflammation. Another possible explanation is that curcumin can impact endothelial function, and thereby affect vascular smooth muscle tone, through increasing nitric oxide bioavailability (Stehouwer, Henry, & Ferreira, 2008). A previous study in postmenopausal women showed that curcumin ameliorated endothelial function (Akazawa et al., 2012). Finally, it is plausible that a combination of the above mechanisms, including inflammatory modulation via anti-inflammatory cytokine IL-10, vascular smooth muscle tone, and improvements in endothelial function, could explain the significant change in brachial PP among the curcumin group.

It has been demonstrated that obesity leads to stiffer arteries (DeVallance et al., 2015) as early as adolescence and extending to old age (Zebekakis et al., 2005) adjusted for BP, ethnicity, and age (Safar, Czernichow, & Blacher, 2006). The mean baseline cfPWV, however, for the curcumin subjects was 6.36 m/s, which is not above reference values for healthy individuals of this 18–35 age group (Reference Values for Arterial Stiffness' Collaboration, 2010). Mean cfPWV for healthy <30 and 30–39 year old individuals is 6.2 m/s and 6.5 m/s, respectively (Reference Values for Arterial Stiffness' Collaboration, 2010). However, an important, yet unexpected finding of the current study is that a higher baseline aortic stiffness measure was related to the responsiveness of the curcumin intervention. On average, the individuals who responded to curcumin treatment entered the study with modestly elevated aortic stiffness (cfPWV: 6.81 ± 0.83 m/s) in contrast to the non-responders (cfPWV: 5.84 ± 0.41 m/s) (Reference Values for Arterial Stiffness' Collaboration, 2010). Additionally, the individual baseline measure predicted responsiveness among the responders, showing that those with the greatest aortic stiffness were more likely to see the greatest improvements ($r = -0.936$, $p = 0.006$). This finding suggests a role for curcumin to ameliorate obesity-related aortic stiffness when cfPWV is above mean reference values of healthy age-matched counterparts. Importantly, aortic stiffness is a strong predictor of CV events, CV mortality, and all-cause mortality (Vlachopoulos et al., 2010), and obesity predisposes individuals to an increased risk of CVD and death (Pérez Pérez et al., 2007). An increase in cfPWV of 1.0 m/s corresponded with a 14% increase in CV events, 15% increase in CV mortality, and 15% increase in all-cause mortality among individuals with a greater age range than herein (Vlachopoulos et al., 2010). Thus, the ability to reduce aortic stiffness in a young, obese population may slow the rate of progression and, therefore, delay and/or prevent future CV-related events later in life.

The lack of overall effect among the curcumin group is consistent with a previous study in postmenopausal women with a lower dose (150 mg/day) and shorter 8-week duration (Sugawara et al., 2012). The lack of a decrease in cfPWV in the non-responders is likely due to lower stiffness values associated with younger age; a previous study in mice showed that curcumin had the potential to reduce aortic stiffness to levels that were not significantly different from young controls but did not have an effect on young mice (Fleenor et al., 2013). The responsiveness to curcumin as determined by cfPWV among individuals with higher baseline values may be partially explained by inflammatory response to the treatment, though this likely occurs through a different pathway than the functional inflammatory response associated with changes in brachial PP postulated above. These data suggest reductions in aortic stiffness in the responders could be explained by the increase in anti-inflammatory cytokine IL-13 ($p = 0.052$, group by time interaction), which was driven by the effect in the responders ($p = 0.018$, 12 weeks v. baseline). The understanding of the role of IL-13 in vascular function is still in its infancy, though previous work has suggested IL-13 might protect against vascular pathologies through vasoactive properties (Tang, Spitzbarth, Kuhn, Chaitidis, & Campbell, 2003) or modulation of collagen fibers (Cardilo-Reis et al., 2012). Therefore, the effects of IL-13 may occur through functional and/or structural modification(s) of the artery.

In rabbits, IL-13 regulated vascular tone through the beneficial increase in vaso-relaxation of the aorta (Tang et al., 2003). In mice, IL-13 has been reported to play a protective role against atherosclerosis, which is a distinct but related arterial pathology, by ameliorating atherosclerotic lesions through modulation and structural re-organization of collagen fibers (Cardilo-Reis et al., 2012). This may provide insight in models of aortic stiffness, where imbalance and improper functioning of arterial proteins, such as collagen and elastin, cause rigidity and changes in mechanical properties of the arterial wall (Dobrin, Baker, & Gley, 1984). In non-responders, for whom aortic stiffness was already at lower, healthier levels, proper vaso-reactivity of the aorta or normalized collagen content may have already been present, leading to no additional benefit on aortic stiffness from curcumin. Thus, the present study indicates the necessity of future studies to determine the potential role of anti-inflammatory IL-13 in the modification of aortic stiffness by either functional or structural adaptations.

Despite the novel findings of the present pilot study, the small sample size and lack of mechanistic insight for the beneficial effects of curcumin are limitations. Furthermore, the 12 week duration of this study may not have been a long enough intervention period to see changes in arterial stiffness that have accumulated over a lifetime; thus, a longer intervention period may have resulted in a more favorable effect. Moreover, the effect of a higher dose of curcuminoids, in comparison with the current dose of 193 mg/day, should be addressed in future studies to see if additional favorable modifications would have resulted. While changes in physical activity were not monitored throughout this study, participants were instructed to maintain regular lifestyle habits, including physical activity and exercise, throughout the duration of the study. It should also be noted that this study was conducted in a young, apparently healthy population without hypertension; therefore, older or diseased populations with higher arterial stiffness measures could potentially see a greater effect, but further studies will need to investigate this possibility. Additionally, further investigations should examine the potentially additive effect of curcumin and exercise as found in previous studies (Akazawa et al., 2013; Sugawara et al., 2012).

5. Conclusions

The present study provides initial evidence for the novel curcumin compound, CurQfen®, to de-stiffen arteries in young, obese males with elevated baseline aortic stiffness to enhance cardiovascular health. Importantly, the anti-inflammatory properties of curcumin, when administered in a bioavailable formulation, are implicated in the amelioration of aortic stiffness. Reduced aortic stiffness is likely to cause a reduction in CV risk and mortality. These preliminary findings also suggest a potential mechanistic role of anti-inflammatory IL-13 in the amelioration of aortic stiffness that warrants further investigation.

Disclosures

Dr. Charnigo discloses having been a co-investigator on grants from AstraZeneca pertaining to Crestor and Brilinta. Dr. KK belongs to the company Akay who owns the patent and trademark for CurQfen®. Dr. Fleenor has been a principal investigator on a grant from AstraZeneca related to Saxagliptin.

Acknowledgements

We would like to thank the staff in the CCTS for technical support with the study. We would also like to thank Drs. Mark Abel, Jody Clasey, and Travis Thomas for their insightful discussions and suggestions related to this study. This study was funded by Akay Flavours & Aromatics Pvt. Ltd., Arvle and Ellen Turner Thacker Research Fund, and was supported by the National Center for Advancing Translational Sciences, National Institutes of Health, through grant number UL1TR000117. The content is solely the responsibility of the authors and does not necessarily represent the official views of the NIH.

Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.jff.2016.12.013.

References

Aggarwal, B. B., & Harikumar, K. B. (2009). Potential therapeutic effects of curcumin, the anti-inflammatory agent, against neurodegenerative, cardiovascular, pulmonary, metabolic, autoimmune and neoplastic diseases. *The International Journal of Biochemistry & Cell Biology, 41*(1), 40–59.

Akazawa, N., Choi, Y., Miyaki, A., Tanabe, Y., Sugawara, J., Ajisaka, R., & Maeda, S. (2012). Curcumin ingestion and exercise training improve vascular endothelial function in postmenopausal women. *Nutrition Research, 32*(10), 795–799.

Akazawa, N., Choi, Y., Miyaki, A., Tanabe, Y., Sugawara, J., Ajisaka, R., & Maeda, S. (2013). Effects of curcumin intake and aerobic exercise training on arterial compliance in postmenopausal women. *Artery Research, 7*(1), 67–72.

Bastard, J.-P., Maachi, M., Lagathu, C., Kim, M. J., Caron, M., Vidal, H., ... Feve, B. (2006). Recent advances in the relationship between obesity, inflammation, and insulin resistance. *European Cytokine Network, 17*(1), 4–12.

Cardilo-Reis, L., Gruber, S., Schreier, S. M., Drechsler, M., Papac-Milicevic, N., Weber, C., ... Binder, C. J. (2012). Interleukin-13 protects from atherosclerosis and modulates plaque composition by skewing the macrophage phenotype. *EMBO Molecular Medicine, 4*(10), 1072–1086.

Cecelja, M., & Chowienczyk, P. (2012). Role of arterial stiffness in cardiovascular disease. *JRSM Cardiovascular Disease, 1*(4), 11.

DeVallance, E., Fournier, S. B., Donley, D. A., Bonner, D. E., Lee, K., Frisbee, J. C., & Chantler, P. D. (2015). Is obesity predictive of cardiovascular dysfunction independent of cardiovascular risk factors&quest. *International Journal of Obesity, 39*(2), 244–253.

Dewar, A. M., Clark, R. A., Singer, A. J., & Frame, M. D. (2011). Curcumin mediates both dilation and constriction of peripheral arterioles via adrenergic receptors. *Journal of Investigative Dermatology, 131*(8), 1754–1760.

Dobrin, P. B., Baker, W. H., & Gley, W. C. (1984). Elastolytic and collagenolytic studies of arteries: Implications for the mechanical properties of aneurysms. *Archives of Surgery, 119*(4), 405–409.

Duvoix, A., Blasius, R., Delhalle, S., Schnekenburger, M., Morceau, F., Henry, E., ... Diederich, M. (2005). Chemopreventive and therapeutic effects of curcumin. *Cancer Letters, 223*(2), 181–190.

Fiorentino, D. F., Zlotnik, A., Mosmann, T., Howard, M., & O'garra, A. (1991). IL-10 inhibits cytokine production by activated macrophages. *The Journal of Immunology, 147*(11), 3815–3822.

Fleenor, B. S. (2013). Large elastic artery stiffness with aging: Novel translational mechanisms and interventions. *Aging and Disease, 4*(2), 76.

Fleenor, B. S., Sindler, A. L., Marvi, N. K., Howell, K. L., Zigler, M. L., Yoshizawa, M., & Seals, D. R. (2013). Curcumin ameliorates arterial dysfunction and oxidative stress with aging. *Experimental Gerontology, 48*(2), 269–276.

Gaballa, M. A., Jacob, C. T., Raya, T. E., Liu, J., Simon, B., & Goldman, S. (1998). Large artery remodeling during aging biaxial passive and active stiffness. *Hypertension, 32*(3), 437–443.

Gonzales, A. M., & Orlando, R. A. (2008). Curcumin and resveratrol inhibit nuclear factor-kappaB-mediated cytokine expression in adipocytes. *Nutrition & Metabolism, 5*(1), 1.

Greenberg, A. S., & Obin, M. S. (2006). Obesity and the role of adipose tissue in inflammation and metabolism. *The American Journal of Clinical Nutrition, 83*(2), 461S–465S.

Gupta, S. C., Patchva, S., & Aggarwal, B. B. (2013). Therapeutic roles of curcumin: Lessons learned from clinical trials. *The AAPS Journal, 15*(1), 195–218.

Jablonski, K. L., Donato, A. J., Fleenor, B. S., Nowlan, M. J., Walker, A. E., Kaplon, R. E., ... Seals, D. R. (2015). Reduced large elastic artery stiffness with regular aerobic exercise in middle-aged and older adults: Potential role of suppressed nuclear factor κ B signalling. *Journal of Hypertension, 33*(12), 2477.

Kenward, M. G., & Roger, J. H. (1997). Small sample inference for fixed effects from restricted maximum likelihood. *Biometrics*, 983–997.

Krishnakumar, I., Ravi, A., Kumar, D., Kuttan, R., & Maliakel, B. (2012). An enhanced bioavailable formulation of curcumin using fenugreek-derived soluble dietary fibre. *Journal of Functional Foods, 4*(1), 348–357.

McEniery, C., & Wilkinson, I. (2005). Large artery stiffness and inflammation. *Journal of Human Hypertension, 19*(7), 507–510.

Murphy, S. L., Xu, J., & Kochanek, K. D. (2013). Deaths: Final data for 2010. *National Vital Statistics Reports: From the Centers for Disease Control and Prevention, National Center for Health Statistics, National Vital Statistics System, 61*(4), 1–117.

Nakmareong, S., Kukongviriyapan, U., Pakdeechote, P., Kukongviriyapan, V., Kongyingyoes, B., Donpunha, W., ... Phisalaphong, C. (2012). Tetrahydrocurcumin alleviates hypertension, aortic stiffening and oxidative stress in rats with nitric oxide deficiency. *Hypertension Research, 35*(4), 418–425.

Napoli, C., de Nigris, F., & Palinski, W. (2001). Multiple role of reactive oxygen species in the arterial wall. *Journal of Cellular Biochemistry, 82*(4), 674–682.

Ogden, C. L., Carroll, M. D., Kit, B. K., & Flegal, K. M. (2014). Prevalence of childhood and adult obesity in the United States, 2011–2012. *JAMA, 311*(8), 806–814.

Pérez Pérez, A., Ybarra Muñoz, J., Blay Cortés, V., & de Pablos Velasco, P. (2007). Obesity and cardiovascular disease. *Public Health Nutrition, 10*(10A), 1156–1163.

Reference Values for Arterial Stiffness' Collaboration. (2010). Determinants of pulse wave velocity in healthy people and in the presence of cardiovascular risk factors: 'Establishing normal and reference values'. *European Heart Journal, 31*(19), 2338–2350.

Ross, R. (1999). Atherosclerosis—An inflammatory disease. *New England Journal of Medicine, 340*(115–126).

Safar, M. E., Czernichow, S., & Blacher, J. (2006). Obesity, arterial stiffness, and cardiovascular risk. *Journal of the American Society of Nephrology, 17*(4 Suppl. 2), S109–S111.

Scuteri, A., Najjar, S. S., Morrell, C. H., & Lakatta, E. G. (2005). The metabolic syndrome in older individuals: Prevalence and prediction of cardiovascular events the cardiovascular health study. *Diabetes Care, 28*(4), 882–887.

Stehouwer, C., Henry, R., & Ferreira, I. (2008). Arterial stiffness in diabetes and the metabolic syndrome: A pathway to cardiovascular disease. *Diabetologia, 51*(4), 527–539.

Sugawara, J., Akazawa, N., Miyaki, A., Choi, Y., Tanabe, Y., Imai, T., & Maeda, S. (2012). Effect of endurance exercise training and curcumin intake on central arterial hemodynamics in postmenopausal women: Pilot study. *American Journal of Hypertension, 25*(6), 651–656.

Tang, X., Spitzbarth, N., Kuhn, H., Chaitidis, P., & Campbell, W. B. (2003). Interleukin-13 upregulates vasodilatory 15-lipoxygenase eicosanoids in rabbit aorta. *Arteriosclerosis, Thrombosis, and Vascular Biology, 23*(10), 1768–1774.

Thangapazham, R. L., Sharma, A., & Maheshwari, R. K. (2006). Multiple molecular targets in cancer chemoprevention by curcumin. *The AAPS Journal, 8*(3), E443–E449.

Vlachopoulos, C., Aznaouridis, K., & Stefanadis, C. (2010). Prediction of cardiovascular events and all-cause mortality with arterial stiffness: A systematic review and meta-analysis. *Journal of the American College of Cardiology, 55*(13), 1318–1327.

Vlachopoulos, C., Dima, I., Aznaouridis, K., Vasiliadou, C., Ioakeimidis, N., Aggeli, C., ... Stefanadis, C. (2005). Acute systemic inflammation increases arterial stiffness and decreases wave reflections in healthy individuals. *Circulation, 112*(14), 2193–2200.

Wellen, K. E., & Hotamisligil, G. S. (2003). Obesity-induced inflammatory changes in adipose tissue. *Journal of Clinical Investigation, 112*(12), 1785.

Wildman, R. P., Mackey, R. H., Bostom, A., Thompson, T., & Sutton-Tyrrell, K. (2003). Measures of obesity are associated with vascular stiffness in young and older adults. *Hypertension, 42*(4), 468–473.

Zebekakis, P. E., Nawrot, T., Thijs, L., Balkestein, E. J., van der Heijden-Spek, J., Van Bortel, L. M., ... Staessen, J. A. (2005). Obesity is associated with increased arterial stiffness from adolescence until old age. *Journal of Hypertension, 23*(10), 1839–1846.