Letter to the Editor

Curcuminoids Target Decreasing Serum Adipocyte-fatty Acid Binding Protein Levels in Their Glucose-lowering Effect in Patients with Type 2 Diabetes*

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Whether supplementation of curcuminoids decreases serum adipocyte-fatty acid binding protein (A-FABP) level and whether this decrease benefits glucose control is unclear. One-hundred participants (*n*=50 administered curcuminoids, *n*=50 administered placebo) from our previous report on the effect of curcuminoids on type 2 diabetes in a 3-month intervention were assessed for levels of serum A-FABP, oxidative stress, and inflammatory biomarkers. Curcuminoids supplementation led to significant decreases in serum A-FABP, C-reactive protein (CRP), tumor necrosis factor-α, and interleukin-6 levels. Curcuminoids supplementation also significantly increased serum superoxide dismutase (SOD) activity. The change in serum A-FABP levels showed positive correlations with changes in levels of glucose, free fatty acids (FFAs), and CRP in subjects supplemented with curcuminoids. Further stepwise regression analysis showed that A-FABP was an independent predictor for levels of FFAs, SOD, and CRP. These results suggest that curcuminoids may exert anti-diabetic effects, at least in part, by reductions in serum A-FABP level. A-FABP reduction is associated with improved metabolic parameters in human type 2 diabetes.

Adipocyte-fatty acid binding protein (A-FABP) is a major cytoplasmic protein produced by adipocytes and plays an important role in mediating intracellular fatty acid trafficking. In animal studies, A-FABP deficiency improves glucose and lipid metabolism in both obese and apoE$^{-/-}$ mice[1]. However, only limited information is available regarding the role of A-FABP in human disease. Recently, A-FABP was found to be closely associated with metabolic syndrome[2]. Circulating A-FABP can be used as a marker to predict the incidence of metabolic syndrome and type 2 diabetes, independently of adiposity and insulin resistance. Studies from human investigations also reported that serum A-FABP level was positively correlated with serum lipids, C-reactive protein (CRP), and interleukin-6 (IL-6) levels in both obese and type 2 diabetic patients[2]. These results indicate that A-FABP might play a role in both metabolic and inflammatory pathways involved in metabolic syndrome.

Curcuminoids are natural compounds that have a broad range of health benefits, including anti-diabetic functions[3]. Studies have shown that curcumin exhibits an anti-diabetic effect through promoting fatty acid uptake and β-oxidation and inhibition of fatty acid synthesis in the adipose, liver, and skeletal muscle tissues of diabetic animals. Moreover, the anti-diabetic effect of curcuminoids is also partly due to suppression of inflammation and oxidative stress, which involves nuclear factor-kappa B and peroxisome proliferator-activated receptor gamma. Studies on the anti-diabetic effects of curcuminoids in human diabetes remain limited. Our group recently found that curcuminoids lowered blood glucose, triglycerides, and free fatty acid acids (FFAs) levels in patients with type 2 diabetes[4].

Based on the results of these studies, we hypothesized that A-FABP may help to promote the anti-diabetic effect of curcuminoids on the regulation of lipid metabolism, oxidative stress, and inflammation. Second, we assessed whether a decrease in serum A-FABP level benefits glucose control and evaluated its possible mechanism in human diabetes.

The subjects were from our previously reported study to investigate the effect of curcuminoids on type 2 diabetes (ECTD) including 100 participants (*n*=50 administered curcuminoids, *n*=50

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administered placebo)[4]. Eligible participants were overweight/obese with type 2 diabetes. Detailed methods as well as inclusion/exclusion criteria were provided in ECTD study[4]. The study was approved by the ethics committee of Harbin Medical University (ref: 2009007) and was conducted in accordance with the Declaration of Helsinki (http://www.controlled-trials.com/, registered number: ISRCTN85826075). Written informed consent was obtained from all participants.

Eligible subjects were randomized into 2 groups. The subjects in curcuminoids group received a 150 mg-curcuminoids-capsule twice daily for a total intake of 300 mg/d curcuminoids. The subjects in placebo group took capsules with placebo instead of curcuminoids at the same frequency and amount. The capsules used in the 2 groups were identical in appearance. During the intervention, the subjects were asked to maintain their habitual diet, lifestyle and original drug treatment. Subjects were interviewed every 2 weeks and the compliance was assessed according to whether they maintained their original lifestyle, diet, medications, and intake of capsules as requested.

Body weight, blood pressure, and waist circumference were measured. Body mass index (BMI) was calculated as the weight in kilograms divided by the square of height in meters (kg/m^2). Blood was collected following an overnight fasting. The serum concentrations of total cholesterol, triglycerides, HDL-C, LDL-C, glucose, and HbA1c (%) were measured by using ROCHE Modular P800 Automatic Biochemical Analyzer (Roche Diagnostics). Serum A-FABP level was also assessed by ELISA method (R&D System). Serum FFAs concentrations were determined by GC-MS as previous described[4]. Serum CRP, tumor necrosis factor-α (TNF-α), and IL-6 were determined using ELISA method with commercial kits kits (R&D System). Serum superoxide dismutase (SOD) and glutathione peroxidase (SGH-Px) activity and malondialdehyde (MDA) concentration were measured with commercial kits using enzymatic methods (Jiancheng Technology, Nanjing, China).

We described patient characteristics using means±SD for continuous variables and numbers (percentage) for categorical variables. Mean levels of continuous variables at baseline and follow-up between the two groups were compared using independent-samples *t* test and analysis of covariance (ANCOVA), respectively. ANCOVA was adjusted for age, sex, smoking history, lipid-lowering drug use, physical activity level, blood pressure, diabetes duration, drug treatment, and baseline values. Correlations between A-FABP and other parameters were analyzed using Pearson correlation and Partial Pearson correlation analysis after adjustments for age, sex, BMI, and drug treatment. A stepwise multi-linear regression analysis was performed to identify whether A-FABP is a predictor of selected serum markers. $P<0.05$ was considered as statistically significant.

The baseline characteristics of the patients with type 2 diabetes were comparable between the two groups[4]. Serum A-FABP level showed positive correlations with BMI, waist circumference, systolic blood pressure, diastolic blood pressure, HbA1c (%), levels of glucose, triglycerides, total cholesterol, LDL-C, FFAs, CRP, TNF-α, and IL-6 (Table 1). However, A-FABP level was negatively correlated with HDL-C level and SOD activity (Table 1). The correlations found before (model 1) and after (model 2) A-FABP correction for age, sex, BMI, and drug treatment were very similar (Table 1).

In the ECTD study, we have reported that significant decreases were observed in fasting glucose (8.17±2.06 *vs.* 7.28±1.77 mmol/L, $P<0.01$), HbA1c (%) (7.99±2.86 *vs.* 7.02±2.04, $P=0.031$), FFAs (2393.2±378.1 *vs.* 2104.8±248.3 mmol/L, $P<0.01$), and triglycerides (2.11±0.75 *vs.* 1.78±0.56 mmol/L, $P=0.018$) after curcuminoids supplementation[4]. In this study, supplementation of curcuminoids led to significant decreases in serum A-FABP (34.50±7.02 *vs.* 26.18±10.03 ng/mL, $P<0.001$), CRP (2.56±1.10 *vs.* 2.09±1.02 mg/L, $P<0.001$), TNF-α (2.14±0.84 *vs.* 1.72±0.74 pg/mL, $P=0.047$), and IL-6 (3.90±2.04 *vs.* 2.68±1.63 pg/mL, $P<0.001$), after adjusted for baseline values, age, sex, smoking history, physical activity level, diabetes duration, and drug treatment. Curcuminoids supplementation also significantly increased serum SOD activity (81.87±22.59 *vs.* 94.68±23.76 U/mL, $P=0.005$). Curcuminoids supplementation had no significant effects on serum GSH-Px activity and MDA concentration.

The change in serum A-FABP level showed positive correlations with the change in levels of glucose, FFAs, and CRP after adjusted for age, sex, BMI, and drug treatment (Table 2). Further stepwise regression analysis showed that A-FABP was an independent predictor for FFAs, SOD, and CRP (Table 3).

In the present study, we found for the first time that serum A-FABP level was significantly decreased by curcuminoids supplementation and the change in

Table 1. Correlation between Serum A-FABP Level and Various Metabolic Parameters in the Patients before Intervention

Metabolic Parameters	A-FABP (ng/mL) (*n*=100)			
	Model 1*		Model 2#	
	r	*P* Value	*r*	*P* Value
BMI (kg/m^2)	0.249	0.013		
Waist circumference (cm)	0.534	<0.001	0.470	<0.001
Systolic blood pressure (mmHg)	0.259	0.009	0.250	0.015
Diastolic blood pressure (mmHg)	0.218	0.029	0.319	0.002
Glucose (mmol/L)	0.479	<0.001	0.396	<0.001
HbA1c (%)	0.295	0.003	0.220	0.030
Triglycerides (mmol/L)	0.390	<0.001	0.248	0.015
Total cholesterol (mmol/L)	0.292	0.003	0.185	0.073
HDL-C (mmol/L)	-0.383	<0.001	-0.314	0.002
LDL-C (mmol/L)	0.460	<0.001	0.349	0.001
FFAs (mmol/L)	0.439	<0.001	0.328	0.001
CRP (mg/L)	0.522	<0.001	0.352	<0.001
TNF-α (pg/mL)	0.300	0.002	0.133	0.200
IL-6 (pg/mL)	0.558	<0.001	0.421	<0.001
SOD (U/mL)	-0.276	0.005	-0.228	0.021
GSH-Px (μmol/L)	-0.148	0.143	-0.125	0.228
MDA (nmol/mL)	0.032	0.752	0.002	0.985

Note. *Pearson correlation coefficient. #Partial Pearson correlation coefficient adjusted for age, sex, BMI, and drug treatment.

Table 2. Correlation between the Change in Serum A-FABP Level and Changes in Various Metabolic Parameters in the Subjects Supplemented with Curcuminoids

Change in Metabolic Parameters	Change in A-FABP (ng/mL) (*n*=50)			
	Model 1*		Model 2#	
	r	*P* Value	*r*	*P* Value
Glucose (mmol/L)	0.461	0.001	0.374	0.010
HbA1c (%)	0.271	0.057	0.215	0.148
Triglycerides (mmol/L)	0.236	0.099	0.196	0.184
FFAs (mmol/L)	0.327	0.020	0.358	0.015
CRP (mg/L)	0.391	0.005	0.382	0.006
TNF-α (pg/mL)	-0.119	0.411	-0.103	0.513
IL-6 (pg/mL)	0.178	0.216	0.144	0.327
SOD (U/mL)	-0.300	0.034	-0.268	0.067

Note. *Pearson correlation coefficient. #Partial Pearson correlation coefficient adjusted for age, sex, BMI, and drug treatment.

Table 3. Stepwise Regression Analysis regarding the Relationship between the Metabolic Risk Factors and A-FABP after the Intervention

Dependent Variable	Step	Independent Variable	r^2 Cumulative	*t*	*P* Value
FFAs	1	A-FABP	0.189	3.205	0.002
	2	Intervention	0.251	-2.843	0.005
SOD	1	Intervention	0.072	4.215	<0.001
	2	A-FABP	0.183	-3.619	<0.001
CRP	1	A-FABP	0.055	2.272	0.025
	2	Intervention	0.096	-2.103	0.038

serum A-FABP level was positively correlated with the change in serum glucose level in diabetic patients, independently of age, sex, BMI, and other drug treatment. These results suggest that curcuminoids administration decreased serum A-FABP level, resulting in blood glucose control in type 2 diabetes.

One of our hypotheses in this study was that A-FABP regulates fatty acid metabolism via the anti-diabetic effect of curcuminoids, which was supported by our finding that curcuminoids supplementation decreased serum A-FABP level and was positively correlated with the change in serum FFA levels in patients with type 2 diabetes; A-FABP was the primary predictor for FFAs level, which was responsible for 18.9% of the variance in our multivariable regression analysis. In a normal physiological state, serum FFAs are mainly generated by lipolysis of adipocytes in adipose tissue. In adipocytes, A-FABP forms a 1:1 complex with hormone-sensitive lipase on a regulatory docking domain and such an interaction positions the A-FABP to bind to FFAs and facilitate lipolysis[5]. Circulating A-FABP is secreted by adipocytes *in vivo* as well as by cultured adipocytes[6]. We hypothesized that circulating A-FABP possibly reflects the extent of lipolysis in adipose tissue. Baar et al.[7] found that adipocytes obtained from A-FABP-deficient mice exhibited decreased lipolytic efficiency and a reduction in fatty acid release, suggesting that A-FABP mediates the efflux of fatty acids. Reduced fatty acid release into the circulation probably reduced the flux of FFAs to the liver and muscle, thereby decreasing inhibition of the normal insulin-signaling cascade by FFAs. Moreover, in A-FABP-/- mice, reduced FFAs efflux from adipocytes led to an increase in peripheral glucose oxidation[7]. The results of a previous study showed that A-FABP can be successfully targeted by an orally active small molecule inhibitor to generate a profile reminiscent of genetic deficiency both *in vitro* and *in vivo*[8]. In our study, we hypothesized that curcuminoids play a role as an A-FABP inhibitor in adipocytes, thereby reducing fatty acid release from adipose tissue and increasing peripheral glucose oxidation, which contribute to glucose-lowering effects.

A second hypothesis was that A-FABP modulates oxidative stress and inflammation via the anti-diabetic effect of curcuminoids. Serum A-FABP level was recently found to be positively correlated with serum CRP and IL-6 levels in both obese patients and those with type 2 diabetes[2]. In the present study, the change in serum A-FABP level was positively correlated with the change in serum CRP level and showed a trend of negative correlation with SOD activity in type 2 diabetic patients supplemented with curcuminoids, independently of age, sex, BMI, and other drug treatment. Also, stepwise regression analysis demonstrated that A-FABP was a predictor for SOD and CRP, accounting for 18.3% and 5.5% of the variance, respectively. These results collectively support our hypothesis that A-FABP probably links curcuminoids with their effect on oxidative stress and inflammation. Previous studies have shown that A-FABP is also expressed in macrophages, thereby mediating inflammatory cytokine production and cholesterol ester accumulation. Deletion of FABPs in adipocytes resulted in reduced expression of inflammatory cytokines in macrophages, whereas the same deletion in macrophages led to enhanced insulin signaling and glucose uptake in adipocytes[9]. The results of these experiments indicated that neither macrophages nor adipocytes individually could account for the total impact of FABPs on systemic metabolism and suggested that interactions between these two cell types, particularly in adipose tissue, were critical for the inflammatory basis of metabolic deterioration. In the present study, the decrease in serum inflammation markers was likely due to the inhibition of A-FABP in adipocytes following curcuminoids administration, leading to reduced production and secretion of inflammatory markers from macrophages. In addition, adipocytes secrete several proteins that impact whole-body insulin sensitivity, including leptin, adiponectin, resistin, TNF-α, and IL-6. In a previous study[10], TNF-α mRNA levels were reduced in A-FABP-null adipocytes, indicating that the reduction in A-FABP level by curcuminoids supplementation, at least in part, decreased the production and secretion of inflammation markers by adipocytes.

In conclusion, the results of this study have shown, for the first time, that curcuminoids may exert anti-diabetic effects partly by reducing serum A-FABP level, as a reduction in serum A-FABP level has been associated with improved metabolic parameters in type 2 diabetes.

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