

## Supplement of bamboo extract lowers serum monocyte chemoattractant protein-1 concentration in mice fed a diet containing a high level of saturated fat

Jason K. Higa, Wanyu Liu, Marla J. Berry and Jun Panee\*

Department of Cell and Molecular Biology, John A. Burns School of Medicine, University of Hawaii at Manoa, 651 Ilalo Street BSB 222, Honolulu, HI 968 13, USA

(Received 24 May 2010 – Revised 10 March 2011 – Accepted 22 March 2011 – First published online 7 June 2011)

### Abstract

Monocyte chemoattractant protein-1 (MCP-1) is an inflammatory chemokine up-regulated in obese subjects, contributing to the development of type 2 diabetes. The present study investigated the inhibitory effect of an ethanol–water extract from bamboo (BEX, *Phyllostachys edulis*) on the blood concentration of MCP-1. C57BL/6J mice were fed a standard diet or a high-fat diet with or without the BEX supplement (11 g dry mass/17 000 kJ) for 6 months. A total of ten mice were used in each group. Body weight and food consumption were measured weekly. After euthanasia, the weight of visceral fat and circulating MCP-1 concentration were measured. In comparison with the standard control group, the high-fat control group had increased body weight, abdominal fat storage and serum MCP-1 concentration by 60% ( $P < 0.001$ ), 266% ( $P < 0.001$ ) and 180% ( $P < 0.01$ ), respectively. In comparison with the high-fat control group, the high-fat BEX group showed a 3% decrease in body weight ( $P < 0.01$ ), 24% decrease in mesenteric fat depot ( $P < 0.01$ ) and 49% decrease in serum MCP-1 concentration ( $P < 0.05$ ). The present study suggests that the BEX supplement in the high-fat diet ameliorates elevated MCP-1 concentrations in the blood, and whether this is related to modulated endocrine properties of the visceral fat is to be studied.

**Key words:** Monocyte chemoattractant protein-1: High-fat diet: Bamboo extract: Mesenteric fat

Monocyte chemoattractant protein-1 (MCP-1/chemokine (C–C motif) ligand 2) is a member of the C–C chemotactic cytokine (chemokine) family, produced by multiple cell types constitutively or after induction<sup>(1)</sup>. The circulating level of MCP-1 was found to be approximately 50% higher in obese mice<sup>(2)</sup> and in human subjects with type 2 diabetes<sup>(3)</sup> in comparison with controls. MCP-1 recruits monocytes into adipose tissues and enhances obesity-associated chronic inflammation<sup>(4)</sup> and insulin resistance<sup>(5)</sup>. It also facilitates the expansion and remodelling of the adipose tissue during the development of obesity through an angiogenic effect on the endothelial cells<sup>(6)</sup>; furthermore, this chemokine can decrease the liposynthesis ability of adipocytes<sup>(2)</sup> and subsequently elevate the NEFA level in the circulation<sup>(4)</sup>, exerting lipotoxicity in the periphery<sup>(7)</sup>. Therefore, inhibiting MCP-1 overproduction has become a preventive strategy for obesity-induced type 2 diabetes.

Our previous study has shown that an ethanol–water extract from bamboo (BEX, *Phyllostachys edulis*) efficiently protected murine muscle C2C12 cells from lipotoxicity<sup>(8)</sup>, an obesity-related condition leading to inflammation and insulin resistance<sup>(9,10)</sup>. In the present study, it is further revealed

that the BEX as a dietary supplement significantly decreased the circulating level of MCP-1 in mice treated with a diet containing a high level of saturated fat, with concurrence of decreased weight of the mesenteric fat depot.

### Experimental methods

#### Bamboo extract

The BEX used in the present study was provided by Golden Basin LLC (Kailua, HI, USA). It is prepared from fresh leaves and small branches of bamboo (*P. edulis*) in Hunan Province, China, through a patented ethanol–water extraction procedure (Chinese invention patent, CN 1287848A).

#### Animals

Male C57BL/6J mice at 4 weeks of age were purchased from Jackson Laboratories (Bar Harbor, MN, USA). The animals were housed three to four per cage and had *ad libitum* access to water and food. The room temperature was controlled at 20°C, and lighting was turned on and off with 12 h

**Abbreviations:** BEX, bamboo extract; MCP-1, monocyte chemoattractant protein-1.

\* **Corresponding author:** J. Panee, fax +1 808 692 1970, email junchen@hawaii.edu

intervals. Institutional and national guidelines for the care and use of animals were followed, and all experimental procedures involving animals were approved by the Institutional Animal Care and Use Committee at the University of Hawaii (Honolulu, HI, USA).

### Dietary treatment

After 1 week of acclimatisation with regular rodent chow, mice were fed a standard diet (10% energy from fat) or a high-fat diet (60% energy from fat) with or without the BEX supplement (11 g dry mass per 17 000 kJ) for 6 months. A total of ten mice were used in each dietary group. Body weight and food consumption were measured weekly. All diets were purchased from Research Diets (New Brunswick, NJ, USA). The dietary composition is listed in Table 1.

### Measurement of the abdominal fat pads

After euthanasia, epididymal fat, perirenal fat and mesenteric fat were collected and weighed.

### Serum monocyte chemoattractant protein-1 quantification

Blood was collected through cardiac puncture. MCP-1 concentrations in the sera were measured using a Cytometric Bead Array – Mouse MCP-1 Flex Set (BD Biosciences, Bedford, MA, USA).

### Statistical analysis

Statistical analysis was performed by two-way ANOVA with Bonferroni's *post hoc* test using the software Prism 4.0a (GraphPad Software, Inc., La Jolla, CA, USA) and Stata 11.0

(StataCorp LP, College Station, TX, USA) (Table 2). The weekly record of body weight was analysed using linear regression with Huber correction and random-effects regression to account for multiple measurements per mouse.  $P \leq 0.05$  was considered statistically significant.

## Results

Table 2 summarises the major findings of the present study. During the 6 months of treatment, the high dietary fat content increased the daily energy intake by approximately 30% ( $P < 0.0001$ ). The BEX supplement did not affect the energy intake. The body weight of mice at both start and end points is shown. High-fat diets resulted in an average of 60% increase in body weight ( $P < 0.0001$ ) at the end point. When the weekly record of the body weight (data not shown) was analysed, the BEX supplement in the high-fat diet was found to slightly decrease ( $-3\%$ ,  $P < 0.01$ ) the weight gain of mice.

The high dietary fat content also increased the total weight of the abdominal fat by approximately threefold. When the weight of the individual fat depot was analysed, the BEX was found to increase the epididymal fat by 20% (0.37 g,  $P < 0.05$ ) and decrease the mesenteric fat by 24% (0.52 g,  $P < 0.01$ ), but did not affect the total weight of the visceral fat. An interaction between the fat content and the BEX was found to play an important role in regulating the weight of the mesenteric fat depot ( $P < 0.01$ ).

Most interestingly, the BEX supplement dramatically decreased high-fat, diet-induced elevated MCP-1 concentration in the serum ( $-49\%$ ,  $P < 0.05$ ), and whether this is related to modulated endocrine properties of the visceral fat, especially the mesenteric fat, is to be studied.

**Table 1.** Composition of the diets used in the present study

Diets...	Standard control		Standard BEX		High-fat control		High-fat BEX	
	g	kJ	g	kJ	g	kJ	g	kJ
<b>Ingredients</b>								
Casein, 80 mesh	200	3349	200	3349	200	3349	200	3349
L-Cystine	3	50	3	50	3	50	3	50
Maize starch	315	5275	315	5275	0	0	0	0
Maltodextrin 10	35	586	35	586	125	2093	125	2093
Sucrose	350	5862	350	5862	68.8	1151	68.8	1151
Cellulose, BW200	50	0	50	0	50	0	50	0
Soyabean oil	25	942	25	942	25	942	25	942
Lard	20	754	20	754	245	9232	245	9232
Mineral mix S10026	10	0	10	0	10	0	10	0
Dicalcium phosphate	13	0	13	0	13	0	13	0
Calcium carbonate	5.5	38	5.5	38	5.5	38	5.5	38
Potassium citrate, 1 H <sub>2</sub> O	16.5	0	16.5	0	16.5	0	16.5	0
Vitamin mix V10001	10	167	10	167	10	167	10	167
Choline bitartrate	2	0	2	0	2	0	2	0
Bamboo extract (dry mass)	0	0	11	0	0	0	11	0
Water from the bamboo extract	0	0	11	0	0	0	11	0
FD&C Yellow Dye no. 5	0.05	0	0.025	0	0	0	0.025	0
FD&C Red Dye no. 40	0	0	0	0	0	0	0.025	0
FD&C Blue Dye no. 1	0	0	0.025	0	0.05	0	0	0
<b>Total</b>	<b>1055.1</b>	<b>16 986</b>	<b>1077.1</b>	<b>16 986</b>	<b>773.9</b>	<b>16 986</b>	<b>795.9</b>	<b>16 986</b>

BEX, bamboo extract.

**Table 2.** Energy intake, body weight, abdominal fat and serum monocyte chemoattractant protein-1 (MCP-1) concentration in mice

(Mean values and standard deviations)

Group...	Standard control		Standard BEX		High-fat control		High-fat BEX	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Energy intake (kJ/d)*	39.4 <sup>a</sup>	4.0	37.9 <sup>a</sup>	4.5	50.3 <sup>b</sup>	5.3	50.9 <sup>b</sup>	3.6
Body weight (g) (start)	17.8 <sup>a</sup>	2.1	18.2 <sup>a</sup>	1.8	18.0 <sup>a</sup>	0.9	18.4 <sup>a</sup>	1.1
Body weight (g) (end)	28.7 <sup>a</sup>	3.1	28.3 <sup>a</sup>	2.5	47.5 <sup>b</sup>	2.2	44.6 <sup>b</sup>	2.6
Epididymal fat (g)	0.65 <sup>a</sup>	0.26	0.78 <sup>a</sup>	0.38	1.84 <sup>b</sup>	0.24	2.21 <sup>c</sup>	0.37
Perirenal fat (g)	0.17 <sup>a</sup>	0.07	0.24 <sup>a</sup>	0.16	0.85 <sup>b</sup>	0.31	0.74 <sup>b</sup>	0.19
Mesenteric and omental fat (g)	0.22 <sup>a</sup>	0.08	0.30 <sup>a</sup>	0.14	2.17 <sup>b</sup>	0.23	1.65 <sup>c</sup>	0.47
Total abdominal fat (g)	1.04 <sup>a</sup>	0.38	1.32 <sup>a</sup>	0.66	4.86 <sup>b</sup>	0.58	4.69 <sup>b</sup>	0.39
Serum MCP-1 (pg/ml)	16.26 <sup>a</sup>	6.58	19.34 <sup>a</sup>	3.23	45.78 <sup>b</sup>	9.56	23.37 <sup>a</sup>	1.75

BEX, bamboo extract.

<sup>a,b,c</sup> Mean values within a row with unlike superscript letters were significantly different ( $P < 0.05$ ).

\* Energy intake is the average of the weekly measurements.

## Discussion

Using the same diet containing a high level of saturated fat, Yu *et al.*<sup>(11)</sup> treated C57BL/6 mice for 3 months and compared the MCP-1 expression and secretion in four types of adipose tissues: mesenteric, epididymal, perirenal and subcutaneous. While the amounts of MCP-1 released by the epididymal, perirenal and subcutaneous fat depots were approximately the same, this level quadruplicated in the mesenteric fat. Mesenteric adipose tissue-conditioned medium also induced the highest degree of macrophage migration and stimulated pro-inflammatory cytokine production in macrophages. These findings indicate that in comparison with the other fat depots, the mesenteric fat tissue has a more pronounced role in obesity-associated inflammation. The present study showed that the BEX supplement in a high-fat diet decreased the weight of the mesenteric fat by 0.52 g, and therefore it may attenuate MCP-1 secretion from this tissue and subsequently contribute to the decrease in MCP-1 in the circulation. Although the BEX increased the weight of the epididymal fat by 0.37 g, this may not compensate the change caused by the mesenteric fat due to the dramatic difference between the MCP-1 secretion abilities of these two fat depots. This suggests that the BEX may alter the distribution of fat storage in the visceral adipose tissues and lower MCP-1 secretion as a final result. Although white adipose tissue is a major source of MCP-1<sup>(2)</sup>, this chemokine is also produced in other tissues<sup>(12–14)</sup>, and therefore a systematic study is needed to evaluate the tissue-specific effect of BEX.

Other natural products that inhibit the overproduction of MCP-1 in obese/diabetic rodents and in cell culture models include traditional Asian medicine<sup>(15–18)</sup>, extracts from herbs<sup>(19)</sup>, spices<sup>(20,21)</sup>, fruits and vegetables<sup>(22–25)</sup>. Due to the use of different models and experimental procedures, and variable purity of the extracts, it is difficult to perform an accurate comparison between the efficacy of BEX and other reported natural products. The daily dose of BEX used in the present study was 773 mg/kg body weight for mouse, and this corresponds to 63 mg/kg body weight for human subjects (3.8 g/d for a 60 kg adult) when the body surface

area normalisation method is used for an allometric dose translation<sup>(26)</sup>.

The BEX used in the present study consists of approximately 50% water, 20% saccharides, 10% protein and 20% other components. The active component(s) contributing to the effects described earlier are to be further determined. The extraordinary abundance of the raw material is a major advantage of this natural product. *P. edulis* is known for its fast growth, wide geographical distribution and easy propagation. The raw materials (small branches and leaves) used for BEX production are by-products of the bamboo timber industry. Therefore, the present study suggests a potential nutraceutical application of a rich and environmentally friendly natural resource.

## Acknowledgements

We thank Dr Leigh Anne Shafer who is funded by the University of Hawaii RCMI Programme for assistance with statistical analysis. The present study was supported by grant no. R21 AT003874-02 (J. P.) and R21 AT005139-01 (J. P.) from the NCCAM and ORWH, 5G12RR003061-23 from the NCRR, and 5P20 MD000173-08 from the NCMHD. The contents of this paper are solely the responsibility of the authors and do not necessarily represent the official views of the funding agencies or the National Institutes of Health. J. K. H. contributed to the MCP-1 measurement and data analysis; W. L. contributed to the animal management; M. J. B. contributed to the general consultation; J. P. contributed to the study design, data analysis and manuscript writing. The authors declare that there are no conflicts of interest.

## References

1. Deshmane SL, Kremlev S, Amini S, *et al.* (2009) Monocyte chemoattractant protein-1 (MCP-1): an overview. *J Interferon Cytokine Res* **29**, 313–326.
2. Sartipy P & Loskutoff DJ (2003) Monocyte chemoattractant protein 1 in obesity and insulin resistance. *Proc Natl Acad Sci U S A* **100**, 7265–7270.

3. Nomura S, Shouzu A, Omoto S, *et al.* (2000) Significance of chemokines and activated platelets in patients with diabetes. *Clin Exp Immunol* **121**, 437–443.
4. Kamei N, Tobe K, Suzuki R, *et al.* (2006) Overexpression of monocyte chemoattractant protein-1 in adipose tissues causes macrophage recruitment and insulin resistance. *J Biol Chem* **281**, 26602–26614.
5. Fantuzzi G (2005) Adipose tissue, adipokines, and inflammation. *J Allergy Clin Immunol* **115**, 911–919.
6. Salcedo R, Ponce ML, Young HA, *et al.* (2000) Human endothelial cells express CCR2 and respond to MCP-1: direct role of MCP-1 in angiogenesis and tumor progression. *Blood* **96**, 34–40.
7. Unger RH (1995) Lipotoxicity in the pathogenesis of obesity-dependent NIDDM. Genetic and clinical implications. *Diabetes* **44**, 863–870.
8. Panee J, Liu W, Lin Y, *et al.* (2008) A novel function of bamboo extract in relieving lipotoxicity. *Phytother Res* **22**, 675–680.
9. Boden G, She P, Mozzoli M, *et al.* (2005) Free fatty acids produce insulin resistance and activate the proinflammatory nuclear factor-kappaB pathway in rat liver. *Diabetes* **54**, 3458–3465.
10. Shi H, Kokoeva MV, Inouye K, *et al.* (2006) TLR4 links innate immunity and fatty acid-induced insulin resistance. *J Clin Invest* **116**, 3015–3025.
11. Yu R, Kim CS, Kwon BS, *et al.* (2006) Mesenteric adipose tissue-derived monocyte chemoattractant protein-1 plays a crucial role in adipose tissue macrophage migration and activation in obese mice. *Obesity* **14**, 1353–1362.
12. Heymann F, Trautwein C & Tacke F (2009) Monocytes and macrophages as cellular targets in liver fibrosis. *Inflamm Allergy Drug Targets* **8**, 307–318.
13. Wozniak SE, Gee LL, Wachtel MS, *et al.* (2009) Adipose tissue: the new endocrine organ? A review article. *Dig Dis Sci* **54**, 1847–1856.
14. Marino M, Scuderi F, Provenzano C, *et al.* (2008) IL-6 regulates MCP-1, ICAM-1 and IL-6 expression in human myoblasts. *J Neuroimmunol* **196**, 41–48.
15. Zhang H, Chen S, Deng X, *et al.* (2007) The effects of Danggui-Buxue-Tang on blood lipid and expression of genes related to foam cell formation in the early stage of atherosclerosis in diabetic GK rats. *Diabetes Res Clin Pract* **77**, 479–481.
16. Zhang HM, Chen SW, Xie CG, *et al.* (2006) Mechanism of Shenqi compound recipe anti-earlier diabetic atherosclerosis in GK rats. *Zhongguo Zhong Yao Za Zhi* **31**, 1272–1276.
17. Luo P, Tan ZH, Zhang ZF, *et al.* (2008) Scutellarin isolated from *Erigeron multiradiatus* inhibits high glucose-mediated vascular inflammation. *Yakugaku Zasshi* **128**, 1293–1299.
18. Lee YJ, Kang DG, Kim JS, *et al.* (2008) *Buddleja officinalis* inhibits high glucose-induced matrix metalloproteinase activity in human umbilical vein endothelial cells. *Phytother Res* **22**, 1655–1659.
19. Kang MS, Hirai S, Goto T, *et al.* (2009) Dehydroabietic acid, a diterpene, improves diabetes and hyperlipidemia in obese diabetic KK-Ay mice. *Biofactors* **35**, 442–448.
20. Jain SK, Rains J, Croad J, *et al.* (2009) Curcumin supplementation lowers TNF-alpha, IL-6, IL-8, and MCP-1 secretion in high glucose-treated cultured monocytes and blood levels of TNF-alpha, IL-6, MCP-1, glucose, and glycosylated hemoglobin in diabetic rats. *Antioxid Redox Signal* **11**, 241–249.
21. Woo HM, Kang JH, Kawada T, *et al.* (2007) Active spice-derived components can inhibit inflammatory responses of adipose tissue in obesity by suppressing inflammatory actions of macrophages and release of monocyte chemoattractant protein-1 from adipocytes. *Life Sci* **80**, 926–931.
22. Abe D, Saito T, Kubo Y, *et al.* (2010) A fraction of unripe kiwi fruit extract regulates adipocyte differentiation and function in 3T3-L1 cells. *Biofactors* **36**, 52–59.
23. Chacón MR, Ceperuelo-Mallafre V, Maymó-Masip E, *et al.* (2009) Grape-seed procyanidins modulate inflammation on human differentiated adipocytes *in vitro*. *Cytokine* **47**, 137–142.
24. Sugimoto M, Arai H, Tamura Y, *et al.* (2009) Mulberry leaf ameliorates the expression profile of adipocytokines by inhibiting oxidative stress in white adipose tissue in db/db mice. *Atherosclerosis* **204**, 388–394.
25. Zhu J, Yong W, Wu X, *et al.* (2008) Anti-inflammatory effect of resveratrol on TNF-alpha-induced MCP-1 expression in adipocytes. *Biochem Biophys Res Commun* **369**, 471–477.
26. Reagan-Shaw S, Nihal M & Ahmad N (2008) Dose translation from animal to human studies revisited. *FASEB J* **22**, 659–661.