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ORIGINAL ARTICLE

Protective Effect of *Trans*-chalcone Against High-Fat Diet-Induced Pulmonary Inflammation Is Associated with Changes in miR-146a And pro-Inflammatory Cytokines Expression in Male Rats

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Abstract— High-fat diet (HFD) increases the risk of non-communicable inflammatory diseases including pulmonary disorders. *Trans*-chalcone is a chalcone with antioxidant and anti-inflammatory effects. This study aimed to explore the effect of this natural compound and molecular mechanism of its effect on HFD-induced pulmonary inflammation. Twenty-eight male Wistar rats were randomly divided into four main groups ($n = 7$ per each group): control, receiving 10% tween 80; Chal, receiving *trans*-chalcone, HFD, receiving a high-fat emulsion and 10% tween 80; HFD + Chal, receiving a high-fat emulsion and *trans*-chalcone. After 6 weeks, the lungs were dissected, and the expression levels of tumor necrosis factor-α (TNF-α), interleukin-1β (IL-1β), interleukin-6 (IL-6), and miR-146a were determined using real-time PCR. Moreover, histological analysis was done by hematoxylin and eosin staining. Significant elevations in TNF-α, IL-1β, IL-6, and miR-146a expression levels ($P < 0.001$) were observed within the lungs of HFD-fed rats compared with the control. However, oral administration of *trans*-chalcone reduced TNF-α, IL-1β, IL-6 ($P < 0.001$), and miR-146a ($P < 0.05$) expression levels and also improved HFD-induced histological abnormalities. These findings indicate that *trans*-chalcone ameliorates lung inflammatory response and structural alterations. It seems that this beneficial effect is associated with the down-regulation of pro-inflammatory cytokines and miR-146a.

KEY WORDS: *trans*-chalcone; lung; high-fat diet; TNF-α; IL-1β; IL-6.

INTRODUCTION

The prevalence of obesity, a chronic inflammatory condition, has increased dramatically over the past years. Obesity and high-fat diet (HFD) increase the risk of chronic diseases including cancer, diabetes, cardiovascular disease, and respiratory disease [13, 34]. For instance, HFD may induce lung inflammation and remodeling by increasing production of pro-inflammatory cytokines such as tumor necrosis factor-α (TNF-α) [27]. It has been suggested

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that airway remodeling, as a result of chronic inflammation, is a well-known feature of lung inflammatory diseases including chronic obstructive lung disease and asthma [6, 11, 23].

microRNAs (miRNAs) are small non-coding RNA molecules which post-transcriptionally regulate the expression of their target genes [5]. They contain about 22 nucleotides and have important roles in biological processes such as cell differentiation, proliferation, and modulation of immune responses in macrophages [1]. These non-coding RNAs exert central roles in the pathogenesis of various pulmonary diseases. Therefore, miRNAs may serve as biomarkers and therapeutic targets in lung diseases [3, 31]. Especially, miR-146a may be regarded as a therapeutic target for lung inflammation [31, 32]. It has been suggested that inflammatory condition causes up-regulation of this miRNA in a nuclear factor kappa B (NF-κB)-dependent manner [36].

Chalcones, as an important class of flavonoids, exert wide biological activities such as antioxidant, anti-fibrotic, and anti-inflammatory activities [18, 28]. It has been known that *trans* isomer of chalcone is thermodynamically more favorable than *cis* [2]. *Trans*-chalcone is a flavonoid precursor which exerts beneficial effects on HFD-induced obesity [14]. A recent study indicated that a chalcone derivative, L2H21, inhibited lipopolysaccharide (LPS)-induced pulmonary inflammation and acute lung injury in mice [41]. However, the possible role of *trans*-chalcone and mechanism of its action in pulmonary injury induced by HFD remains unclear. Therefore, this study aimed to evaluate the possible miR-146-dependent effect of *trans*-chalcone on pulmonary inflammation in HFD-fed rats.

MATERIALS AND METHODS

Experimental Protocol

Twenty-eight male Wistar rats (210–250 g) were obtained and housed in an environment with controlled conditions (22 ± 2 °C and 12:12 light-dark cycle) with free access to rodent chow and water. This animal experiment was approved by the Ethical Committee of Tabriz University of Medical Sciences (code number: IR.TBZMED.REC.1396.582).

After 1 week of adaptation, rats were randomly divided into four groups ($n = 7$ per group): control (gavaged with 2 ml of 10% Tween 80), Chal (gavaged with 20 mg/kg *trans*-chalcone, which was dissolved in 2 ml of 10% Tween 80 [16]), HFD (gavaged with 10 ml/kg high-fat emulsion + 2 ml of 10% Tween 80), and HFD + Chal (gavaged with 10 ml/kg high-fat emulsion + 20 mg/kg *trans*-chalcone). High-fat emulsion (HFD) was composed of distilled water (300 ml), cholesterol (100 g), corn oil (400 g), saccharose (150 g), tween 80 (36.4 g), total milk powder (80 g), propylene glycol (31.1 g), cooking salt (10 g), mineral mixture (1.5 g), sodium deoxycholate (10 g), and vitamin mixture (2.5 g) [42]. All treatments including 10% Tween 80, *trans*-chalcone [16], and high-fat emulsion [42] were done by once-daily oral gavage for 6 weeks.

At the end of this period, animals were anesthetized by an intraperitoneal injection of ketamine (60 mg/kg)/xylazine (10 mg/kg) combination and lung samples were collected. One part of each sample was fixed in 10% formalin for histological analysis and the other part was stored at − 80 °C for further analyses.

Real-Time PCR

Expression levels of miR-146a and mRNAs including TNF-α, interleukin (IL)-1β, and IL-6 in the lung samples were determined through real-time PCR method. Isolation of total RNAs (miRNA and mRNAs) and synthesis of cDNA in lung samples were done as previously described [17]. Real-time PCR was performed with 2 μl cDNA and PCR master mix (2 μl forward and reverse primers, 12.5 μl SYBR Green PCR Master Mix, and 8.5 μl water) in a final reaction volume of 23 μl [17]. After normalization to housekeeping genes including miR-191 for miR-146a and β-actin for mRNAs (TNF-α, IL-1β, and IL-6), relative quantification expression of each gene was calculated using $2^{-\Delta\Delta Ct}$ method [15]. A List of primer sequences used in the present study are shown in Table 1.

Histological Evaluation

The lung tissues were fixed in a 10% buffered formalin solution. Then, hematoxylin and eosin stained lung sections were evaluated by a blinded pathologist under a light microscope (Axioskop2; Carl Zeiss MicroImaging Inc., Germany). Scoring of histopathological data was performed using these three criteria: lymphocyte infiltration (0 = no, 1 = mild, 2 = moderate, 3 = severe), distension of the alveolar sacs (0 = no, 1 = yes), and thickening mucus in interalveolar septa (0 = no, 1 = yes).

Table 1. The primers sequences for genes

Genes	Primer sequence[a]
L-1β	F: CACCTTCTTTTCCTTCATCTTTG
	R: GTCGTTGCTTGTCTCTCCTTGTA
IL-6	F: TGATGGATGCTTCCA AACTG
	R: GAGCATTGGAAGTTGGGG TA
TNF-α	F: ACTGAACTT CGG GGTGATTG
	R: GCTTGGTGGTTTGCTACGAC
β-actin	F: TACAGCTTCACCACCACAGC
	R: ATGCCACAGGATTCCATACC
	Target sequence[b]
rno-miR-146a-5p	UGAGAACUGAAUUCCAUGGGUU
rno-miR-191a-5p	CAACGGAAUCCCAAAAGCAGCUG

[a] Sequences were derived from NCBI (www.ncbi.nlm.nih.gov)
[b] Sequences were derived from miRBase (www.mirbase.org)

Statistical Analysis

The SPSS 16 software package was used for statistical analyses. All normally distributed data were analyzed using one-way analysis of variance (ANOVA) with *post hoc* Turkey's test. Analysis of non-normally distributed data, obtained by histological scoring, were done by the Kruskal-Wallis followed by Mann-Whitney test as *post hoc* analysis (when a significant statistical difference was observed). Normally distributed data are expressed as mean ± SEM and data without a normal distribution are expressed as median (min–max). P value < 0.05 was considered as statistical significance.

RESULTS

TNF-α, *IL-1β*, *IL-6*, and miR-146a Expressions

In the current study, expression levels of TNF-α, IL-1β, and IL-6 in the lungs of rats in experimental groups are depicted in Fig. 1a–c. Feeding with HFD led to significant ($P < 0.001$) increases in mRNA levels of TNF-α, IL-1β, and IL-6. However, *trans*-chalcone significantly ($P < 0.001$) inhibited these HFD-related changes in HFD + Chal rats. Furthermore, expression of the miR-146a was significantly ($P < 0.001$) increased in the lungs of rats in HFD compared with the control group, while treatment of HFD-fed rats with *trans*-chalcone significantly ($P < 0.05$) reversed this change (Fig. 2).

Histological Findings

The histological study was used to confirm the effects of treatment with HFD and *trans*-chalcone on lung tissue.

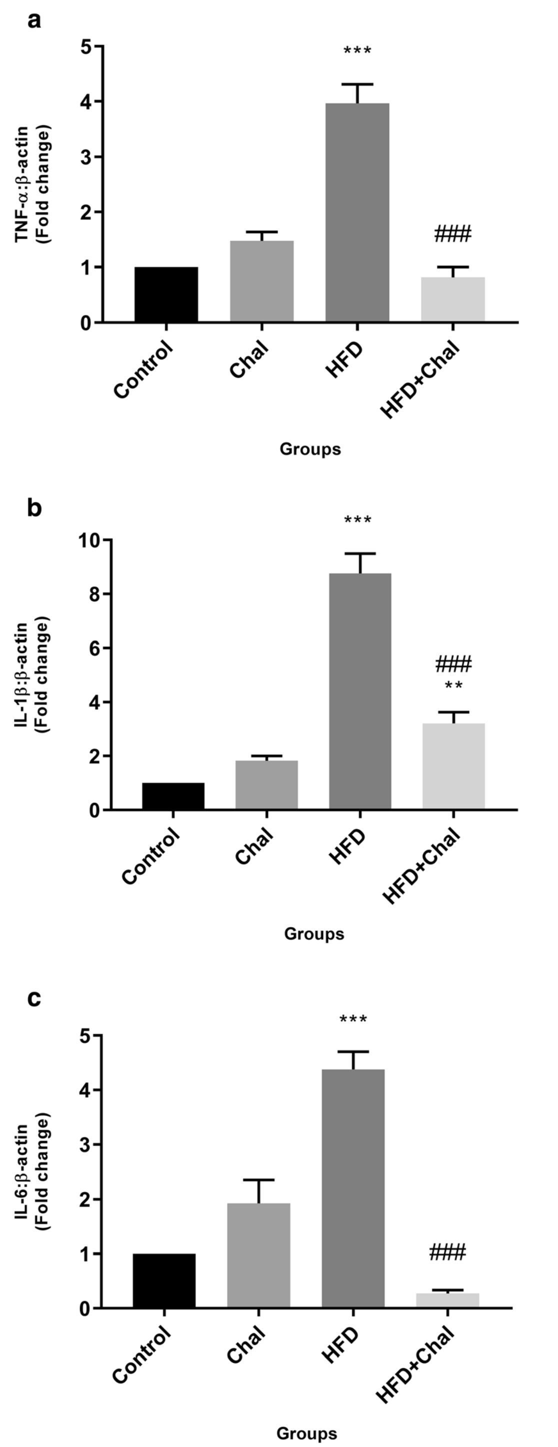

Fig. 1. Pulmonary mRNA levels of TNF-α (**a**), IL-1β (**b**), and IL-6 (**c**) in different study groups (control, receiving 10% tween 80; Chal, receiving *trans*-chalcone; HFD, receiving the high-fat emulsion and 10% tween 80; HFD + Chal, receiving the high-fat emulsion and *trans*-chalcone). Data are expressed as mean ± SEM. **$P < 0.01$ *versus* control, ***$P < 0.001$ *versus* control, ###$P < 0.001$ *versus* HFD.

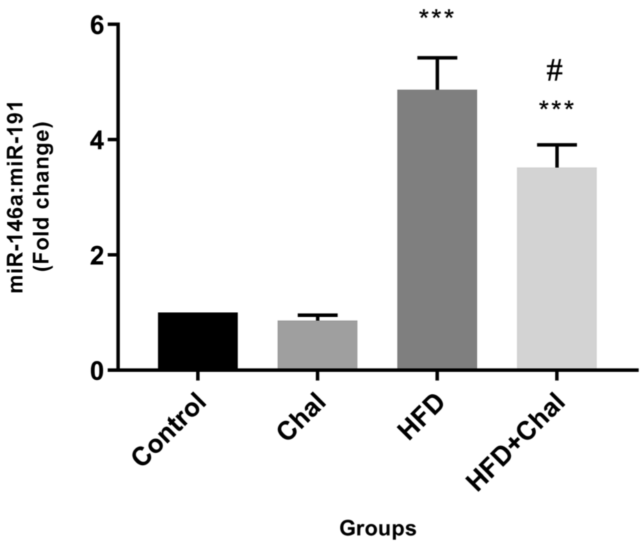

Fig. 2. Pulmonary expression of miR-146a in different groups (control, receiving 10% tween 80; Chal, receiving *trans*-chalcone; HFD, receiving the high-fat emulsion and 10% tween 80; HFD + Chal, receiving the high-fat emulsion and *trans*-chalcone). Data are expressed as mean ± SEM. $^{***}P < 0.001$ *versus* control, $\#P < 0.05$ *versus* HFD.

As shown in Fig. 3, lymphocyte infiltration, distension of the alveolar sacs, and thickening mucus in interalveolar septa were found in the HFD group. According to results of histological analysis, HFD feeding significantly increased lymphocyte infiltration ($P < 0.01$) and thickening mucus in interalveolar septa ($P < 0.05$) scores (Table 2). However, *trans*-chalcone significantly ($P < 0.01$) reduced lymphocyte infiltration in the HFD + Chal group and also reversed HFD-induced thickening mucus in interalveolar septa to normal level (Fig. 3 and Table 2).

Values are expressed as median (min–max)

$^{*}P < 0.05$ *versus* control, $^{**}P < 0.01$ *versus* control, $^{\#\#}P < 0.01$ *versus* HFD

Chal trans-chalcone, *HFD* high-fat diet

DISCUSSION

The main findings of the current study were as follows: (1) long-term exposure to HFD led to histological changes including an increase in lymphocyte infiltration and thickening of mucus in interalveolar septa. (2) HFD increased expression levels of TNF-α, IL-1β, and IL-6 in the lung. (3) Feeding with HFD markedly increased miR-146a expression in the lung tissue. (4) *trans*-chalcone could reduce miR-146a, TNF-α, IL-1β, and IL-6 expressions and also restored HFD-induced structural alterations in the lung of HFD-fed animals.

These findings indicated the involvement of inflammatory signaling pathway in the HFD-induced lung abnormalities. It has been suggested that IL-1ß is an important mediator in immune responses in obesity [24]. This cytokine was up-regulated in the lung of obese animals [4, 21]. Furthermore, IL-1ß could activate the NF-κB and increased the expression of various inflammatory genes [22]. Several previous studies suggested that HFD feeding led to lung inflammatory response, which was manifested by pro-inflammatory cytokine production such as IL-1β, NF-κB, and IL-6, and also by inflammatory cell infiltration [1, 7, 34, 37]. It has been reported that maternal HFD-feeding led to inflammation and remodeling of lung in offspring [34]. Moreover, a previous study showed that direct intra-tracheal TNF-α administration induced expression of several factors involved in lung remodeling such as IL-1β and TNF-α [27]. In agreement with these previous studies, in the present study, HFD-feeding increased the expression levels of TNF-α, IL-1β, IL-6 after 6 weeks, which were accompanied by pulmonary histological abnormalities.

Recently, miRNAs have emerged as key regulators of several biological processes including inflammation along with intrinsic and acquired immunity [8, 29]. Among these small non-coding RNAs, miRNA-146a is an NF-κB-dependent miRNA which negatively regulates inflammatory response [26, 36]. Anti-inflammatory role of this miRNA has been suggested by previous studies [30, 39].

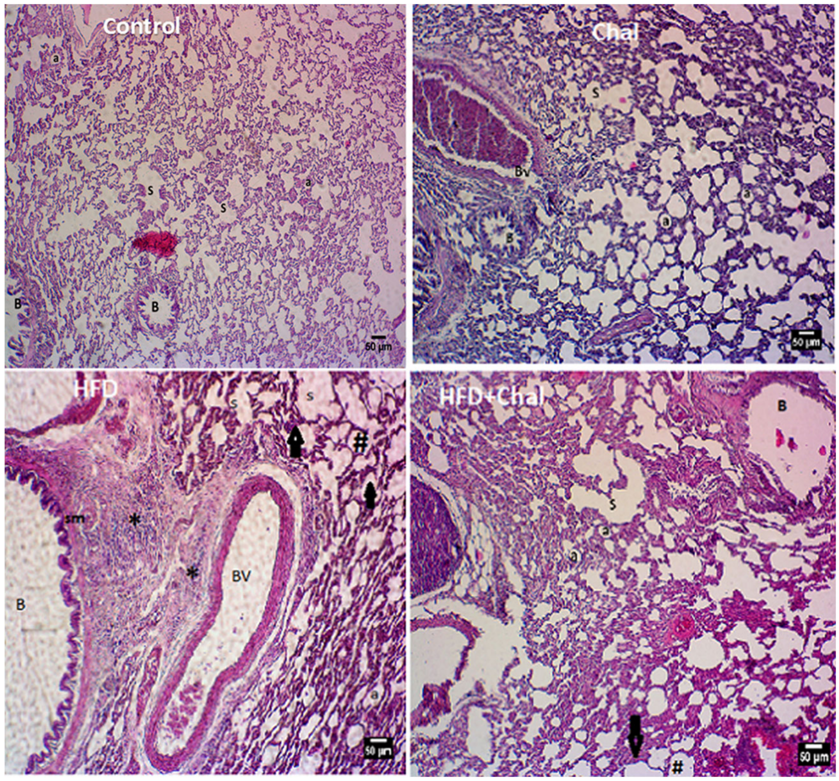

Fig. 3. Lung sections stained with hematoxylin and eosin in study groups (control, receiving 10% tween 80; Chal, receiving *trans*-chalcone; HFD, receiving the high-fat emulsion and 10% tween 80; HFD + Chal, receiving the high-fat emulsion and *trans*-chalcone). Lung tissue in HFD + Chal group showed normal thin interalveolar septa and blood vessels. Furthermore, lymphocyte infiltration (star), distension of the alveolar sacs (square) and thickening mucus in interalveolar septa (arrow) in the HFD + Chal group were decreased compared with the HFD. (a): alveoli, (s): alveolar sacs, (B): respiratory epithelium in bronchial, (sm): smooth muscle (BV), blood vessels bars = 50 μm.

Pro-inflammatory cytokines, such as TNF-α and IL-6, are known targets of miR-146a [10]. Wu et al. indicated that IL-6, an important cytokine that mediates inflammation, was a direct target of miR-146a and increased IL-6 expression was linked to reduced miR-146a expression during lung injury [38]. In agreement with the present study, it has recently been suggested that decreased inflammatory response in idiopathic pulmonary lung fibroblasts was correlated with reduced miR-146a expression [12]. Zheng et al. reported that LPS-induced lung injury was linked to up-regulation of miR-146a and pro-inflammatory cytokines including TNF-α, IL-6, IL-1ß. In their study, manipulation with miR-146a mimic markedly inhibited LPS-mediated TNF-α, IL-1β, and IL-6 production. Therefore, the up-regulation of miR-146a could inhibit inflammatory mediators in LPS induced-acute lung injury [40]. Taken together, these data strongly suggest that miR-146a displays an anti-inflammatory effect in various organs such as lung tissue. Accordingly, up-regulation of this miRNA was observed in adipose tissue following HFD feeding which was positively regulated by TNF-α [8, 19].

To the best of our knowledge, no specific effect of miR-146a in lung injury induced by HFD has been reported. Interestingly, in the present study, HFD feeding up-

Table 2. Histological findings for experimental groups

	Control	Chal	HFD	HFD + Chal
Lymphocyte infiltration	0 (0–0)	0 (0–1)	3 (2–3) **	1 (1–2) **##
Distention of alveolar sacs	0 (0–0)	0 (0–0)	0.5 (0–1)	0 (0–1)
Thickening mucus in interalveolar septa	0 (0–0)	0 (0–0)	1 (0–1) *	0 (0–1)

regulated the expression levels of miR-146a and pro-inflammatory cytokines including TNF-α, IL-6, IL-1ß in the lung. These results suggested that HFD-induced pulmonary inflammation in rats could be due to the local production of pro-inflammatory cytokines. Furthermore, in this study, treatment with *trans*-chalcone alleviated the pulmonary abnormalities in the HFD + Chal group which was evidenced by the improvement of pulmonary histological alterations and also down-regulation of TNF-α, IL-1β, IL-6, and miR-146a expressions.

Growing evidence has suggested that flavonoids, as a large group of polyphenolic compounds, have various biological effects and play preventive roles against several human diseases [18, 20]. Recently, it has been indicated that *trans*-chalcone protected the liver of HFD-fed rats against NASH development [16]. Anti-inflammatory effect of *trans*-chalcone was also reported in gout arthritis [35], hepatic injury [17, 33], and skin damage [25]. Accordingly, *trans*-chalcone ameliorated experimental gout arthritis in mice by mitigating oxidative stress and inflammation markers including NF-κB, TNF-α, and IL-1β [35]. The reduction of these cytokines was associated with the inhibition of neutrophil recruitment as a hallmark of the acute phase of inflammation [35]. Consistent with this study, a recent study suggested that elevation of miR-146a, an inhibitor of inflammation, was a compensatory response post the cerebral ischemia-reperfusion injury, as an inflammatory condition. In the mentioned study, antagonism of this miRNA promoted ischemia-reperfusion injury-induced activation of NF-κB signaling pathway [9]. Therefore, it seems that the elevation of miR-146a is a compensatory response to decrease inflammatory injury under HFD feeding condition. In this study, the anti-inflammatory effect of *trans*-chalcone was concomitant with the down-regulation of miR-146a in HFD-fed rats. To the best of our knowledge, this is the first study to prove that *trans*-chalcone administration alleviates the pulmonary inflammation. This effect is confirmed by preventing histological abnormalities and decreasing mRNA levels of TNF-α, IL-1β, IL-6, and miR-146a in the lung of HFD-fed rats. Certainly, more detailed molecular studies are needed to explore the detailed signaling pathways involved in the effects of different chalcone derivatives on HFD-induced pulmonary injury.

CONCLUSIONS

Trans-chalcone could alleviate HFD-induced pulmonary injury which was reflected by preventing histological abnormities and also by decreasing expression levels of miR-146a and pro-inflammatory inflammatory cytokines including TNF-α, IL-1β, and IL-6 in the lungs of HFD-fed rats.

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COMPLIANCE WITH ETHICAL STANDARDS

Conflict of Interest. The authors declare that they have no conflicts of interest.

REFERENCES

1. Akhavanakbari, G., B. Babapour, M.R. Alipour, R. Keyhanmanesh, M. Ahmadi, and M.R. Aslani. 2019. Effect of high fat diet on NF-κB microRNA146a negative feedback loop in ovalbumin-sensitized rats. *Biofactors* 45 (1): 75–84. https://doi.org/10.1002/biof.1466.
2. Aksöz, B.E., and R. Ertan. 2011. Chemical and structural properties of Chalcones I. *FABAD Journal of Pharmaceutical Sciences* 36: 223–242.
3. Alipoor, S.D., I.M. Adcock, J. Garssen, E. Mortaz, M. Varahram, M. Mirsaeidi, and A. Velayati. 2016. The roles of miRNAs as potential biomarkers in lung diseases. *European Journal of Pharmacology* 791: 395–404. https://doi.org/10.1016/j.ejphar.2016.09.015.
4. Aslani, M.R., R. Keyhanmanesh, A.M. Khamaneh, M.A. Ebrahimi Saadatlou, M. Mesgari Abbasi, and M.R. Alipour. 2016. Lung altered expression of IL-1beta mRNA and its signaling pathway molecules in obese-asthmatic male Wistar rats. *Iranian Journal of Allergy, Asthma and Immunology* 15 (3): 183–197.
5. Bartel, D.P. 2004. MicroRNAs: Genomics, biogenesis, mechanism, and function. *Cell* 116 (2): 281–297.
6. Broide, D. 2008. New perspectives on mechanisms underlying chronic allergic inflammation and asthma in 2007. *Journal of Allergy and Clinical Immunology* 122 (3): 475–480. https://doi.org/10.1016/j.jaci.2008.06.025.
7. Calixto, M.C., L. Lintomen, D.M. Andre, L.O. Leiria, D. Ferreira, C. Lellis-Santos, G.F. Anhe, S. Bordin, R.G. Landgraf, and E. Antunes. 2013. Metformin attenuates the exacerbation of the allergic eosinophilic inflammation in high fat-diet-induced obesity in mice. *PLoS One* 8 (10): e76786. https://doi.org/10.1371/journal.pone.0076786.
8. Chartoumpekis, D.V., A. Zaravinos, P.G. Ziros, R.P. Iskrenova, A.I. Psyrogiannis, V.E. Kyriazopoulou, and I.G. Habeos. 2012.

Differential expression of microRNAs in adipose tissue after long-term high-fat diet-induced obesity in mice. *PLoS One* 7 (4): e34872. https://doi.org/10.1371/journal.pone.0034872.

9. Chu, B., Y. Zhou, H. Zhai, L. Li, L. Sun, and Y. Li. 2018. The role of microRNA-146a in regulating the expression of IRAK1 in cerebral ischemia-reperfusion injury. *Canadian Journal of Physiology and Pharmacology* 96 (6): 611–617. https://doi.org/10.1139/cjpp-2017-0586.
10. Cornett, A.L., and C.S. Lutz. 2014. Regulation of COX-2 expression by miR-146a in lung cancer cells. *Rna* 20 (9): 1419–1430. https://doi.org/10.1261/rna.044149.113.
11. Dournes, G., and F. Laurent. 2012. Airway Remodelling in asthma and COPD: Findings, similarities, and differences using quantitative CT. *Pulmonary Medicine* 2012: 670414–670418. https://doi.org/10.1155/2012/670414.
12. Hadjicharalambous, M.R., B.T. Roux, C.A. Feghali-Bostwick, L.A. Murray, D.L. Clarke, and M.A. Lindsay. 2018. Long non-coding RNAs are central regulators of the IL-1beta-induced inflammatory response in Normal and idiopathic pulmonary lung fibroblasts. *Frontiers in Immunology* 9: 2906. https://doi.org/10.3389/fimmu.2018.02906.
13. Innis, S.M. 2007. Dietary lipids in early development: Relevance to obesity, immune and inflammatory disorders. *Current Opinion in Endocrinology, Diabetes and Obesity* 14 (5): 359–364. https://doi.org/10.1097/MED.0b013e3282be90b9.
14. Jalalvand, F., M.M. Amoli, P. Yaghmaei, M. Kimiagar, and A. Ebrahim-Habibi. 2015. Acarbose versus trans-chalcone: Comparing the effect of two glycosidase inhibitors on obese mice. *Archives of Endocrinology and Metabolism* 59 (3): 202–209. https://doi.org/10.1590/2359-3997000000038.
15. Karimi-Sales, E., S. Jeddi, A. Ebrahimi-Kalan, and M.R. Alipour. 2018. Trans-chalcone enhances insulin sensitivity through the miR-34a/SIRT1 pathway. *Iranian Journal of Basic Medical Sciences* 21 (4): 359–363. https://doi.org/10.22038/ijbms.2018.24300.6063.
16. Karimi-Sales, E., A. Ebrahimi-Kalan, and M.R. Alipour. 2019. Preventive effect of trans-chalcone on non-alcoholic steatohepatitis: Improvement of hepatic lipid metabolism. *Biomedicine & Pharmacotherapy* 109: 1306–1312. https://doi.org/10.1016/j.biopha.2018.10.196.
17. Karimi-Sales, E., S. Jeddi, A. Ebrahimi-Kalan, and M.R. Alipour. 2018. Trans-Chalcone prevents insulin resistance and hepatic inflammation and also promotes hepatic cholesterol efflux in high-fat diet-fed rats: Modulation of miR-34a-, miR-451-, and miR-33a-related pathways. *Food & Function* 9 (8): 4292–4298. https://doi.org/10.1039/c8fo00923f.
18. Karimi-Sales, E., G. Mohaddes, and M.R. Alipour. 2018. Chalcones as putative hepatoprotective agents: Preclinical evidence and molecular mechanisms. *Pharmacological Research* 129: 177–187. https://doi.org/10.1016/j.phrs.2017.11.022.
19. Karkeni, E., L. Bonnet, J. Marcotorchino, F. Tourniaire, J. Astier, J. Ye, and J.F. Landrier. 2018. Vitamin D limits inflammation-linked microRNA expression in adipocytes in vitro and in vivo: A new mechanism for the regulation of inflammation by vitamin D. *Epigenetics* 13 (2): 156–162. https://doi.org/10.1080/15592294.2016.1276681.
20. Kumar, S., and A.K. Pandey. 2013. Chemistry and Biological Activities of Flavonoids: An Overview. *The Scientific World Journal* 2013: 162750–162716. https://doi.org/10.1155/2013/162750.
21. Lu, F.L., R.A. Johnston, L. Flynt, T.A. Theman, R.D. Terry, I.N. Schwartzman, A. Lee, and S.A. Shore. 2006. Increased pulmonary responses to acute ozone exposure in obese db/db mice. *American Journal of Physiology-Lung Cellular and Molecular Physiology* 290 (5): L856–L865. https://doi.org/10.1152/ajplung.00386.2005.
22. Ludigs, K., V. Parfenov, R.A. Du Pasquier, and G. Guarda. 2012. Type I IFN-mediated regulation of IL-1 production in inflammatory disorders. *Cellular and Molecular Life Sciences* 69 (20): 3395–3418. https://doi.org/10.1007/s00018-012-0989-2.
23. Maddox, L., and D.A. Schwartz. 2002. The pathophysiology of asthma. *Annual Review of Medicine* 53: 477–498. https://doi.org/10.1146/annurev.med.53.082901.103921.
24. Martin, M., and K. Resch. 1988. Interleukin 1: More than a mediator between leukocytes. *Trends in Pharmacological Sciences* 9 (5): 171–177.
25. Martinez, R.M., F.A. Pinho-Ribeiro, D.L. Vale, V.S. Steffen, F.T.M.C. Vicentini, J.A. Vignoli, M.M. Baracat, S.R. Georgetti, W.A. Verri Jr., and R. Casagrande. 2017. Trans-chalcone added in topical formulation inhibits skin inflammation and oxidative stress in a model of ultraviolet B radiation skin damage in hairless mice. *Journal of Photochemistry and Photobiology. B, Biology* 171: 139–146. https://doi.org/10.1016/j.jphotobiol.2017.05.002.
26. Meisgen, F., N. Xu Landen, A. Wang, B. Rethi, C. Bouez, M. Zuccolo, A. Gueniche, et al. 2014. MiR-146a negatively regulates TLR2-induced inflammatory responses in keratinocytes. *The Journal of Investigative Dermatology* 134 (7): 1931–1940. https://doi.org/10.1038/jid.2014.89.
27. Naura, A.S., C.P. Hans, M. Zerfaoui, Y. Errami, J. Ju, H. Kim, K. Matrougui, J.G. Kim, and A.H. Boulares. 2009. High-fat diet induces lung remodeling in ApoE-deficient mice: An association with an increase in circulatory and lung inflammatory factors. *Laboratory investigation; a journal of technical methods and pathology* 89 (11): 1243–1251. https://doi.org/10.1038/labinvest.2009.98.
28. Nowakowska, Z. 2007. A review of anti-infective and anti-inflammatory chalcones. *European Journal of Medicinal Chemistry* 42 (2): 125–137.
29. Rodriguez, A., E. Vigorito, S. Clare, M.V. Warren, P. Couttet, D.R. Soond, S. van Dongen, R.J. Grocock, P.P. Das, E.A. Miska, D. Vetrie, K. Okkenhaug, A.J. Enright, G. Dougan, M. Turner, and A. Bradley. 2007. Requirement of bic/microRNA-155 for normal immune function. *Science* 316 (5824): 608–611. https://doi.org/10.1126/science.1139253.
30. Roos, J., E. Enlund, J.B. Funcke, D. Tews, K. Holzmann, K.M. Debatin, M. Wabitsch, and P. Fischer-Posovszky. 2016. miR-146a-mediated suppression of the inflammatory response in human adipocytes. *Scientific Reports* 6: 38339. https://doi.org/10.1038/srep38339.
31. Sato, T., H. Baskoro, S.I. Rennard, K. Seyama, and K. Takahashi. 2015. MicroRNAs as therapeutic targets in lung disease: Prospects and challenges. *Chronic Obstructive Pulmonary Diseases: Journal of the COPD Foundation* 3 (1): 382–388. https://doi.org/10.15326/jcopdf.3.1.2015.0160.
32. Sato, Tadashi, Xiangde Liu, Amy Nelson, Masanori Nakanishi, Nobuhiro Kanaji, Xingqi Wang, Miok Kim, Yingji Li, Jianhong Sun, Joel Michalski, Amol Patil, Hesham Basma, Olaf Holz, Helgo Magnussen, and Stephen I. Rennard. 2010. Reduced miR-146a increases prostaglandin E_2 in chronic obstructive pulmonary disease fibroblasts. *American Journal of Respiratory and Critical Care Medicine* 182 (8): 1020–1029. https://doi.org/10.1164/rccm.201001-0055OC.
33. Singh, H., S. Sidhu, K. Chopra, and M.U. Khan. 2016. Hepatoprotective effect of trans-Chalcone on experimentally induced hepatic injury in rats: Inhibition of hepatic inflammation and fibrosis. *Canadian Journal of Physiology and Pharmacology* 94 (8): 879–887. https://doi.org/10.1139/cjpp-2016-0071.

34. Song, Y., Y. Yu, D. Wang, S. Chai, D. Liu, X. Xiao, and Y. Huang. 2015. Maternal high-fat diet feeding during pregnancy and lactation augments lung inflammation and remodeling in the offspring. *Respiratory Physiology & Neurobiology* 207: 1–6. https://doi.org/10.1016/j.resp.2014.12.003.
35. Staurengo-Ferrari, L., K.W. Ruiz-Miyazawa, F.A. Pinho-Ribeiro, V. Fattori, T.H. Zaninelli, S. Badaro-Garcia, S.M. Borghi, T.T. Carvalho, J.C. Alves-Filho, T.M. Cunha, F.Q. Cunha, R. Casagrande, and W.A. Verri Jr. 2018. Trans-Chalcone attenuates pain and inflammation in experimental acute gout arthritis in mice. *Frontiers in Pharmacology* 9: 1123. https://doi.org/10.3389/fphar.2018.01123.
36. Taganov, K.D., M.P. Boldin, K.J. Chang, and D. Baltimore. 2006. NF-kappaB-dependent induction of microRNA miR-146, an inhibitor targeted to signaling proteins of innate immune responses. *Proceedings of the National Academy of Sciences of the United States of America* 103 (33): 12481–12486. https://doi.org/10.1073/pnas.0605298103.
37. Wang, F., Z. Zuo, K. Chen, J. Fang, H. Cui, G. Shu, Y. Zhou, Z. Chen, C. Huang, and W. Liu. 2018. Histopathological changes caused by inflammation and oxidative stress in diet-induced-obese mouse following experimental lung injury. *Scientific Reports* 8 (1): 14250. https://doi.org/10.1038/s41598-018-32420-3.
38. Wu, W., and Y. Li. 2018. Lung injury caused by paraquat poisoning results in increased interleukin-6 and decreased microRNA-146a levels. *Experimental and Therapeutic Medicine* 16 (1): 406–412. https://doi.org/10.3892/etm.2018.6153.
39. Ye, E., and J.S. Jena. 2016. miR-146a attenuates inflammatory pathways mediated by TLR4/NF-κB and TNFα to protect primary human retinal microvascular endothelial cells grown in high glucose. *Mediators of inflammation* 3958453: 1–9. https://doi.org/10.1155/2016/3958453.
40. Zeng, Z., H. Gong, Y. Li, K. Jie, C. Ding, Q. Shao, F. Liu, Y. Zhan, C. Nie, W. Zhu, and K. Qian. 2013. Upregulation of miR-146a contributes to the suppression of inflammatory responses in LPS-induced acute lung injury. *Experimental Lung Research* 39 (7): 275–282. https://doi.org/10.3109/01902148.2013.808285.
41. Zhang, Y., T. Xu, B. Wu, H. Chen, Z. Pan, Y. Huang, L. Mei, Y. Dai, X. Liu, X. Shan, and G. Liang. 2017. Targeting myeloid differentiation protein 2 by the new chalcone L2H21 protects LPS-induced acute lung injury. *Journal of Cellular and Molecular Medicine* 21 (4): 746–757. https://doi.org/10.1111/jcmm.13017.
42. Zou, Yuhong, Jun Li, Chao Lu, Jianqing Wang, Jinfang Ge, Yan Huang, Lei Zhang, and Yuanyuan Wang. 2006. High-fat emulsion-induced rat model of nonalcoholic steatohepatitis. *Life Sciences* 79 (11): 1100–1107.