Toxicology Research

REVIEW

View Article Online
View Journal | View Issue

Cite this: *Toxicol. Res.*, 2016, **5**, 731

The effects of T-2 toxin on the prevalence and development of Kashin–Beck disease in China: a meta-analysis and systematic review

Danyang Li,†[a] Jing Han,*†[a] Xiong Guo,[a] Chengjuan Qu,[b] Fangfang Yu[a] and Xiaofang Wu[a]

To reveal the influence of T-2 toxin detection rate and detection amount in food samples on Kashin–Beck disease (KBD), and define a linking mechanism between T-2 toxin induced chondrocytes or cartilage damage and KBD pathological changes, seven electronic databases were searched to obtain epidemiological and experimental studies. For epidemiological studies, subgroup analyses of the positive detection rate (PDR) of the T-2 toxin and PDR of the T-2 toxin with concentrations (PDRC of T-2) >100 ng g^{-1} were carried out, together with a histogram of the T-2 toxin concentrations in different food types in KBD and non-KBD areas. For experimental studies, a systematic review of a variety of chondrocyte and cartilage changes and damage induced by the T-2 toxin was performed. As a result, in epidemiological studies, meta-analysis demonstrated that the T-2 toxin PDR and the overall PDRC of T-2 toxin >100 ng g^{-1} showed a slightly significant increase in KBD areas than that in non-KBD areas separately. From the histogram, T-2 toxin accumulation was more serious in endemic areas, especially in wheat flour samples. In experimental studies, the T-2 toxin could induce damage of chondrocytes and cartilage, and inhibit cell proliferation by promoting apoptosis and catabolism as well as intracellular injuries, which is similar to the characteristics of KBD. In conclusion, the amount of T-2 toxin detected has a more significant influence on KBD prevalence and development as compared to the T-2 toxin detection rate. Besides, the T-2 toxin induces chondrocyte and cartilage damage through apoptosis, catabolism promotion and intracellular impairment, which is similar to the KBD change.

Received 14th October 2015,
Accepted 16th February 2016

DOI: 10.1039/c5tx00377f

www.rsc.org/toxicology

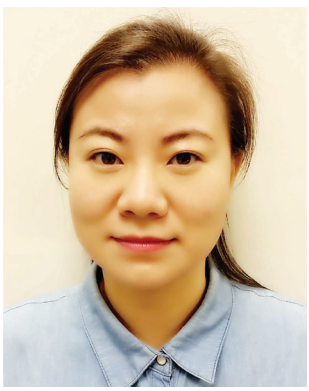
[a]*College of Public Health, Xi'an Jiaotong University Health Science Center, Xi'an, Shaanxi 710061, PR China. E-mail: bbbishop@126.com, 121819866@qq.com, guox@mail.xjtu.edu.cn, 844481760@qq.com, 1028294561@qq.com*
[b]*Department of Integrative Medical Biology, Umeå University, Umeå 90187, Sweden. E-mail: chengjuan.qu@gmail.com*
†These authors contributed equally to this work.

1 Introduction

T-2 toxin, a kind of trichothecene mycotoxin, is produced by the *Fusarium* fungus.[1] In 1968, the T-2 toxin was separated and purified for the first time by Bamburg *et al.*[2] With a wide range

Danyang Li

Danyang Li, Female, China. Postgraduate of the College of Public Health, Xi'an Jiaotong University Health Science Center. Research interests: epidemiology and pathogenesis of Kashin–Beck disease. E-mail address: 121819866@qq.com.



Jing Han

Jing Han, Female, China. Lecturer in the Department of Public Health and the Key Laboratory of Trace Elements and Endemic Diseases, Health Science Center, Xi'an Jiaotong University. Areas of research work include (but not limited to) pathogenesis of selenium deficiency and T-2 toxin in the occurrence and development of Kashin–Beck disease (KBD); selenium supplements for the prevention and treatment of KBD; scaffolds for cartilage and bone tissue remolding. E-mail address: bbbishop@126.com.

of distribution in many parts of the world,[3] the T-2 toxin can be detected in approximately 20% of the food samples from 12 European Union countries.[4] Meanwhile, it has been reported that the T-2 toxin was found in up to 65% of corn samples in New Zealand.[5] Dietary ingestion is claimed as the most common route for human exposure to the T-2 toxin. Moreover, T-2 toxin contamination shows no specificity to food samples, and can occur in a number of field crops (wheat, maize, barley and oats) and processed grains (malt, beer and bread).[1] The T-2 toxin is demonstrated to have a variety of toxic effects on both experimental animals and humans, including dermal toxicity, lethal effects with disruption of the central nervous system, inhibition of protein, DNA and RNA synthesis[6] as well as damage of chondrocytes and cartilage.

Kashin–Beck disease (KBD), an endemic, chronic and deformed osteoarthropathic disease, was first reported in 1849.[7] KBD mostly occurs from northeastern to southwestern China, south-eastern Siberia and North Korea.[8] In China, there are about 0.7 million patients and 105 million residents living in endemic areas that are at risk.[9] It is reported that KBD can affect the growth of articular cartilage, and further lead to apoptosis and necrosis of chondrocytes. The common syndromes of KBD are joint pain, stiffness in the morning, motion restriction of the elbow and finger joint, joint enlargement and joint space narrowing.[10] The etiology of KBD is still unclear. In China, the proposed risk factors include selenium deficiency, organic acid contamination in drinking water, and fungal contamination of staple grains.[11]

Previous epidemiological studies have confirmed that the concentration of the T-2 toxin in endemic food samples remains at a high level (2.0–1549.4 ng g^{-1}, with an average of 468.7 ng g^{-1}).[8] In addition, it is also reported that the pathologic changes of the cartilage in chicks fed with food containing the T-2 toxin are quite similar to KBD patients in animal studies.[8] However, it is still difficult to confirm that the T-2 toxin is one of the important etiological factors for KBD, because discrepancies exist in the detection rate and the amount of T-2 toxin detected from the staple food in KBD endemic and non-endemic areas (in China, national criteria of WS/T 207-2010 (http://www.moh.gov.cn/zwgkzt/s9500/201006/47920.shtml) and GB 16395-2011 (http://www.moh.gov.cn/zwgkzt/s9500/201207/55322.shtml) were applied for the diagnosis of KBD and the determination and classification of KBD endemic areas respectively). Since lots of experimental studies have been performed to investigate the mechanism of T-2 toxin in chondrocytes or cartilage damage at present, a comprehensive and systematic review is really needed for better understanding the effects of T-2 toxin on the prevalence and development of KBD.

Therefore, a meta-analysis and systematic review of the effects of T-2 toxin on the prevalence and development of KBD are carried out in the present study. This review will focus on the influence of T-2 toxin detection rate and detection amount in food samples on KBD prevalence and development, as well as the role of the T-2 toxin on chondrocyte or cartilage damage in human or animal subjects and its mechanisms.

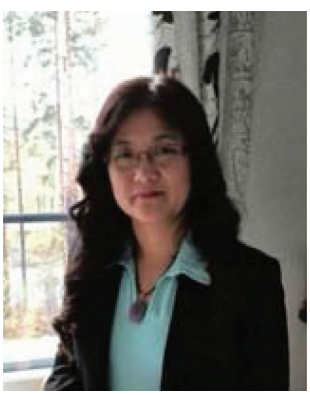
2 Materials and methods

2.1 Search strategy

With respect to the search strings: for epidemiological studies, search strings of "KBD" or "Kashin–Beck disease", "T-2 toxin" and "Endemic detection" were used; and for experimental studies, search strings of "cartilage" or "chondrocyte" and "T-2 toxin" were applied. Seven electronic databases: MEDLINE, Web of Knowledge, EMBASE, Google Scholar, CNKI (Chinese National Knowledge Infrastructure), CBM (Chinese Biomedical

Xiong Guo

Xiong Guo, Male, China. Professor in the Department of Public Health, and director of the Key Laboratory of Trace Elements and Endemic Diseases, Health Science Center, Xi'an Jiaotong University. Research interests: clinical diagnosis, molecular pathogenesis and prevention of Kashin–Beck disease (KBD), the relationship between low Se as well as other elements, toxins and KBD, and the prevention effect of the supplement Se on KBD; chondrocyte apoptosis, cell differentiation, environmental factors and gene interaction, differentially expressed proteins for KBD. E-mail address: guox@mail.xjtu.edu.cn.



Chengjuan Qu

Chengjuan Qu, Female, Finland. Department of Integrative Medical Biology, Umeå University, Umeå, Sweden. A senior researcher with more than 10-year experience in cartilage tissue engineering with primary chondrocytes, and mesenchymal and pluripotent stem cells. Teaching experience in anatomy and stem cell biology. Docent in Tissue Engineering, Department of Applied Physics, the University of Eastern Finland, Kuopio, Finland. Doctor's thesis on Articular Cartilage Proteoglycan and Biosynthesis, Department of Anatomy, the University of Eastern Finland, Kuopio, Finland. Eight-year clinical experience in diagnosis and examination in Radiology, Tianjin, China. E-mail address: chengjuan.qu@gmail.com.

Literature Database), and the Wan Fang database were used independently for the search process together with other relevant published studies. There were no restrictions on the language, date, design and publication of the studies. The last update search was conducted on May 29th, 2015.

2.2 Included/excluded criteria

All studies following the search strategy could be divided into epidemiological studies and experimental studies and both of them could be initially included in this article if: (1) they were written in English or Chinese; (2) they had original data and results; (3) for epidemiological studies, they should be related to KBD and the T-2 toxin, the specimens should be food samples, positive detection rates (PDRs) or average content of T-2 toxin should be obtained from KBD endemic and non-endemic areas (intervention and control groups) without any other interventions; (4) for experimental studies, they should address only the effect of T-2 toxin on chondrocyte or cartilage damage, and the research studies on T-2 toxin plus other interventions would be excluded. Studies would be excluded if they failed to meet any one of the criteria.

2.3 Study selection

Firstly, all included titles were screened by three reviewers (LDY, HJ and YFF) in order to remove duplicate studies. Then the abstracts of the selected studies were reviewed if they met the selection criteria. Any articles that did not match the standards were excluded. And after full-text articles were assessed for eligibility, some of them were eliminated because of data duplication or nonconformity to the criteria.

2.4 Methodical evaluation

For the epidemiological studies, after being carefully reviewed, all the included studies were found to be cross-sectional studies. Thus the AHRQ (Agency for Healthcare Research and Quality) standard[12] was applied for assessing the studies. According to the standard, 11 items (Table 1) were evaluated by answering with "Yes", "No" or "Unclear" respectively, including the source of information, the character of the subjects, the quality assessment of the articles and so on.

Experimental studies were divided into *in vitro* studies and *in vivo* studies. Due to the lack of an agreed evaluation standard at present, the "Evidence Pyramid"[13] and the grading system of the previous studies[14,15] were used. For the *in vitro* studies, the articles were evaluated according to the following standards: (A) systematic reviews (including meta-analyses) of studies *in vitro*; (B) with comparable baseline; (C) baseline unknown; and (D) no comparable baseline. For the *in vivo* studies, the evaluation standards used were the following: (A) systematic reviews (including meta-analyses) of studies in animals; (B) randomized controlled studies, or inbred animal studies; (C) controlled studies; and (D) non-controlled studies.

2.5 Data extraction and collection

For the epidemiological studies, data were extracted from cross-sectional studies after all the selected articles had been reviewed, including study design, location, total number of food samples, types of investigated food in each area, the number of samples with detectable T-2 toxin, T-2 toxin content >100 ng g^{-1} and the distribution (*i.e.*, medians, means) of T-2 toxin in different types of food samples.

For the experimental studies, because of the heterogeneity across the data, descriptive methods and data extraction tables were used for extracting experimental data from every study following PICO (P: sources, I: interventions, C: control study, O: outcomes) standards. Data extraction was performed by two independent reviewers (LDY and HJ); any disagreement was resolved by consensus.

2.6 Data analysis

In epidemiological studies of selected cross-sectional articles, meta-analysis (subgroup analysis) of the PDR of T-2 toxin and PDR of T-2 toxin with concentrations (PDRC of T-2 toxin) >100 ng g^{-1} in KBD and non-KBD areas was performed according to food types by using Stata 12.0, the relative risks (RRs) with 95% confidence intervals (CIs) were estimated. The heterogeneity was quantified by the I^2 statistic among different studies.

Fangfang Yu

Fangfang Yu, Male, China. PhD candidate in occupational and environmental health at Xi'an Jiaotong University. Research interests: epidemiology and pathogenesis of Kashin–Beck disease. E-mail address: 844481760@qq.com

Xiaofang Wu

Xiaofang Wu, female, China. Postgraduate of Xi'an Jiaotong University. Major in public health. Research interest is cell toxicity. E-mail address: 1028294561@qq.com.

Table 1 Methodological quality of cross-sectional studies according to the AHRQ standard

	Luo *et al.* 1992[17]	Yang *et al.* 1995[18]	Sun *et al.* 1997[19]	Feng *et al.* 2004[20]	Liu *et al.* 2004[21]	Bao *et al.* 2005[22]	Sun *et al.* 2012[23]
(1) Define the source of information (survey, record review)	Y	Y	Y	Y	Y	Y	Y
(2) List inclusion and exclusion criteria for exposed and unexposed subjects (cases and controls) or refer to previous publications	Y	Y	Y	Y	Y	Y	Y
(3) Indicate time period used for identifying patients	Y	Y	Y	U	Y	Y	Y
(4) Indicate whether or not subjects were consecutive if not population-based	Y	Y	Y	Y	Y	Y	Y
(5) Indicate if evaluators of subjective components of study were masked to other aspects of the status of the participants	U	U	U	U	U	U	U
(6) Describe any assessments undertaken for quality assurance purposes (*e.g.*, test/retest of primary outcome measurements)	U	Y	Y	Y	U	U	U
(7) Explain any patient exclusions from analysis	U	U	U	U	U	U	U
(8) Describe how confounding was assessed and/or controlled	U	U	U	U	U	U	Y
(9) If applicable, explain how missing data were handled in the analysis	U	U	U	U	U	U	U
(10) Summarize patient response rates and completeness of data collection	Y	Y	Y	Y	Y	Y	Y
(11) Clarify what follow-up, if any, was expected and the percentage of patients for which incomplete data or follow-up was obtained	U	U	U	U	U	U	U

AHRQ: Agency for Healthcare Research and Quality; Y: yes; U: unclear.

A "Fixed-effect" model was used when the heterogeneity was statistically insignificant, otherwise a "Random-effect" model was used (when $P < 0.05$) to pool RRs. Low, moderate and high heterogeneity were considered when I^2 = 25%, 50%, 75% separately. In addition, a histogram of the T-2 toxin concentrations in various food types from endemic and non-endemic regions was shown by using Microsoft Excel 2003.

In the experimental studies, we reviewed the effects of T-2 toxin on chondrocytes and cartilage from humans and animals. In *in vitro* studies, the discrepancies of the morphological and ultrastructural changes of chondrocytes, cell viability and proliferative activity discrepancies, as well as the metabolism, apoptosis of chondrocytes and other changes in chondrocytes were estimated. Furthermore, the morphological and radiological changes of chondrocytes and cartilage, intracellular changes of chondrocytes and metabolism of the extracellular matrix in cartilage were investigated as well. The supposed toxic mechanism of the T-2 toxin on the prevalence and development of KBD, including chondrocytes and cartilage damage through apoptosis, catabolism promotion and intracellular impairment, was proposed by drawing a conclusion from the extracted data.

3 Results

3.1 Search results and study quality

A total of 1999 citations were initially included in this article. After the titles or abstracts were reviewed, 82 articles were enrolled for full text reviewing. Finally, 72 articles were selected and assessed against the exclusion criteria, including seven epidemiological articles and 65 experimental articles [33 *in vitro* studies and 33 *in vivo* studies (one article covers both the *in vitro* and *in vivo* studies)[16]] (Fig. 1).

The methodological quality of all included cross-sectional studies of the epidemiological studies were basically in accordance with the selection requirements, as most of the studies were assessed as having five or six "Yes" answers to the items of the AHRQ standard (Table 1). Meanwhile, for experimental studies, all the *in vitro* studies were evaluated as grade B with a comparable baseline according to the previously mentioned criteria. Additionally, 29 of the *in vivo* studies were randomized controlled studies (RCTs), and four were controlled studies.

3.2 Accumulation of T-2 toxin in food samples of epidemiological studies

3.2.1 Characteristics of epidemiological studies. The characteristics of all included 15 epidemiological studies in seven articles[17–23] are shown in Table 2. Most of the investigations were performed from 1990 to 2010 in the Northwest and Northeast of China. Four kinds of food including wheat flour (six studies), wheat (two studies), corn flour (five studies) and rice (two studies), were investigated in these studies. Ten food studies showed the results of the PDR of the T-2 toxin with a maximum rate of 100% in five KBD and one non-KBD areas.[19,22] The highest content of T-2 toxin in the average of wheat flour samples in endemic regions was 468.7 ng g^{-1} [23] and 152.1 ng g^{-1} in the control regions,[19] respectively.

3.2.2 Meta-analysis of PDR of T-2 toxin in epidemiological studies. Subgroup analysis of eight studies in five articles[18–22] was pooled to measure the difference of PDR of the T-2 toxin between endemic and normal areas (Fig. 2). The heterogeneity of the studies was examined with the "Fixed-effect model", which showed no statistically significant differences in the heterogeneity of the studies within the different subgroups (overall: $P = 0.795$, $I^2 = 0.0\%$; wheat flour: $P = 0.671$, $I^2 = 0.0\%$; corn flour: $P = 0.494$, $I^2 = 0.0\%$; rice: only one study). The

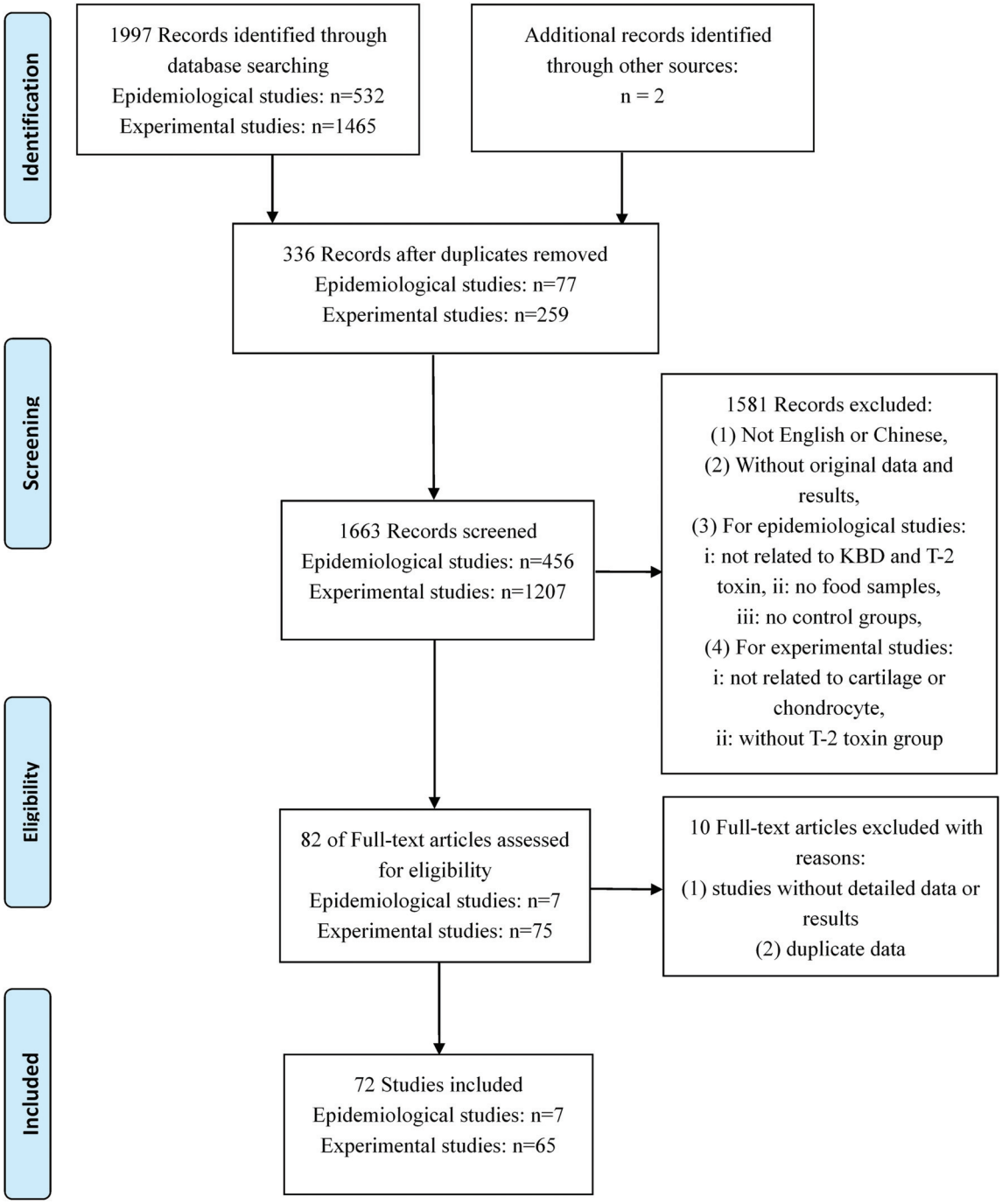


Fig. 1 Flow chart of the study selection process.

overall PDR of the T-2 toxin in endemic regions was slightly higher than that in control regions [Pooled RR = 1.27, 95% CI (1.10, 1.46)] indicating a significant difference in efficacy (Z = 3.26, P = 0.001). In addition, the T-2 toxin detection rate in wheat flour was a bit higher in KBD areas than that in control areas, but no obvious differences were observed in the T-2 toxin detection rate in the corn flour or rice in KBD areas when compared with that in control areas [wheat flour: RR = 1.26, 95% CI (1.08, 1.46); corn flour: RR = 1.37, 95% CI (0.97, 1.93); rice: RR = 0.36, 95% CI (0.02, 5.30)]. Furthermore, the efficacy showed a significant difference in wheat flour between KBD areas and control areas (wheat flour: Z = 3.03, P = 0.002; corn flour: Z = 1.81, P = 0.070; rice: Z = 0.74, P = 0.459).

3.2.3 Meta-analysis of PDRC of T-2 toxin >100 ng g^{-1} in epidemiological studies. A total of four studies in three articles[20,21,23] were included for assessing the PDRC of T-2 toxin >100 ng g^{-1} in different subgroups for meta-analysis (Fig. 3). Since the heterogeneity of studies was insignificant

Table 2 Baseline characteristics of included cross-sectional studies of T-2 toxin exposure in food samples

			Endemic areas					Non-endemic areas				
			Number of samples			T-2 toxin (ng g^{-1})		Number of samples			T-2 toxin (ng g^{-1})	
Ref.	Sites	Food type	Total	Positive	PDR (%)	PDRC > 100	Average	Total	Positive	PDR (%)	PDRC > 100	Average
Luo *et al.* 1992[17]	Xi'an city, Shanxi, Shandong, Jilin, Qinghai and Neimenggu provinces	Wheat	16	0	0	—	—	7	0	0	—	—
		Corn flour	67	0	0	—	—	10	—	—	—	—
Yang *et al.* 1995[18]	Sichuan and Shaanxi provinces	Wheat flour	15	10	66.67	8	468.7	15	10	66.67	3	84.2
		Corn flour	8	4	50	3	276.3	7	4	57.14	0	23.9
		Rice	3	0	0	—	0	15	5	33.33	—	3.1
Sun *et al.* 1997[19]	Fuyu and Shuangcheng counties	Wheat flour	10	10	100	—	278.4	5	5	100	—	40.3
		Corn flour	5	5	100	—	122.0	5	4	80	—	152.1
Feng *et al.* 2004[20]	Heilongjiang province and Fuyu village	Wheat flour	27	21	77.78	7	120.64	130	80	61.53	8	58.74
		Corn flour	25	13	52.00	—	23.73	130	43	33.07	—	30.41
		Rice	130	9	6.92	—	17.2	—	—	—	—	—
Liu *et al.* 2004[21]	Fengtian and Linmao villages, North East and North China areas	Wheat flour	27	21	77.78	8	120.64	130	80	61.53	12	58.74
		Corn flour	25	13	52.00	—	23.73	—	—	—	—	—
Bao *et al.* 2005[22]	Nenjiang county and Shitougou village	Wheat flour	16	16	100	—	8.58	15	10	66.67	—	84.2
Sun *et al.* 2012[23]	Xinghai and Tongde counties	Wheat flour	171	171	100	19	47.47	30	—	—	—	12.23
		Wheat	153	153	100	41	78.91	—	—	—	—	—

PDR: positive detection rate; PDRC: positive detection rate of concentrations.

within different subgroups (overall: $P = 0.900$, $I^2 = 0.0\%$; wheat flour: $P = 0.815$, $I^2 = 0.0\%$; corn flour: only one study), the "Fixed-effect model" was applied. The overall PDRC of T-2 toxin >100 ng g^{-1} was much higher in KBD areas than that in normal areas with pooled RR = 3.472, 95% CI (2.045, 5.895), which indicated a significant difference in efficacy ($Z = 4.61$, $P < 0.001$), meanwhile, the PDRC of the T-2 toxin >100 ng g^{-1} was significantly higher in wheat flour than that in corn flour between endemic regions and non-endemic regions [wheat flour: RR = 3.32, 95% CI (1.95, 5.66); corn flour: RR = 6.22, 95% CI (0.38, 102.93)] with a significant difference in efficacy (wheat flour: $Z = 4.43$, $P < 0.001$; corn flour: $Z = 1.28$, $P = 0.202$).

3.2.4 Difference of T-2 toxin average contents in epidemiological studies. The differences of T-2 toxin contents in different groups were compared with a histogram made from the nine studies in six articles (Fig. 4).[18–23] Almost in every study, the average contents of T-2 toxin were much higher in endemic areas than that in normal areas. According to the Food and Agriculture Organization (FAO) standard related to food contamination with the T-2 toxin (the maximum detection of T-2 toxin <100 ng g^{-1}),[24] the average contents of the T-2 toxin in five studies were above 100 ng g^{-1} (three wheat flour samples and two corn flour samples in endemic areas, and one corn flour sample in a non-endemic area) in all nine studies. More seriously, the average contents of the T-2 toxin in three food samples (two wheat flour samples and one corn flour sample) from endemic areas were more than 200 ng g^{-1},[18,19] which exceeded the human tolerance per day based on the standard.[25] The T-2 toxin contamination in food samples, especially in the wheat flour samples was obviously existent in the endemic areas.

3.3 Effects of T-2 toxin on chondrocytes or cartilage in experimental studies

3.3.1 Effects of T-2 toxin on chondrocytes in *in vitro* studies

Morphological observations of chondrocyte damage and cell proliferation. A total of 12 *in vitro* studies[26–37] were involved in the assessment of the damage effects of the T-2 toxin on chondrocyte morphology. As shown in Table 3, the T-2 toxin in different doses could induce the damage of the cell structure in the human fetus, Wistar rats and rabbits with a decrease in cell density and increase of cell separation, and incomplete cytomembrane when observed by an inverted/light microscope. Scanning electron microscopy (SEM) images showed that collagen microfibrils and cytoskeleton were decreased in chondrocytes from a chicken embryo treated with the T-2 toxin. Furthermore, the results of transmission electron microscopy showed that the nucleus, cytoplasmic and endoplasmic reticulum damage could be found in most chondrocytes of the human fetus, Wistar rat and rabbit after the co-culturing of chondrocytes with different doses of T-2 toxin for 4–5 days. Membrane damage could also be detected in rabbit and chicken chondrocytes from these three studies.[33–35] The same inhibitory effect on the cell viability and proliferative

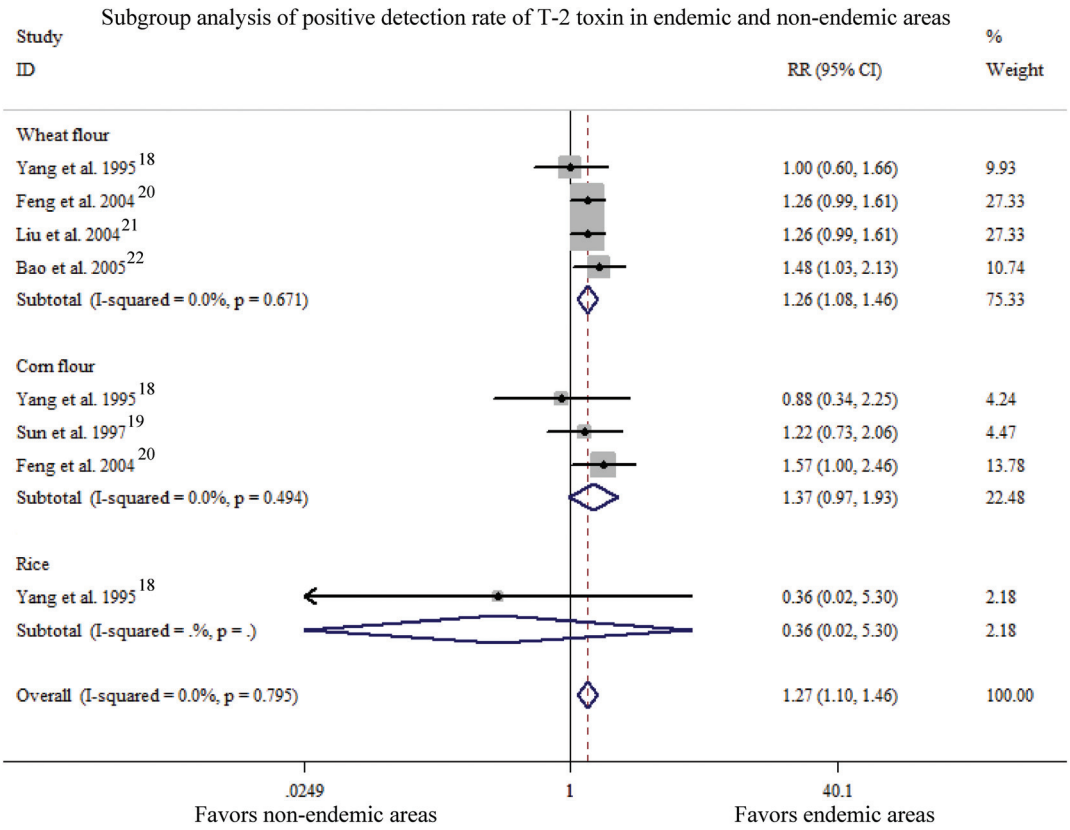


Fig. 2 Subgroup analysis of the positive detection rate of the T-2 toxin in endemic and non-endemic areas.

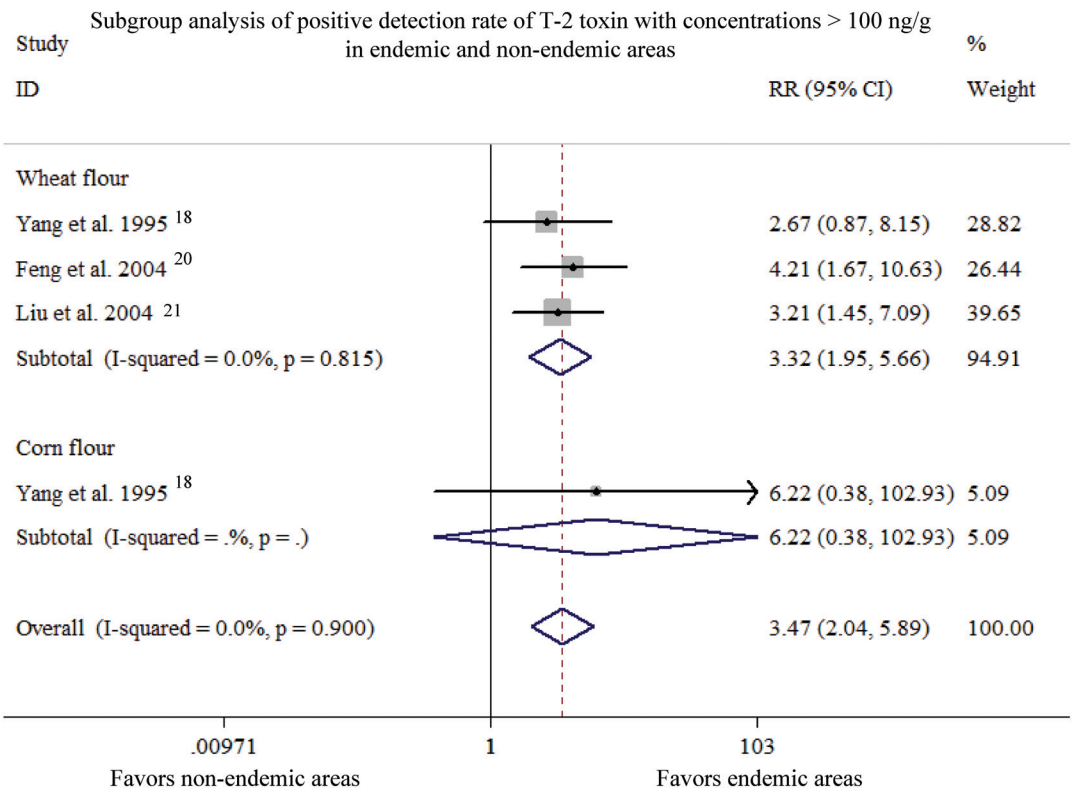


Fig. 3 Subgroup analysis of the positive detection rate of the T-2 toxin with concentrations >100 ng g^{-1} in endemic and non-endemic areas.

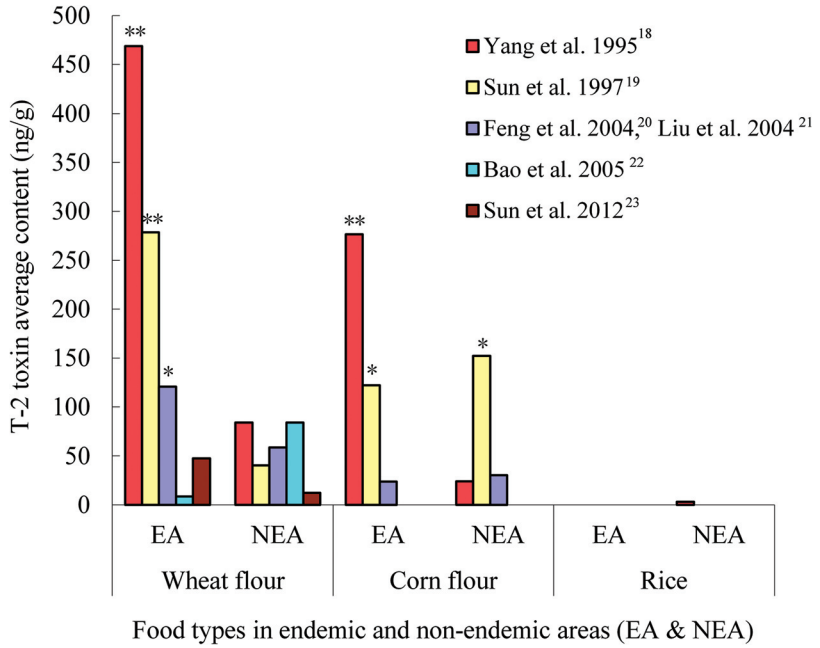


Fig. 4 Histogram of the T-2 toxin content in endemic and non-endemic areas (EA: endemic areas; NEA: non-endemic areas; *: T-2 toxin average content >100 ng g^{-1}; **: T-2 toxin average content >200 ng g^{-1}).

activity of chondrocytes could be seen in the 14 *in vitro* studies (Table 4).[27,28,38–49] This effect was independent of the concentration of the T-2 toxin.

Apoptosis of chondrocytes. The results of 10 studies[26–28,39,40,42,44,46,50,51] were included in the analysis of apoptosis of chondrocytes, and shown in Table 5. In less than five days of T-2 toxin intervention, the apoptotic rate of chondrocytes in humans, human fetus and broiler chicken was significantly increased in a concentration-dependent manner, when analyzed by flow cytometry (FCM) analysis. The mRNA and protein levels of Fas and p53 were increased in human or human fetus chondrocytes after being treated with T-2 toxin. In the Bcl-2 family, Bax mRNA and protein expression were up-regulated, whereas Bcl-xL expression was down-regulated after treatment with the T-2 toxin. The ratio of Bcl-2/Bcl-xL at the protein level was consistent in different studies. Moreover, both caspase-9 and caspase-3 at the protein and mRNA levels increased after T-2 toxin treatment. In addition, JNK, p38 and mitochondrial pathways were involved in mediating the apoptosis by the T-2 toxin.

Metabolism of chondrocytes. The metabolic inhibition of T-2 toxin-treated chondrocytes was found in the 13 *in vitro* studies (Table 6).[16,29,38,41,43,45–47,49,52–55] After T-2 toxin intervention, the expression of matrix metalloproteinases (MMPs, MMP-1, 3, 13) at the gene and protein levels, aggrecanase-1, 2 mRNAs and a disintegrin and metalloproteinase with thrombospondin motifs 4, 5 (ADAMTS 4, 5) proteins, and pro-inflammatory factors such as IL-1β, IL-6 and TNF-α were increased. Meanwhile, tissue inhibitors of metalloproteinase 1–3 (TIMP 1–3), alpha-2-Macroglobulin (α_2M), collagens (total collagen, type I, II, IX), proteoglycan (PG) and aggrecan were reduced both at the protein and mRNA levels, while collagen X expression at the mRNA and protein levels was still controversial. Additionally, other factors such as CD44, hyaluronan synthetase 2 (HAS2) and integrins at the mRNA and protein levels were also changed.

Other intracellular changes in chondrocytes (Table 7). Alteration of DNA and proteins. A total of four studies[29,30,32,56] related to DNA and protein alteration showed that the T-2 toxin caused DNA damage and the content reduction of DNA, matrix proteins and glucuronic acid (GLcUA) in a concentration-dependent manner (Table 7).

Mitochondrial damage. All four *in vitro* studies[35–37,40] referred to the damage of mitochondria and showed that the T-2 toxin destroyed the antioxidant defense system, including the inhibition of glutathione peroxidase (GPx) activity and intracellular glutathione (GSH) content. The T-2 toxin increased the reactive oxygen species (ROS), but reduced the levels of mitochondrial transmembrane potential ($\Delta\Psi_m$) and cellular adenosine triphosphate (ATP) in a dose-dependent manner. Furthermore, the activities of complexes III–V, H^+-ATP enzyme and cytochrome C oxidase rather than complexes I, II, citrate synthase and succinate dehydrogenase were restrained by the T-2 toxin in chondrocytes from the human and chick embryo.

Oxidative stress. The two studies[46,49] related to oxidative stress indicated that the levels of ROS and malondialdehyde (MDA) were increased after exposure to the T-2 toxin, while the activities of alkaline phosphatase (ALP) and GSH were decreased. Simultaneously, up-regulated activities of catalase (CAT) and superoxide dismutase (SOD) (two important anti-oxidases) by the T-2 toxin were observed.

Table 3 Morphological damage in chondrocytes

		Interventions		Outcomes						
						TEM (Damage of)				
Ref.	Sources	T-2 toxin	Time	LM	SEM	Membrane	Mitochondria	Endoplasmic reticulum	Nucleus	Cytoplasm
Chen *et al*. 2005[26]	Human fetus	1, 10, 20 μg L^{-1}	5 d				Y	Y	Y	Y
Chen *et al*. 2006[27]	Human fetus	10 ng mL^{-1}	5 d						Y	Y
Chen *et al*. 2006[28]	Human fetus	1, 10, 20 ng mL^{-1}	5 d				Y	Y	Y	Y
Li *et al*. 2008[29]	Human fetus	0.01 μg mL^{-1}	18 d	Nucleus fragmentation↑, integral cytomembrane↓, cell ghosts↑						
Huo *et al*. 1998[30]	Wistar rat	0.0005, 0.001, 0.005 mg L^{-1}	2 d, 4 d	Cell density↓				Y	Y	Y
Wang *et al*. 2005[31]	Wistar rat	0.5, 1.0 μg L^{-1}	1 d	Cell falls off↑			Y	Y	Y	Y
Cao *et al*. 1994[32]	Rabbit	0.005, 0.01, 0.02 μg mL^{-1}	2 d, 4 d	Cell density↓, cell falls off↑						
Cao *et al*. 1995[33]	Rabbit	0.005, 0.01, 0.02 μg mL^{-1}	4 d	Cell proliferation↓, cell density↓		Y	Y	Y	Y	Y
Cao *et al*. 1995[34]	Rabbit	0.005, 0.0 l, 0.02 μg mL^{-1}	4 d	Cell density↓, cytoplasmic granules↑, irregular cells↑		Y		Y	Y	Y
Li *et al*. 1993[35]	Chick embryo	0.01 ppm	5 d		Collagen microfibrils↓, cytoskeleton↓	Y				
Li *et al*. 1993[36]	Chick embryo	0.01, 0.04 ppm	4 d		Collagen microfibrils↓, cytoskeleton↓					
Lin *et al*. 1994[37]	Chick embryo	0.01, 0.04 ppm	4 d		Collagen microfibrils↓, cytoskeleton↓					

Y: yes; ↑: increased; ↓: decreased; LM: light microscope; SEM: scanning electron microscope; TEM: transmission electron microscope. Membrane: segmental defects and membrane protein particle reduction. Mitochondria: vacuolar degeneration, medullary change, and cristae fracture. Endoplasmic reticulum: cystic dilatation. Nucleus: nuclear condensation, nuclear membrane thickening, defect, and uneven distribution of chromatin. Cytoplasm: the number of organelle reduction and fuzzy, the number of cytoplasmic lysosomes, vacuoles, medullary structure increase, some constituents in the cytoplasmic dissolution.

Table 4 Cell viability and proliferative activity of chondrocytes

		Interventions		Outcomes	
Ref.	Sources	T-2 toxin	Time	Cell viability (MTT assay)	Proliferation (Cell counting)
Wang *et al.* 2012[38]	Human (C28/I2)	1.5625–400 ng ml^{-1}	2–5 d	↓	
Han *et al.* 2013[39]	Human	1–500 ng ml^{-1}	2–5 d	↓	
Liu *et al.* 2014[40]	Human	1–100 ng ml^{-1}	3–5 d	↓	
Yang *et al.* 2001[41]	Human fetus	1–8 μg l^{-1}	3–7 d		↓
Yang *et al.* 2001[42]	Human fetus	5, 10, 20, 40 μg l^{-1}	3–7 d		↓
Chen *et al.* 2006[27]	Human fetus	1, 10, 20 ng ml^{-1}	3–5 d	↓	
Chen *et al.* 2006[43]	Human fetus	0.001–8 mg l^{-1}	3–5 d	↓	
Chen *et al.* 2006[28]	Human fetus	1–8000 ng ml^{-1}	2–5 d	↓	
Chen *et al.* 2008[44]	Human fetus	1–8000 ng ml^{-1}	3–5 d	↓	
Chen *et al.* 2011[45]	Human fetus	1–8000 ng ml^{-1}	3–5 d	↓	
He *et al.* 2011[46]	Broiler chicken	10, 100, 1000 nm/5, 50, 500, 5000 nmol l^{-1}	3, 6, 9 d/48, 72 h	↓	
Liu *et al.* 2008[47]	Zelanian rabbit	1, 10, 20, 100 μg l^{-1}	1–5 d	↓	
Liu *et al.* 2011[48]	Zelanian rabbit	1, 10, 20, 100 μg l^{-1}	1–5 d	↓	
Tian *et al.* 2012[49]	Murine (ATDC5)	10, 20, 40, 80 μg l^{-1}	6, 12, 24 h	↓	

↓: Decreased.

Nitric oxide (NO) synthesis. As shown in studies by Yang *et al.* and Chen *et al.*,[28,57] NO was increased in a time-dependent manner after the exposure to the T-2 toxin. The expression of inducible nitric oxide synthase (iNOS) also had a significant promotion when treated with the T-2 toxin.

3.3.2 Effects of T-2 toxin on cartilage in *in vivo* studies

Morphological observation in cartilage. Morphological changes in cartilage after T-2 toxin treatment were investigated in 25 *in vivo* studies (Table 8),[58–82] which were mainly histological and radiological changes. The histological changes included: damage of the epiphyseal growth plate, articular cartilage and chondrocyte necrosis in cartilage after 7-day exposure to T-2 toxin, which could be classified as a short term toxic effect of the T-2 toxin; and the injury of the epiphyseal growth plate, articular cartilage and chondrocyte in 1–6 months, which would be the consequences of the subchronic toxicity effect of the T-2 toxin. However, no effects of T-2 toxin treatment have been found in two studies, which showed no damage on the epiphyseal growth plate after T-2 toxin treatment.[73,78] In addition, a study by Pang *et al.*[72] reported a reduction of the bone mineralization rate after 4 week exposure to the T-2 toxin in SD rats' cartilage. On the other hand, the radiological changes involved in all four studies[66,68–70] showed that T-2 toxin treatment caused significant damage of the epiphyseal growth plate in the cartilage of Wistar rats after eight weeks exposure.

Intracellular changes in cartilage (Table 9). Cell growth and metabolism. The inhibition effects of the T-2 toxin on cell growth and metabolism[79,80,83,84] were confirmed in four studies (Table 9). The contents of DNA and protein were decreased by 100 μg per kg BW per d T-2 toxin exposure for 5 or 8 weeks. During the exposure of 1.0 mg per kg BW per d T-2 toxin for 1 week on the cartilage of chicks, DNA fragmentation was increased. However, the results were still controversial, and the studies by Sun and Sun *et al.*[79,83] showed an insignificant change of DNA fragmentation after five or eight weeks of 100 μg per kg BW per d T-2 toxin intervention.

Oxidative stress. As shown in Table 9, oxidative stress response was changed with an increase of MDA and thiobarbituric acid reactive substance (TBARS) contents in the cartilage of SD rats fed with 100, 200 ng per g BW per d T-2 toxin in four weeks. Glutathione peroxidase (GSH-Px), glutathione peroxidase (GPX), SOD and CAT at the protein and mRNA levels were decreased.

Apoptosis. With 200 ng per g BW per d T-2 toxin treatment for 30 days, Bax (an apoptosis regulator) at mRNA levels was up-regulated, whereas Bcl-2, as an anti-apoptotic protein was down-regulated. The expression of p53 and caspase-3 was increased in the costal cartilage of SD rats after T-2 toxin treatment.

Metabolism of the extracellular matrix in cartilage. Changes in the cartilage matrix metabolism[16,59–64,68,73,75,76,81,88,89] induced by the T-2 toxin are listed in Table 10. In SD rat cartilage, the T-2 toxin at a concentration of 100–200 ng per g BW per d promoted the expression of MMP-13, IL-6, IL-lβ and TNF-α in four weeks. In cartilage from Wistar rats, different doses of the T-2 toxin significantly decreased the total collagen at the beginning of the first week. Meanwhile, changes of collagens with the increase, breakage and desquamation of collagen fibers were observed in the cartilage from Wistar rats after 6 months, but fibrils appeared at 3 months for SD rats. Furthermore, type II collagen was reduced, while type I collagen was increased in the cartilage ECM of chicks when exposed to 100–600 μg per g BW per d T-2 toxin. Proteoglycan and its components (sulfate groups, hexosamine and glucuronic acid) were decreased in the cartilage of Wistar rats after 3–6 months of T-2 toxin intervention. Similarly in the cartilage from SD rats and chicks fed with T-2 toxin, total PG, sulfated glycosaminoglycan (sGAG), keratan sulfate and chondroitin sulfate were also decreased in 4–9 weeks.

Table 5 Apoptosis in chondrocytes

		Interventions		Outcomes				
Ref.	Sources	T-2 toxin	Time	Apoptosis (FCM)	Fas, P53	Bcl-2 family	Caspases	Others
Yang *et al.* 2001[42]	Human fetus	5, 10, 20, 40 μg l^{-1}	16 h	Y				Apoptosis according to TUNEL staining↑
Chen *et al.* 2005[26]	Human fetus	1, 10, 20 μg /l/10 μg l^{-1}	5 d/1, 3, 5 d	Y		Bcl-2 (P)↑, Bax (P)↑, Bax/Bcl-2 (P)↑		
Chen *et al.* 2006[27]	Human fetus	1, 10, 20 ng ml^{-1}	5 d	Y		Bcl-2 (P)↑, Bcl-2 (R) (–), Bax (P, R)↑, Bax/Bcl-2 (P)↑		
Chen *et al.* 2006[28]	Human fetus	1, 10, 20 ng ml^{-1}	5 d	Y	Fas (P)↑			NO↑, iNOS↑
Chen *et al.* 2008[44]	Human fetus	1, 10, 20 ng ml^{-1}	5 d		Fas (P, R)↑ P53 (P, R)↑	Bcl-xL (P, R)↓, Bcl-2 (P, R) (–), Bax (P, R)↑, Bax/Bcl-2 (P)↑, Bax/Bcl-xL (P)↑	Procaspase-3 (P)↑ Caspase-3 (P, R)↑	
Yang *et al.* 2008[50]	Human fetus	1, 10, 20 μg l^{-1}	5 d		P53 (P, R)↑	Bcl-xL (P)↓, Bcl-xL (R) (–)	Caspase-3 (P, R)↑	
Yang *et al.*, 2009[51]	Human fetus	1, 10, 20 μg l^{-1}	5 d		P53 (P, R)↑	Bcl-xL (P)↓, Bcl-xL (R) (–)	Caspase-3 (P, R)↑	
Han *et al.* 2013[39]	Human	20 ng ml^{-1}	3 d/24 h	Y				AFT2, JNK and p38↑
Liu *et al.* 2014[40]	Human	1, 10, 20 ng ml^{-1}	5 d	Y			Caspase-3, 9 (P)↑	Cytochrome c release↑
He *et al.* 2011[46]	Broiler chicken	5, 50, 500 nmol l^{-1}	48 h	Y			Caspase-3 (P, R)↑	Mitochondrial membrane potential↓, pathological aggregation of calcium↑, ROS↑, GPx↑

Y: yes; ↑: increased; ↓: decreased; (–): unchanged; P: protein; R: mRNA; FCM: flow cytometry.

Table 6 Metabolism of chondrocytes

		Interventions		Outcomes					
Ref.	Sources	T-2 toxin	Time	MMPs, aggrecanase	TIMPs,α2M	ILs, TNFs	Collagens	PG, aggrecan	Others
Yang *et al.* 2001[41]	Human fetus	8 μg l^{-1}	2 d			IL-1β↑, IL-6↑			
Li *et al.* 2004[52]	Human fetus	—	5 d/15 d						CD44 (R, P)↓
Chen *et al.* 2006[43]	Human fetus	1, 10, 20 μg l^{-1}	5 d				Type II (P, R)↓	Aggrecan (P, R)↓	
Li *et al.* 2008[29]	Human fetus	0.01 μg ml^{-1}	5 d	Aggrecanase-2 (R)↑		IL-1β↑, TNF-α↑		Aggrecan (R)↓, HA (P)↓	CD44 (R, P)↓, sCD44 (P)↑, HAS-2 (R)↓
Chen *et al.* 2011[45]	Human fetus	1, 10, 20 ng ml^{-1}/ 10 ng ml^{-1}	5 d/14 d	MMP-1 (P, R)↑, MMP-13 (P, R)↑	TIMP1–2 (R)↓, a2M (P, R)↓		Type II (P)↓		
Yu *et al.* 2012[53]	Human fetus	l, 10, 20 μg l^{-1}	5 d	Aggrecanase-1, 2 (R)↑				Aggrecan (P)↓	
Lu *et al.* 2012[54]	Human fetus	0.01 μg ml^{-1}	21 d	MMP1, 3 (P)↑	TIMP1, 3 (P)↓, α2M (P)↓		Type II (P)↓, type X (P)↑	Aggrecan (P)↓	
Wang *et al.* 2012[38]	Human (C28/I2)	1, 6, 12 ng ml^{-1}	3 d						Integrins αv↑, β1↑, α2↓α5↓, β5↓, α1, α3, α6, α10, β3 (R) (–)
Chen *et al.* 2014[16]	Human (C28/I2)	20, 40 μg l^{-1}	24 h	MMP-13 promoter↑					
Cao *et al.* 2007[55]	Wistar rat	0.4, 0.8, 1.6, 3.2 μg l^{-1}	24 h	MMP-13 (P)↑					
Tian *et al.* 2012[49]	Murine (ATDC5)	20 μg l^{-1}/10–80 μg l^{-1}	24 h/1–48 h	MMP-3, 9, 12, 13 (P)↑, ADAMTS4, 5 (P)↑			Type I, II, IX, X (P)↓	Aggrecan (P)↓	HIF-2α (P, R)↑, IκB-α (P)↓, SOX9, Runx2, HIF-1α (R) (–)
He *et al.* 2011[46]	Broiler chicken	1, 10, 100, 1000 nmol l^{-1}	3, 6, 9 d				Total collagen (P)↓ type X (R)↓	PG (P)↓	VEGF, Runx2 (R)↓
Liu *et al.* 2008[47]	Zelanian rabbit	1, 10, 20, 100 μg l^{-1}	5 d	MMP-3 (R)↑				Aggrecan (R)↓	

↑: Increased; ↓: decreased; (–): unchanged; P: protein; R: mRNA; HA: hyaluronic acid; sCD44: soluble CD44.

Table 7 Other intracellular changes in chondrocytes

Ref.	Sources	Interventions: T-2 toxin	Interventions: Time	Outcomes
Alteration of DNA and proteins				
Li *et al.* 2008[29]	Human fetus	0.01 μg ml^{-1}	5 d	DNA content↓
Cao *et al.* 1994[32]	Rabbit	0.005, 0.01, 0.02 μg ml^{-1}	4 d	DNA content↓, GLcUA content in matrix↓
Huo *et al.* 1998[30]	Rabbit	0.0005, 0.001, 0.005 mg l^{-1}	4 d	DNA content↓, protein content ↓
Wang *et al.* 2006[56]	Wistar rat	1, 10, 100 μg l^{-1}	24 h	DNA damage↑
Mitochondria damage				
Liu *et al.* 2014[40]	Human	1, 10, 20 ng ml^{-1}	5 d	Citrate synthase (–), complexes I, II (–), III–V↓, $\Delta\Psi_m$↓, ATP↓, ROS↑, GSH↓, GPx↓
Li *et al.* 1993[36]	Chick embryo	0.004, 0.01, 0.04 ppm	5 d	H^+-ATP enzyme↓, cytochrome C oxidase↓, succinate dehydrogenase (–)
Li *et al.* 1993[35]	Chick embryo	0.01 ppm	5 d	H^+-ATP enzyme↓, cytochrome C oxidase↓, succinate dehydrogenase (–)
Lin *et al.* 1994[37]	Chick embryo	0.004, 0.01, 0.04 ppm	4 d	H^+-ATP enzyme↓, cytochrome C oxidase↓, succinate dehydrogenase (–)
Oxidative stress				
He *et al.* 2011[46]	Broiler chicken	5, 50, 500 nmol l^{-1}	48 h	ROS↑, MDA↑, CAT↑, SOD↑, ALP↓, GSH↓
Tian *et al.* 2012[49]	Murine (ATDC5)	10, 20, 40 μg l^{-1}	1–24 h	ROS↑
NO synthesis				
Chen *et al.* 2006[28]	Human fetus	1, 10, 20 ng ml^{-1}	2 d, 5 d	NO↑, iNOS↑
Yang *et al.* 2008[57]	Human fetus	1, 10, 20 μg l^{-1}	2 d, 5 d	NO↑, iNOS↑

↑: Increased; ↓: decreased; (–): unchanged.

4. Discussion

4.1 Interpretation of the discrepancy of T-2 toxin detection rate and amount

In general, subgroup meta-analysis showed that the overall PDR of T-2 toxin and PDRC of T-2 toxin >100 ng g^{-1} in food samples was higher in endemic areas, especially in wheat powder. Moreover, T-2 toxin contamination in wheat flour was more serious in KBD endemic areas as compared to non-endemic areas.

A recent study by the meta-analysis of community-based trials of changing grains has demonstrated its benefits for the prevention and treatment of KBD in China,[90] which verified that local food might be one of the factors for KBD incidence. As T-2 toxin contamination was the most investigated food contamination in KBD regions, more attention should be paid to the causes of accumulating T-2 toxin as well as the methods of controlling and reducing T-2 toxin in staple food. First of all, because of the climate and soil situation in KBD areas, local residents preferred to cultivate wheat and corn,[8,91] and use wheat flour as their main staple food. However, these areas were marked by cold temperature and a humid environment,[8,91,92] which provided suitable conditions for T-2 toxin synthesis.[93–95] Thus, it would be better for local people to use rice for their staple food, which was also proposed in the study by Sun *et al.*[96] Secondly, in local endemic areas, inadequate food farming, harvesting and processing procedures also increased the opportunity of T-2 toxin propagation.[97–99] When most of the cereals and foodstuffs were placed in a moist storage environment and bad sanitary conditions, they might induce more production of the poisoned T-2 toxin.[92,97–101] Therefore, the environment for grain processing and storage should be improved such as by improving hygienic conditions, increasing ventilation and reducing wheat flour storage.[23]

In addition to KBD areas, Yang *et al.*[102] reported that up to 80% of wheat samples from seven provinces in China were contaminated by T-2 toxins in 1992. Our present results indicated that the PDR of the T-2 toxin was up to 60% in most non-endemic survey sites, and PDRC above 100 ng per g T-2 toxin was found in food samples from three non-endemic regions. This phenomenon suggested that the T-2 toxin might easily be generated in food, not only in KBD areas, but also in non-KBD areas. However, there were many standards for evaluating T-2 toxin contamination. When assessed by the FAO standard, the PDRC of the T-2 toxin at 100 ng g^{-1} in food was claimed as heavy T-2 toxin pollution. While according to the World Health Organization (WHO) standard, the maximum tolerable daily intake of the T-2 toxin was less than 60 ng per kg of body weight per day (which equaled a daily consumption of 500 g staple food containing 7.2 ng per g T-2 toxin for an 60 kg adult).[24] Thus, due to the difference between the above two standards, a more reliable standard should be formulated in order to determine T-2 toxin contamination for further steps.

4.2 Interpretation of the results from *in vitro*, *in vivo* and KBD studies

4.2.1 Comparison of morphological and ultrastructure damage. The effects of the T-2 toxin in both *in vitro* and *in vivo* studies including the damage of the chondrocyte morphology, nucleus, cytoplasm, organelle, and membrane were investigated. The T-2 toxin caused a short term and subchronic toxicity to chondrocytes and induced damage at the subcellular, cellular and tissue levels without species specificity. When

Table 8 Morphological and radiological changes in cartilage

Ref.	Sources	Interventions: T-2 toxin	Interventions: Time	Outcomes: Damage of epiphyseal growth plate	Outcomes: Damage of articular cartilage	Outcomes: Chondrocyte necrosis	Outcomes: Retardation of bone mineralization
Histology changes							
Wang *et al*. 2007[58]	Wistar rats	10 μg per kg BW per d/0.1, 0.6 μg per kg BW per d	7/90 d	Y		Y	
Kang *et al*. 2009[59]	Wistar rats	1 mg per kg BW per d	2, 4 w	Y		Y	
Wang *et al*. 2009[60]	Wistar rats	100 ng g^{-1}	3, 6 m		Y		
Yao *et al*. 2010[61]	Wistar rats	1 mg per kg BW per d	2, 4 w	Y			
Yao *et al*. 2010[62]	Wistar rats	10 mg per kg BW per d	4 w	Y		Y	
Yan *et al*. 2010[63]	Wistar rats	0.04 mg per kg BW per d	1, 2, 4 w	Y		Y	
Meng *et al*. 2011[64]	Wistar rats	100, 200, 300 μg kg^{-1}	6 m		Y	Y	
Wang *et al*. 2011[65]	Wistar rats	100 ng g^{-1}	6, 10 m	Y		Y	
Yan *et al*. 2011[66]	Wistar rats	0.04 mg per kg BW per d	4, 8, 12 w	Y			
Sa *et al*. 2012[67]	Wistar rats	100 ng kg^{-1}	3, 5 m		Y	Y	
Kang *et al*. 2013[68]	Wistar rats	0.1 mg per kg BW per d	8, 12 w	Y			
Yan *et al*. 2014[69]	Wistar rats	0.04 mg per kg BW per d	4, 8, 12 w	Y			
Liao *et al*. 2014[70]	Wistar rats	—	12 w	Y			
Sa *et al*. 2015[71]	Wistar rats	100 ng kg^{-1}	5 m			Y	
Pang *et al*. 2000[72]	SD rats	0.267 mg per kg BW per d	31 d				Y
Chen *et al*. 2010[73]	SD rats	100, 200 ng per g BW per d	12 w	N	Y		
Chen *et al*. 2012[74]	SD rats	100, 200 ng per g BW per d	4 w		Y		
Guan *et al*. 2013[75]	SD rats	100, 200 ng per g BW per d	4 w	Y	Y		
Zhou *et al*. 2014[76]	SD rats	100, 200 ng per g BW per d	4 w		Y		
Yang *et al*. 1994[77]	Chicks	100 μg per kg BW per d	5 w	Y			
Bai *et al*. 1996[78]	Chicks	100 μg per kg BW per d	30 d	N			
Sun 1997[79]	Chicks	100 μg per kg BW per d	5 w	Y		Y	
Liu *et al*. 1998[80]	Chicks	1.0 mg per kg BW per d	7 d			Y	
Wang *et al*. 2006[81]	Chicks	100, 600 μg per kg BW per d	5 w	Y		Y	
Peng *et al*. 1993[82]	Chick embryos	0.1, 0.5 μg	8 d			Y	
Radiology changes							
Yan *et al*. 2011[66]	Wistar rats	0.04 mg per kg BW per d	8, 12 w	Y			
Kang *et al*. 2013[68]	Wistar rats	0.1 mg per kg BW per d	8, 12 w	Y			
Yan *et al*. 2014[69]	Wistar rats	0.04 mg per kg BW per d	8, 12 w	Y			
Liao *et al*. 2014[70]	Wistar rats	—	12 w	Y			

Y: yes; N: no; BW: body weight. **Histology changes:** *Damage of epiphyseal growth plate*: irregular proliferative cell layers, shorter and sparser cell columns, focal necrosis in the hypertrophic zone, lamellar necrosis in the hypertrophic or proliferative zones, cell accumulation embedded to metaphysis; *Damage of articular cartilage*: a nest-like proliferation of chondrocytes, formation of multiple chondral cell clusters and granulation tissue in the deep zone of articular cartilage, focal cell necrosis close to the deep zone, abnormal calcification in the necrotic area; *Chondrocyte necrosis*: karyopyknosis, chromatic agglutination, organelle reduction, mitochondrial swelling *etc*.; *Retardation of bone mineralization*: bone mineralization rate reduction, osteoid formation. **Radiology changes:** *Damage of epiphyseal growth plate*: epiphyseal plate swelling, blurring, thinning, uneven signal.

Table 9 Intracellular damage in cartilage

Ref.	Sources	Interventions: T-2 toxin	Interventions: Time	Outcomes
Cell growth and metabolism				
Sun *et al*. 1995[83]	Chicks	100 μg per kg BW per d	8 w	DNA content↓, protein content↓, DNA fragmentation (−)
Sun 1997[79]	Chicks	100 μg per kg BW per d	5 w	DNA content↓, protein content↓, DNA fragmentation (−)
Liu *et al*. 1998[80]	Chicks	1.0 mg per kg BW per d	7 d	DNA fragmentation↑
Liu *et al*. 1998[84]	Chicks	1.0, 2.0 mg per kg BW per d	1 w	DNA fragmentation↑
Oxidative stress				
Chen *et al*. 2012[74]	SD rats	100, 200 ng per g BW per d	4 w	TBARS↑, T-AOC↓, SOD↓, CAT↓, GPX↓, SOD mRNA↓, CAT mRNA↓, GPX mRNA↓
Xue *et al*. 2013[85]	SD rats	100, 200 ng per g BW per d	30 d	MDA↑, T-AOC↓, SOD↓, CAT↓, GSH-Px↓, SOD mRNA↓, CAT mRNA↓, GPX mRNA↓
Xue *et al*. 2014[86]	SD rats	100, 200 ng per g BW per d	4 w	MDA↑, T-AOC↓, SOD↓, CAT↓, GSH-Px↓
Apoptosis				
Yang *et al*. 2011[87]	SD rats	200 ng per g BW per d	30 d	P53 mRNA↑, Bax mRNA↑, Bcl-2 mRNA↓, caspase-3 mRNA↑

↑: Increased; ↓: decreased; (−): unchanged; BW: body weight.

Table 10 Metabolism of the cellular matrix in cartilage

Ref.	Sources	Interventions		Outcomes			
		T-2 toxin	Time	MMPs	ILs, TNFs	Collagens	PG, PG components
Mo *et al.* 1994[88]	Wistar rats	0.2 mg per kg BW per 2 d	100 d			Total collagen↓ (SP)	Sulfate groups↓ (SP), hexosamine↓ (SP), glucuronic acid↓ (SP)
Kang *et al.* 2009[59]	Wistar rats	1 mg per kg BW per d	2, 4 w			Total collagen↓ (MS)	
Wang *et al.* 2009[60]	Wistar rats	100 ng g^{-1}	3, 6 m			Collagen fibers appear↑ (W/VG), collagen fibers breakage and desquamation↑ (SEM)	PG↓ (SEM)
Yan *et al.* 2010[63]	Wistar rats	0.04 mg kg^{-1} d^{-1}	1, 2, 4 w			Total collagen↓ (MS)	
Yao *et al.* 2010[61]	Wistar rats	1 mg per kg BW per d	2, 4 w			Total collagen↓ (MS)	
Yao *et al.* 2010[62]	Wistar rats	10 mg per kg BW per d	4 w			Total collagen↓ (MS)	
Meng *et al.* 2011[64]	Wistar rats	100, 200, 300 μg kg^{-1}	6 m			Collagen fibers breakage↑ (SEM)	PG↓ (SEM)
Kang *et al.* 2013[68]	Wistar rats	0.1 mg per kg BW per d	8, 12 w			Total collagen↓ (MS)	
Chen *et al.* 2010[73]	SD rats	100, 200 ng per g BW per d	12 w			Fibrils appear↑ (HE)	
Guan *et al.* 2013[75]	SD rats	100, 200 ng per g BW per d	4 w				sGAG↓ (TB)
Chen *et al.* 2014[16]	SD rats	100 μg per kg BW per d	30 d	MMP-13↑ (IH)			
Zhou *et al.* 2014[76]	SD rats	100, 200 ng per g BW per d	4 w		IL-6↑, IL-1β↑, TNF-α↑, IL-6 mRNA↑, IL-1β mRNA↑ TNF-α mRNA↑		sGAG↓ (TB)
Hu *et al.* 1996[89]	Chicks	0.4 mg per kg BW	9 w			Type I↑, type II↓ (IH)	Keratan sulfate↓, chondroitin sulfate↓ (HC)
Wang *et al.* 2006[81]	Chicks	100, 600 μg per kg BW per d	5 w			Type II↓ (W/VG)	PG↓ (AB)

↑: Increased; ↓: decreased; (−): unchanged; BW: body weight; SP: spectrophotometry; MS: Masson's staining; W/VG: Weigert/Van Gieson staining; HE: hematoxylin & eosin staining; TB: toluidine blue staining; AB: alcian blue staining; HC: histochemical staining; IH: immunohistochemistry; SEM: scanning electron microscope.

compared with the characteristics of KBD patients, some changes of chondrocytes and cartilages induced by the T-2 toxin were quite similar such as focal chondronecrosis in the hypertrophic zone of the growth plate and in the deep zone of the articular cartilage,[103,104] suggesting that the T-2 toxin-induced chondrocyte and cartilage damage was probably one of the pathological factors of KBD. Therefore, understanding the complexities of the toxic mechanism should be crucial for the prevention and treatment of KBD. In addition, the mechanism of chondrocyte and cartilage damage induced by the T-2 toxin could be associated with apoptosis, metabolism alteration and intracellular changes.

4.2.2 Comparison of proliferation and alterations of antioxidant capacity. The results from MTT and cell counting showed a restriction effect of the T-2 toxin on the viability and proliferation of chondrocytes. Both in the chondrocytes and cartilage, the contents of DNA and proteins were suppressed in a time and dose-dependent behavior, indicating inhibition of chondrocyte proliferation and metabolism. Besides, the increase of superoxide with decreased antioxidant ability might be responsible for oxidative stress. ROS, MDA, and TBARS were the factors mediating lipid peroxidation activated by the T-2 toxin. In contrast, antioxidants such as GSH and T-AOC were restrained, which reflected the loss of antioxidant capacity. The antioxidases such as CAT, SOD and GSH-Px were restrained in *in vivo* studies, while CAT and SOD were increased in *in vitro* studies, which is probably due to the difference of the oxidative stress extent in different chondrocytes and cartilage. In KBD patients, it was reported that TBARS was elevated, while antioxidant enzymes such as T-AOC, SOD, CAT and GPX, were suppressed in the serum,[74,105] which were similar to the changes in the T-2 toxin-intervened chondrocyte or cartilage. Meanwhile, ROS was increased as one of the mitochondrial apoptotic factors by T-2 toxin treatment. The T-2 toxin restrained the activities of complexes, H^+-ATP enzyme and cytochrome C oxidase, a manifestation of mitochondrial respiratory chain repression. A previous study has demonstrated that mitochondrial damage played an important role in the pathogenesis of KBD.[106] Therefore, all the consequences mentioned above indicated the connection of chondrocytes change between T-2 toxin exposure and KBD.

4.2.3 Comparison of apoptosis changes. As mentioned above, the T-2 toxin induced apoptosis in chondrocytes from humans and animals. The T-2 toxin was able to up-regulate Fas and p53 as a pro-apoptotic factor.[107,108] The expression of factors of the Bcl-2 family as an important regulator of apoptosis was altered,[109,110] especially the expression of Bax in the mRNA and protein levels as well as the ratio of Bax/Bcl-2 and Bax/Bcl-xL at the protein level. A previous study has shown that the ratio of pro-apoptotic and anti-apoptotic proteins in the Bcl-2 family might be the core factor of the apoptosis process,[111] so the increase of heterodimerization of the Bcl-2 family indicated chondrocytes apoptosis induced by the T-2 toxin. Under the condition of Bcl-2 family changes, the activity of caspases, especially caspase-3, was finally enhanced to

mediate apoptosis indispensably.[112,113] As concluded, the T-2 toxin might induce Fas and p53 up-regulation following Bcl-2 family and caspase alteration, which results in chondrocyte apoptosis. In KBD patients, previous studies have demonstrated that the expression of Fas, Bax, Bcl-2 and caspases in chondrocytes also rose,[114–117] thus, the mechanism of chondrocyte apoptosis induced by the T-2 toxin is linked to KBD pathogenesis. Besides, the T-2 toxin also caused other mechanisms related to apoptosis such as NO and mitochondrial-related pathways which needed more experiments to confirm. Furthermore, the NO content and iNOS expression were elevated in the serum of KBD patients as well as in the chondrocytes after exposure to the T-2 toxin.[118]

4.2.4 Comparison of metabolism and ECM degradations. The cartilage matrix consists of several PGs, glycoproteins and collagens, most of which are secreted by chondrocytes. Based on our results, the T-2 toxin perturbs the synthesis of PG and collagens, especially total collagen and type II collagen in *in vitro* and *in vivo* studies, thereby promoting an excessive catabolism over anabolism. In the cartilage, the collagen changed after exposure to the T-2 toxin, which demonstrated a metabolic disturbance in the ECM. MMPs, aggrecanases, and ADAMTSs are the most important enzymes of matrix proteolysis. As reported, the degradation of type II collagen and aggrecan was accelerated as a result of the elevated expression of MMP-13 induced by the T-2 toxin.[119] Simultaneously, the T-2 toxin triggered up-regulation of aggrecanase-1, 2 activities, which could directly affect the aggrecan degradation. TIMPs and α_2M are both inhibitors of the MMPs. After T-2 toxin treatment, cartilage degradation was accelerated because of decreased TIMP 1–3 and α_2M expression. Moreover, the T-2 toxin enhanced pro-inflammatory factors including TNF-α, IL-1β and IL-6. All of them act as a kind of catabolic cytokine resulting in matrix degradation. Some other molecules such as CD44 and integrins related to the chondrocyte metabolism were also influenced by the T-2 toxin, as certified in chondrocyte catabolism promotion. In summary, after the cartilage or chondrocytes being exposed to the T-2 toxin, MMPs and α_2M were increased while TIMPs and aggrecanases were decreased, which caused the degradation of collagens and PG in ECM as a result. Interestingly, matrix degradation was also found in the development of KBD, including a low type II collagen expression[120,121] and decreased PG.[10,122] MMP-13 was elevated in the articular cartilage of both KBD[121] and OA.[123] Pro-inflammatory factors were also increased in the synovial fluid[124] and serum of KBD patients.[125] All of them showed similar alterations in the chondrocytes and cartilage in both KBD and T-2 toxin intervention.

4.3 Suggestions for further studies

Nevertheless, there are still some limitations to be addressed. For epidemiological studies, data collection among these papers was insufficient. The overall methodological quality of the included studies needed to be improved. So far, all studies on the T-2 toxin were cross-sectional studies, which lacked continuous and systemic investigation, although most of them could be traced back to at least 10 years in the Northeast of China. Therefore, high-quality and well-designed experiments are required. It is suggested that survey locations could be expanded in more KBD regions and focus more on T-2 toxin concentration in different food types with a unified measurement control condition. In addition, the results may be limited by potential bias in that few studies referred to the evaluation of confounding factors. Thus, some information, such as the effect of evaluators of subjective components, and the handling of missing data from analysis should be revealed in further studies. As is known, KBD may be influenced by many factors such as low selenium, iodine of the grains and other mycotoxins such as moniliformin (MON) and deoxynivalenol (DON). More details should be provided when measuring the T-2 toxin content in food. In addition, the relationship between the T-2 toxin and other factors still needs to be investigated in future studies.

For experimental studies included in this article, they were almost at the B level with regard to quality but the evaluation standard was insufficient. Further standards need to be improved to assess the relevant experimental studies accurately. According to our results, the T-2 toxin could destroy the chondrocytes and cartilage through a variety of pathways including apoptosis, changes of metabolism, DNA and protein, oxidative stress, mitochondrial damage and NO synthesis. Some of these pathways are linked to each other, such as the connection of mitochondrial dysfunction and apoptosis,[109,126,127] matrix destruction[128,129] as well as apoptosis and metabolism degradation.[130,131] Additionally, some factors, such as ROS and pro-inflammatory factors are thought to have effects on different pathways. ROS can play an important role in apoptosis,[132,133] matrix degradation,[133] and is considered a mediating factor of intracellular regulation. Other studies demonstrated that pro-inflammatory factors were able to enhance NO[134] and iNOS[57] production and induce chondrocyte apoptosis as well.[135,136] However, whether the T-2 toxin has direct or indirect effects on these connected pathways and the involved factors are not completely confirmed yet. Moreover, since the T-2 toxin in the body is metabolized to HT-2,[137] some results could be different between *in vitro* and *in vivo* experiments with T-2 toxin exposure. Hence, it is necessary to clarify different toxic effects of T-2 and HT-2 toxins on *in vitro* experiments as well. Finally, cartilage is not the only targeted organ of the T-2 toxin, some articles[83,137] reported that the T-2 toxin could result in damage in other organs such as the heart, liver, *etc*. causing diseases such as Keshan disease, alimentary toxic aleukia (ATA)[138] and osteoarthritis.[79] Thus, an overall review of the effect of the T-2 toxin on these organs and diseases is also needed to be investigated in further studies.

In order to confirm the etiology of KBD, the most convincing evidence is in accordance with the results from the cohort and case-control studies in epidemiology. But no studies have directly shown the causality of the T-2 toxin and KBD at present. Further confirmation of the etiologic relationship is needed in subsequent epidemiological investigations. Moreover, with further investigations resulting in the definition of

clear clinical signs of the T-2 toxin detection rate in KBD patients, we may draw a more reasonable conclusion about the effects of the T-2 toxin on KBD prevalence. However, no data on the T-2 toxin concentration in the human body has been obtained in any of the studies yet. This review indicates a high-degree of similarity in the pathology and mechanism of the T-2 toxin and KBD. Combining the summarized results of cross-sectional studies and experimental studies, the T-2 toxin is a likely cause for KBD prevalence. But to some extent, the conclusion is still preliminary. Current experimental studies have only provided a possible explanation for the effect of the T-2 toxin on the pathogenesis of KBD based on similar comparison results, and a correlation between KBD and T-2 toxin is simply presented in cross-sectional, *in vitro* and *in vivo* studies, which excludes population-based studies due to ethical constraints. Our present results may provide a new insight for better understanding the effect of the T-2 toxin on the etiology and pathogenesis of KBD.

Conflict of interests

The authors declare no conflicts of interest. The author's affiliation is as shown on the cover page. The authors are solely responsible for the writing and content of the paper.

Acknowledgements

We thank Professor Eng San Thian from the National University of Singapore for his assistance in the editing of this manuscript. This study was supported by the National Natural Science Foundation of China (81402639, 81472924), the China Postdoctoral Science Foundation (2014M562423) and the Shaanxi Province Natural Science Basic Research Program for Youths (2015JQ8310).

References

1 S. Q. Lv, Z. L. Wang, S. M. Lv and W. Pang, T-2 toxin research, *Chin. J. Control Endem. Dis.*, 1996, **11**, 282–285 (in Chinese).
2 J. R. Bamburg, N. V. Riggs and F. M. Strong, The structure of toxins from two strains of *Fusarium tricinctum*, *Tetrahedron*, 1968, **24**, 3329–3336.
3 World Health Organization and International Programme on Chemical Safety, *Selected Mycotoxins: Ochratoxins, Trichothecenes*, Ergot, World Health Organization, Geneva, 1990.
4 R. C. Schothorst and H. P. van Egmond, Report from SCOOP task 3.2.10 "collection of occurrence data of Fusarium toxins in food and assessment of dietary intake by the population of EU member states": subtask: trichothecenes, *Toxicol. Lett.*, 2004, **153**, 133–143.
5 H. M. Hussein, R. A. Franich, M. Baxter and I. G. Andrew, Naturally occurring Fusarium toxins in New Zealand maize, *Food Addit. Contam.*, 1989, **6**, 49–57.
6 Y. T. Huo and J. L. Cao, The position of Mycotoxins in the etiology of KBD, *Foreign Med. Sci., Sect. Medgeogr.*, 1997, **18**, 55–58, 65 (in Chinese).
7 S. Y. Zhang and X. Y. Mo, *Biochemistry of cartilage and bone cartilage disease*, Shaanxi Science and Technology Press, Xi'an, 1996 (in Chinese).
8 J. B. Yang, Research report on the etiology of Kaschin-Beck disease (KBD), *Chin. J. Endemiol.*, 1995, **14**, 201–204 (in Chinese).
9 Y. H. An, X. F. Jia, X. F. Li, J. He, S. B. Han and H. Zhang, Geological environment characteristics and etiology research on Kashin–Beck disease in China, *Geol. China.*, 2010, **37**, 539–563 (in Chinese).
10 J. Cao, S. Li, Z. Shi, Y. Yue, J. Sun, J. Chen, Q. Fu, C. E. Hughes and B. Caterson, Articular cartilage metabolism in patients with Kashin-Beck disease: an endemic osteoarthropathy in China, *Osteoarthritis Cartilage*, 2008, **16**, 680–688.
11 X. Guo, Progression and prospect of etiology and pathogenesis of Kashin-Beck disease, *J. Xi'an Jiaotong Univ., Med. Sci.*, 2008, **29**, 481–488 (in Chinese).
12 A. Rostom, C. Dube, A. Cranney, N. Saloojee, R. Sy, C. Garritty, M. Sampson, L. Zhang, F. Yazdi, V. Mamaladze, I. Pan, J. McNeil, D. Moher, D. Mack and D. Patel, Celiac Disease. Rockville (MD): Agency for Healthcare Research and Quality (US); 2004 Sep. (Evidence Reports/Technology Assessments, No. 104.) Appendix D. Quality Assessment Forms, http://www.ncbi.nlm.nih.gov/books/NBK35156, (accessed 2004).
13 Center SDM: EBM Evidence Pyramid, http://library.downstate.edu/ebmdos/2100.htm, (accessed 2001).
14 Z. Xiao, C. W. Li, J. Shan, L. Luo, L. Feng, J. Lu, S. F. Li, D. Long and Y. P. Li, Mechanisms of renal cell apoptosis induced by cyclosporine A: a systematic review of *in vitro* studies, *Am. J. Nephrol.*, 2011, **33**, 558–566.
15 Z. Xiao, J. Shan, C. W. Li, L. Luo, J. Lu, S. F. Li, D. Long and Y. P. Li, Mechanisms of cyclosporine-induced renal cell apoptosis: a systematic review, *Am. J. Nephrol.*, 2013, **37**, 30–40.
16 J. H. Chen, J. L. Cao, Z. L. Wang, T. Y. Ma, M. Y. Wang, Y. He, Z. T. Yang and C. Chen, Correlation of matrix metalloproteinases and Kashin-Beck disease, *Chin. J. Endemiol.*, 2014, **33**, 357–362 (in Chinese).
17 Y. Luo, J. S. Zheng, J. S. Yang, F. Liu, T. Yoshizawa, S. Y. Zhang, B. J. Zhang, K. C. Liu, S. S. Zhai, R. Sha and H. Wen, Determination of fusarium mycotoxins in corn and wheat from Kaschin-Beck disease areas, *Chin. J. Control Endem. Dis.*, 1992, **7**, 71–75, 127 (in Chinese).
18 J. B. Yang, D. J. Sun and Z. W. Wang, Determination of T-2 toxin in the staple food from the sick families in Kashin-Beck disease (KBD) areas, *Chin. J. Endemiol.*, 1995, **14**, 146–149 (in Chinese).

19 D. J. Sun, Y. Q. Liu and Q. W. Li, Determination of T-2 toxin in staple food from a Kashin-Beck disease (KBD) area and non-KBD areas in Heilongjiang Province, *Chin. J. Endemiol.*, 1997, **16**, 207–209 (in Chinese).

20 J. Feng, Y. H. Cao, S. P. Wang, B. Gao and X. N. Sun, Report on the level of T-2 toxin in cereals sampled from markets of Heilongjiang Province, *Chin. J. Endemiol.*, 2004, **23**, 560–561 (in Chinese).

21 N. Liu, W. S. Bao, D. A. Li, J. Feng, B. Gao, X. N. Sun and Q. Deng, Contaminative status of T-2 and citreoviridln toxin in cereal, *Chin. J. Endemiol.*, 2004, **23**, 237–239 (in Chinese).

22 W. S. Bao, J. Feng, Y. H. Cao, X. N. Sun and Q. Deng, Investigation on Kaschin-Beck disease in Shitougou Village of Nanjiang County, *Chin. J. Endemiol.*, 2005, **24**, 318–319.

23 L. Y. Sun, Q. Li, F. G. Meng, Y. Fu, Z. J. Zhao and L. H. Wang, T-2 toxin contamination in grains and selenium concentration in drinking water and grains in Kaschin–Beck disease endemic areas of Qinghai Province, *Biol. Trace Elem. Res.*, 2012, **150**, 371–375.

24 Joint FAO/WHO Expert Committee on Food Additives (2001: Geneva, Switzerland), World Health Organization and International Programme on Chemical Safety, Evaluation of certain mycotoxins in food, Fifty-sixth report of the Joint FAO/WHO Expert Committee on Food Additives, World Health Organization, Geneva, 2002.

25 A. Thuvander, T. Möller, H. E. Barbieri, A. Jansson, A. C. Salomonsson and M. Olsen, Dietary intake of some important mycotoxins by the Swedish population, *Food Addit. Contam.*, 2001, **18**, 696–706.

26 J. H. Chen, Y. L. Chu, Z. T. Yang, J. L. Cao, Z. L. Shi, S. Y. Li, X. Guo and Z. L. Wang, Effect of T-2 toxin on chondrocyte apoptosis and Bcl-2/Bax expression, *J. Xi'an Jiaotong Univ., Med. Sci.*, 2005, **26**, 130–134 (in Chinese).

27 J. H. Chen, Y. L. Chu, J. L. Cao, Z. T. Yang, X. Guo and Z. L. Wang, T-2 toxin induces apoptosis, and selenium partly blocks, T-2 toxin induced apoptosis in chondrocytes through modulation of the Bax/Bcl-2 ratio, *Food Chem. Toxicol.*, 2006, **44**, 567–573.

28 J. H. Chen, Y. L. Chu, J. L. Cao, Z. T. Yang, Z. L. Shi, X. Guo and Z. L. Wang, Effect of NO and Fas pathway on T-2 induced apoptosis in chondrocytes, *J. Sichuan Univ., Med. Sci. Edn*, 2006, **37**, 583–586 (in Chinese).

29 S. Y. Li, J. L. Cao, Z. L. Shi, J. H. Chen, Z. T. Zhang and C. E. Hughes, Promotion of the articular cartilage proteoglycan degradation by T-2 toxin and selenium protective effect, *J. Zhejiang Univ., Sci., B*, 2008, **9**, 22–33.

30 Y. T. Huo, J. L. Cao and X. Guo, Experimental study of the critical value and Se protection of T-2 toxin damage to chondrocytes, *Chin. J. Endemiol.*, 1998, **17**, 143–146 (in Chinese).

31 L. H. Wang and L. Zhang, Investigation on the ultrastructures of rat chondrocytes exposed to mini-dose T-2 toxin in vitro, *Chin. J. Endemiol.*, 2005, **24**, 291–293 (in Chinese).

32 J. L. Cao, Y. M. Xiong and S. Y. Zhang, Effect of T-2 toxin on the growth and metabolism of cultural chondrocytes, *Chin. J. Endemiol.*, 1994, **13**, 268–270 (in Chinese).

33 J. L. Cao, Y. M. Xiong, S. Y. Zhang and D. X. Mo, Experimental study of mycotoxins DON, T-2 and NIV in cultured cartilage, *Chin. J. Control Endem. Dis.*, 1995, **10**, 69–71 (in Chinese).

34 J. L. Cao, Y. M. Xiong, S. Y. Zhang and D. X. Mo, Ultrastructural observation of T-2 toxin on cultured chondrocytes, *J. Xi'an Jiaotong Univ., Med. Sci.*, 1995, **16**, 249–251 (in Chinese).

35 S. G. Li, L. Y. Wu, S. Sun, J. Hong, H. F. Ji, Q. W. Lu, Z. H. Lin and F. Y. Yang, The antagonistic effect of Se on the T-2 toxin-induced changes of ultrastructure and function of cultured chicken embryo chondrocyte, *Chin. Biochem. J.*, 1993, **9**, 81–86 (in Chinese).

36 S. G. Li, S. Sun, L. Y. Wu, H. F. Ji, J. Hong and Z. H. Lin, The effect of T-2 toxin on the extracellular matrix and the enzymes of mitochondrial inner membrane of cultured chicken embryo chondrocytes, *Prog. Biochem. Biophys.*, 1993, **20**, 364–368 (in Chinese).

37 Z. H. Lin, S. G. Li, L. Y. Wu, S. Sun and Q. W. Lu, Antagonistic effect of Se on the T-2 toxin-induced changes in the ultrastructure and mitochondrial function of cultured chicken embryonic chondrocytes, *J. Clin. Biochem. Nutr.*, 1994, **17**, 119–132.

38 J. L. Wang, M. X. Luo, J. Li, J. H. Chen, Q. Fu, W. Wang, Z. T. Zhang and J. L. Cao, Effect of T-2 toxin on integrins expression and antagonistic role of selenium, *J Xi'an Jiaotong Univ., Med. Sci.*, 2012, **33**, 271–275 (in Chinese).

39 J. Han and X. Guo, Down-regulation of ATF2 in the inhibition of T-2-toxin-induced chondrocyte apoptosis by selenium chondroitin sulfate nanoparticles, *J. Nanopart. Res.*, 2013, **15**, 1–8.

40 J. T. Liu, L. L. Wang, X. Guo, Q. J. Pang, S. X. Wu, C. Y. Wu, P. Xu and Y. D. Bai, The role of mitochondria in T-2 toxin-induced human chondrocytes apoptosis, *PLoS One*, 2014, **9**, e108394.

41 T. F. Yang, Z. Q. Jia and B. Shen, Effect of T-2 toxin on apoptosis of fetus chondrocytes, *Chin. J. Endemiol.*, 2001, **20**, 84–86 (in Chinese).

42 T. F. Yang, B. C. Zhao and G. L. Wang, Effect of T-2 toxin on IL-1β and IL-6 secretion in human fetal chondrocytes, *Chin. J. Endemiol.*, 2001, **20**, 322–324 (in Chinese).

43 J. H. Chen, J. L. Cao, Y. L. Chu, Z. T. Yang, Z. L. Shi, H. L. Wang, X. Guo and Z. L. Wang, Protective effect of selenium against T-2 toxin-induced inhibition of chondrocyte aggrecan and collagen II synthesis, *J. South Med. Univ.*, 2006, **26**, 381–385 (in Chinese).

44 J. H. Chen, J. L. Cao, Y. L. Chu, Z. L. Wang, Z. T. Yang and H. L. Wang, T-2 toxin-induced apoptosis involving Fas, p53, Bcl-xL, Bcl-2, Bax and caspase-3 signaling pathways in human chondrocytes, *J. Zhejiang Univ. Sci. B*, 2008, **9**, 455–463.

45 J. H. Chen, Y. L. Chu, J. L. Cao, W. Wang, J. Y. Liu and J. L. Wang, Effects of T-2 toxin and selenium on chondro-

cyte expression of matrix metalloproteinases (MMP-1, MMP-13) , α2-macroglobulin (α2M) and TIMPs, *Toxicol. In Vitro*, 2011, **25**, 492–499.

46 S. J. He, *Study on the mechanism of toxicity of T-2 toxin on primary cultured chondrocytes from chicken tibial growth plate*, Nanjing Agricultural University, 2011 (in Chinese).

47 B. Liu, C. Y. Lu, Q. Wang, X. Y. Gong and D. C. Chen, The effect of T-2 toxin on expressions of aggrecan and matrix metalloproteinase-3 in Zelanian rabbit chondrocyte, *Bone*, 2008, **43**, S113–S114.

48 B. Liu and D. C. Chen, The effect of T-2 toxin on proliferation in rabbit chondrocyte, *China Pharm.*, 2011, **20**, 12–13 (in Chinese).

49 J. Tian, J. D. Yan, W. Wang, N. N. Zhong, L. F. Tian, J. Sun, Z. X. Min, J. Ma and S. M. Lu, T-2 toxin enhances catabolic activity of hypertrophic chondrocytes through ROS-NF-κB-HIF-2α pathway, *Toxicol. In Vitro*, 2012, **26**, 1106–1113.

50 Z. T. Yang, Z. L. Wang, J. H. Chen, X. W. Tan, J. L. Cao, X. Guo and Y. M. Xiong, Effect of T-2 toxin on P53, Bcl-xL and Caspase-3 expression in chondrocytes, *Chin. J. Control Endem. Dis.*, 2008, **23**, 412–415 (in Chinese).

51 Z. T. Yang, J. H. Chen, Z. L. Wang, J. L. Cao, X. Guo, Y. M. Xiong and X. W. Tan, Effect of T-2 toxin and selenium on P53, Bcl- xL and Caspase-3 protein expression in chondrocytes, *J. Environ. Health*, 2009, **26**, 283–286 (in Chinese).

52 S. Y. Li, J. L. Cao, Z. L. Shi, P. H. Cao and W. B. Li, Effect of T-2 toxin on chondrocyte CD44 expression, *Chin. J. Endemiol.*, 2004, **23**, 527–529 (in Chinese).

53 B. Q. Yu, J. L. Cao, J. H. Chen, Z. L. Shi, W. Y. Wang, Z. T. Yang, T. Y. Ma and S. J. Wang, Effects of T-2 toxin and selenium on expression of aggrecanase in human chondrocyte, *Chin. J. Endemiol.*, 2012, **31**, 46–50 (in Chinese).

54 M. L. Lu, J. L. Cao, F. Q. Liu, S. Y. Li, J. H. Chen, Q. Fu, Z. T. Zhang, J. Y. Liu, M. X. Luo, J. L. Wang, J. Li and B. Caterson, The effects of mycotoxins and selenium deficiency on tissue-engineered cartilage, *Cells Tissues Organs*, 2012, **196**, 241–250.

55 Y. H. Cao, S. P. Wang, Y. Hui and N. Liu, Effects of T-2 toxin on the expression of matrix metalloproteinase 13 in chondrocytes in vivo, *Chin. J. Endemiol.*, 2007, **26**, 599–602 (in Chinese).

56 L. H. Wang, H. J. Yao and J. B. Yang, Investigation on DNA damage of rat articular chondrocyte induced by mini-dose T-2 toxin in vitro, *Chin. J. Control Endem. Dis.*, 2006, **21**, 212–214 (in Chinese).

57 Z. T. Yang, X. Guo, J. H. Chen, Z. L. Wang, J. L. Cao, Y. M. Xiong and X. W. Tan, Effects of T-2 toxin on NO production and iNOS expression in chondrocytes, *Shaanxi Med. J.*, 2008, **37**, 1115–1117, 1146 (in Chinese).

58 L. H. Wang, W. G. Wang and J. B. Yang, Investigation on histopathology and ultrastructure damages of rat articular growth plate cartilage damage induced by low-dose T-2 toxin, *Chin. J. Endemiol.*, 2007, **26**, 596–598 (in Chinese).

59 P. D. Kang, D. L. Yan, Y. F. Yao, X. B. Li, J. Yang, B. Shen, Z. K. Zhou and F. X. Pei, Compare study of rat bone and joint development effected by T2 toxin and KBD-affected feed of epidemic district in the A'ba autonomous region of the P. R. China, *Chin. J. Bone Joint Surg.*, 2009, **2**, 404–410 (in Chinese).

60 L. H. Wang, Y. X. Shi, Y. Fu, W. C. Ma and Q. Jia, The biomarkers role of type II collagen C-terminal telopeptide and deoxypyridinoline in cartilage injury of experimental rats, *Contemp. Med.*, 2009, **15**, 1–3 (in Chinese).

61 Y. F. Yao, P. D. Kang, X. B. Li, J. Yang, B. Shen, Z. K. Zhou and F. X. Pei, Study on the effect of T-2 toxin combined with low nutrition diet on rat epiphyseal plate growth and development, *Int. Orthop.*, 2010, **34**, 1351–1356.

62 Y. F. Yao, P. D. Kang, X. B. Li, J. Yang, B. Shen, Z. K. Zhou and F. X. Pei, Effect of T-2 toxin on growth and development of rat knee epiphyseal plate and metaphyseal bone in normal and low nutritional status, *Chin. J. Endemiol.*, 2010, **29**, 475–479 (in Chinese).

63 D. L. Yan, P. D. Kang, J. Yang, B. Shen, Z. K. Zhou, L. J. Duan, J. Y. Deng, H. Huang and F. X. Pei, The effect of Kashin-Beck disease-affected feed and T-2 toxin on the bone development of Wistar rats, *Int. J. Rheum. Dis.*, 2010, **13**, 266–272.

64 F. G. Meng, W. C. Ma and L. H. Wang, Morphology damages of rat articular cartilage induced by different doses of T-2 toxin, *Chin. J. Endemiol.*, 2011, **30**, 498–501 (in Chinese).

65 L. H. Wang, Y. Fu, Y. X. Shi and W. G. Wang, T-2 toxin induces degenerative articular changes in rodents: link to Kaschin-Beck disease, *Toxicol. Pathol.*, 2011, **39**, 502–507.

66 D. L. Yan, P. D. Kang, Y. S. Li, J. Yang, B. Shen, Z. K. Zhou, J. Y. Deng and F. X. Pei, Radiographic findings of Wistar rats fed with T-2 toxin and Kashin-Beck disease-affected diet, *Int. J. Rheum. Dis.*, 2011, **14**, 92–97.

67 R. L. Sa, W. W. Man and L. H. Wang, Role of type II collagen in protecting and preventing articular cartilage damage induced by T-2 toxin in rats, *Chin. J. Endemiol.*, 2012, **31**(3), 292–295 (in Chinese).

68 P. Kang, Y. Yao, J. Yang, B. Shen, Z. Zhou and F. Pei, An animal model of Kashine-Beck disease induced by a low-nutrition diet and exposure to T-2 toxin, *Osteoarthritis Cartilage*, 2013, **21**, 1108–1115.

69 D. L. Yan, Y. C. Song, B. Shen, P. D. Kang and F. X. Pei, Magnetic resonance imaging in the tibial epiphyseal growth plate development of Wistar rat, *J. Orthop. Surg. Res.*, 2014, **9**, 39.

70 J. C. Liao, X. B. Yang, Y. Li, F. X. Pei, P. D. Kang and F. B. Gao, MRI evaluation of the effect of Kashin–Beck disease-affected feed and T-2 toxin on the rat knees, *Jt., Bone, Spine*, 2014, **81**, 267–268.

71 R. L. Sa and L. H. Wang, Protective effect of collagen-II on articular cartilage damage induced by T-2 toxin

in rats, *Chin. J. Public Health*, 2015, **31**, 603–605 (in Chinese).

72 W. Pang, L. J. Wang, Z. L. Wang and H. Y. Bi, Effect of T-2 toxin on bone mineralization of rats, *Chin. J. Control Endem. Dis.*, 2000, **15**, 263–265 (in Chinese).

73 J. H. Chen, Z. L. Wang, H. J. Yang, S. H. Xue, D. Q. Song, L. Dong, Z. T. Yang, X. W. Tan, W. Wang, B. Q. Yu and T. Y. Ma, Histopathology of chondronecrosis in knee articular cartilage of rat at T-2 toxin and selenium deficiency conditions, *Chin. J. Control Endem. Dis.*, 2010, **25**, 98–101 (in Chinese).

74 J. H. Chen, S. H. Xue, S. Y. Li, Z. L. Wang, H. J. Yang, W. Wang, D. Q. Song, X. R. Zhou and C. Chen, Oxidant damage in Kashin-Beck disease and a rat Kashin-Beck disease model by employing T-2 toxin treatment under selenium deficient conditions, *J. Orthop. Res.*, 2012, **30**, 1229–1237.

75 F. Guan, S. Y. Li, Z. L. Wang, H. J. Yang, S. H. Xue, W. Wang, D. Q. Song, X. R. Zhou, W. Zhou, J. H. Chen, B. Caterson and C. Hughes, Histopathology of chondronecrosis development in knee articular cartilage in a rat model of Kashin–Beck disease using T-2 toxin and selenium deficiency conditions, *Rheumatol. Int.*, 2013, **33**, 157–166.

76 X. R. Zhou, Z. L. Wang, J. H. Chen, W. Wang, D. Q. Song, S. Y. Li, H. J. Yang, S. H. Xue and C. Chen, Increased levels of IL-6, IL-1β, and TNF-α in Kashin–Beck disease and rats induced by T-2 toxin and selenium deficiency, *Rheumatol. Int.*, 2014, **34**, 995–1004.

77 J. B. Yang, D. J. Sun and L. Jin, Observation on T-2 toxin causing younger chickens to suffer from multiple necrosis in the cartilage of knee joint, *Chin. J. Endemiol.*, 1994, **13**, 1–2 (in Chinese).

78 X. W. Bai, S. M. Lv, S. Bai, S. Y. Zhang, H. Y. Bi, B. Zheng, F. J. Zhang, Z. L. Wang and S. Q. Lv, Experiment of T-2 toxin-induced KBD model in chicks: pathological morphology of tibia, *Chin. J. Control Endem. Dis.*, 1996, **11**, 149–151 (in Chinese).

79 D. J. Sun, Epidemiological study on the etiology of osteoarthrosis caused by T-2 toxin produced by fusarium in the grain, *J. Rheumatol.*, 1997, **2**, 20–24.

80 N. Liu and Z. H. Ren, Electron microscopie observation on chondrocyte injury induced by T-2 toxin, *Chin. J. Endemiol.*, 1998, **17**, 238–240 (in Chinese).

81 L. H. Wang, W. G. Wang and J. B. Yang, Histochemical investigation on the damage of chick articular cartilage induced by T-2 toxin, *Chin. J. Endemiol.*, 2006, **25**, 271–274 (in Chinese).

82 S. Q. Peng, X. L. Yu, B. Z. Wang, Y. Yang, Z. F. Zheng and J. S. Yang, Injurious effect of fusarium toxin on articular chondrocytes and protective effect of selenium, *Chin. J. Control Endem. Dis.*, 1993, **8**, 258–259, 319 (in Chinese).

83 D. J. Sun, J. B. Yang, Y. H. Zhang and Q. W. Ji, In vivo inhibitory effects of T-2 toxin on synthesis of protein and DNA in broiler chickens tissues, *Chin. J. Endemiol.*, 1995, **14**, 363–365 (in Chinese).

84 N. Liu and Z. H. Ren, Study on DNA damage caused by T-2 toxin, *Chin. J. Endemiol.*, 1998, **17**, 72–74 (in Chinese).

85 S. H. Xue, Z. L. Wang, J. H. Chen, H. J. Yang, D. Q. Song and C. H. Zhao, Effects of T-2 toxin on activities and gene expression of antioxidant enzymes in selenium deficient rat's articular cartilage, *Acta Nutr. Sin.*, 2013, **35**, 64–67 (in Chinese).

86 S. H. Xue, Z. L. Wang, J. H. Chen, H. J. Yang, D. Q. Song and C. H. Zhao, Effect of T-2 toxin on antioxidant enzymes of rat tissues under low selenium condition, *Chin. J. Control Endem. Dis.*, 2014, **29**, 256–257 (in Chinese).

87 H. J. Yang, Z. L. Wang, J. H. Chen, D. Q. Song, S. H. Xue, X. R. Zhou, Q. Chen, X. W. Tan, Z. T. Yang and T. Y. Ma, Effects of T-2 toxin on the mRNA expression of apoptosis•-related gene in articular chondrocytes of selenium-deficiency rats, *J. Xi'an Jiaotong Univ., Med. Sci.*, 2011, **32**, 272–278 (in Chinese).

88 X. Y. Mo, S. Q. Peng, J. S. Yang and F. Y. Zhang, Effect of Selenium on metabolic abnormalities of cartilage matrix induced by T-2 toxin in rat, *Chin. J. Endemiol.*, 1994, **13**, 83–85 (in Chinese).

89 M. S. Hu, B. L. Yuan, S. Z. Yu and J. S. Yang, Pathological study on the effects of T-2 toxin and low selenium diet on the collagen and proteoglycan of chicken cartilage, *Bull. Acad. Mil. Med. Sci.*, 1996, **10**, 26–29 (in Chinese).

90 J. Han, F. F. Yu, Z. P. Chang, B. Yang, C. J. Qu, T. T. Zhou, R. Y. Liu and X. Guo, Changing Grains for the Prevention and Treatment of Kashin-Beck Disease in Children: a Meta-analysis, *Biomed. Environ. Sci.*, 2015, **28**, 308–311.

91 J. B. Yang, Mechanisms in occurrence and prevalence of Kashin-Beck disease (KBD), *Chin. J. Endemiol.*, 1998, **17**, 201–206 (in Chinese).

92 J. B. Yang, Continued annotation on "Chinese strategy for control of Kaschin-Beck disease", *Chin. J. Endemiol.*, 2004, **23**, 3–6 (in Chinese).

93 S. G. Edwards, Investigation of Fusarium mycotoxins in UK wheat production, HGCA Project Report No. 413, London, 2007.

94 H. M. Muller, J. Reimann, U. Schumacher and K. Schwadorf, Natural occurrence of Fusarium toxins in oats harvested during five years in an area of southwest Germany, *Food Addit. Contam.*, 1998, **15**, 801–806.

95 Q. W. Li, D. A. Li, X. Q. Meng and X. D. Li, Experimental studies on elementary factors of Fusarium's growth and toxin production, *Chin. J. Endemiol.*, 1998, **17**, 355–358 (in Chinese).

96 D. J. Sun, J. B. Yang and X. D. Li, Determination of T-2 toxin in grain samples from markets in Harbin City using indirect competitive ELISA method based on monoclonal antibody, *J. Harbin Med. Univ.*, 1995, **29**, 283–285 (in Chinese).

97 Q. W. Li, D. A. Li, X. B. Tang, X. D. Li and G. Jiang, Report of T-2 toxin content in flour of KBD family in Xinghai county of Qinghai province, *Chin. J. Endemiol.*, 1999, **18**, 30–31 (in Chinese).

98 Y. Xie, G. J. Sun, C. L. Xiong, S. K. Wang, H. Wang and J. S. Wang, Determination of T-2 toxin content in staple food from KBD families in Xinghai county, Qinghai province, *Chin. J. Food Hyg.*, 2005, **17**, 157–159 (in Chinese).

99 F. G. Meng, Q. Li, Y. Fu, Z. J. Zhao, L. W. Zhou, H. Wang, H. Liu, D. A. Li and L. H. Wang, Investigation of state and influence factors of children's Kaschin-Beck disease in Xinghai county of Qinghai province in 2009, *Chin. J. Endemiol.*, 2012, **31**, 426–429 (in Chinese).

100 X. C. Wang, X. D. Liu, J. C. Liu, G. Wang and K. Y. Wan, Contamination level of T-2 and HT-2 toxin in cereal crops from Aba area in Sichuan Province, China, *Bull. Environ. Contam. Toxicol.*, 2012, **88**, 396–400.

101 Y. Fu, F. G. Meng, J. Y. Deng, X. Y. Fu, H. Huang, D. A. Li and L. H. Wang, Investigation on the selenium and T-2 toxin level in Kaschin-Beck disease relative active regions in Aba state of Sichuan province in 2008, *Chin. J. Endemiol.*, 2010, **29**, 325–329 (in Chinese).

102 C. H. Yang, X. Y. Luo, R. Ji and C. Liu, A survey of T-2 toxin in wheat by an indirect enzyme-linked immunosorbent assay, *Acta Microbiol. Sin.*, 1992, **32**, 450–455.

103 G. Xiong, Diagnostic, clinical and radiological characteristics of Kashin-Beck disease in Shaanxi Province, PR China, *Int. Orthop.*, 2001, **25**, 147–150.

104 F. L. Ren, X. Guo, R. J. Zhang, S. J. Wang, H. Zuo, Z. T. Zhang, D. Geng, Y. Yu and M. Su, Effects of selenium and iodine deficiency on bone, cartilage growth plate and chondrocyte differentiation in two generations of rats, *Osteoarthritis Cartilage*, 2007, **15**, 1171–1177.

105 W. Wang, S. Wei, M. Luo, B. Yu, J. Cao, Z. Yang, Z. Wang, M. B. Goldring and J. Chen, Oxidative stress and status of antioxidant enzymes in children with Kashine-Beck disease, *Osteoarthritis Cartilage*, 2013, **21**, 1781–1789.

106 F. Zhang, X. Guo, W. Wang, H. Yan and C. Li, Genome-wide gene expression analysis suggests an important role of hypoxia in the pathogenesis of endemic osteochondropathy Kashin-Beck disease, *PLoS One*, 2011, **6**, e22983.

107 M. Bennett, K. Macdonald, S. W. Chan, J. P. Luzio, R. Simari and P. Weissberg, Cell surface trafficking of Fas: a rapid mechanism of p53-mediated apoptosis, *Science*, 1998, **282**, 290–293.

108 P. Waring and A. Müllbacher, Cell death induced by the Fas/Fas ligand pathway and its role in pathology, *Immunol. Cell Biol.*, 1999, **77**, 312–317.

109 M. O. Hengartner, The biochemistry of apoptosis, *Nature*, 2000, **407**, 770–776.

110 S. Cory and J. M. Adams, The Bcl2 family: regulators of the cellular life-or-death switch, *Nat. Rev. Cancer*, 2002, **2**, 647–656.

111 E. Yang and S. J. Korsmeyer, Molecular thanatopsis: a discourse on the BCL2 family and cell death, *Blood*, 1996, **88**, 386–401.

112 A. G. Porter and R. U. Jänicke, Emerging roles of caspase-3 in apoptosis, *Cell Death Differ.*, 1999, **6**, 99–104.

113 K. S. Na, B. C. Park, M. Jang, S. Cho, do. H. Lee, S. Kang, C. K. Lee, K. H. Bae and S. G. Park, Protein disulfide isomerase is cleaved by caspase-3 and -7 during apoptosis, *Mol. Cells*, 2007, **24**, 261–267.

114 J. T. Liu, X. Guo, W. J. Ma, Y. G. Zhang, P. Xu, J. F. Yao and Y. D. Bai, Mitochondrial function is altered in articular chondrocytes of an endemic osteoarthritis, Kashine-Beck disease, *Osteoarthritis Cartilage*, 2010, **18**, 1218–1226.

115 Y. Wang, X. Guo, Z. T. Zhang, M. Wang and S. J. Wang, Expression of Caspase-8 and Bcl-2 in the cartilage loose bodies in patients with Kashin-Beck disease, *J. South Med. Univ.*, 2011, **31**, 1314–1317 (in Chinese).

116 S. J. Wang, X. Guo, F. L. Ren, Y. G. Zhang, Z. T. Zhang, F. J. Zhang and D. Geng, Comparison of apoptosis of articular chondrocytes in the pathogenesis of Kashin-Beck disease and primary osteoarthritis, *Zhongguo Yi Xue Ke Xue Yuan Xue Bao*, 2006, **28**, 267–270 (in Chinese).

117 S. J. Wang, X. Guo, H. Zuo, Y. G. Zhang, P. Xu, Z. G. Ping, Z. Zhang and D. Geng, Chondrocyte apoptosis and expression of Bcl-2, Bax, Fas, and iNOS in articular cartilage in patients with Kashin-Beck disease, *J. Rheumatol.*, 2006, **33**, 615–619.

118 B. D. Zhang, X. Guo, G. L. Bai, Z. G. Ping, H. Zuo, F. L. Reng, G. Y. Xu and D. Geng, The changes of nitric oxide, NO synthase and sFas/APO-1 in serum among the patients with Kashin-Beck disease, *Chin. J. Endemiol.*, 2004, **23**, 172–175 (in Chinese).

119 R. Visse and H. Nagase, Matrix metalloproteinases and tissue inhibitors of metalloproteinases: structure, function, and biochemistry, *Circ. Res.*, 2003, **92**, 827–839.

120 C. Y. Wu, R. H. Lei, M. Tiainen, S. X. Wu, Q. Zhang, F. X. Pei and X. Guo, Disordered glycometabolism involved in pathogenesis of Kashin-Beck disease, an endemic osteoarthritis in China, *Exp. Cell Res.*, 2014, **326**, 240–250.

121 W. Wang, X. Guo, J. H. Chen, P. Xu and M. J. Lammi, Morphology and phenotype expression of types I, II, III, and X collagen and MMP-13 of chondrocytes cultured from articular cartilage of Kashin-Beck Disease, *J. Rheumatol.*, 2008, **35**, 696–702.

122 S. Li, J. Cao, B. Caterson and C. E. Hughes, Proteoglycan metabolism, cell death and Kashin-Beck disease, *Glycoconjugate J.*, 2012, **29**, 241–248.

123 L. Troeberg and H. Nagase, Proteases involved in cartilage matrix degradation in osteoarthritis, *Biochim. Biophys. Acta*, 2012, **1824**, 133–145.

124 W. S. Tong and T. F. Yang, IL-1 and TNF bioassay in synovial fluid of patients with Kashin- Beck disease, *Chin. J. Control Endem. Dis.*, 2000, **15**, 71–72.

125 D. L. Yan, P. D. Kang, B. Shen, J. Yang, Z. K. Zhou and L. J. Duan, Serum levels of IL-1β, IL-6 and TNF-α in rats fed with Kashin-Beck disease-affected diet, *Int. J. Rheum. Dis.*, 2010, **13**, 406–411.

126 J. Estaquier, F. Vallette, J. L. Vayssiere and B. Mignotte, The mitochondrial pathways of apoptosis, *Adv. Exp. Med. Biol.*, 2012, **942**, 157–183.
127 C. Wang and R. J. Youle, The role of mitochondria in apoptosis, *Annu. Rev. Genet.*, 2009, **43**, 95–118.
128 K. N. Reed, G. Wilson, A. Pearsall and V. I. Grishko, The role of mitochondrial reactive oxygen species in cartilage matrix destruction, *Mol. Cell. Biochem.*, 2014, **397**, 195–201.
129 B. Cillero-Pastor, I. Rego-Pérez, N. Oreiro, C. Fernandez-Lopez and F. J. Blanco, Mitochondrial respiratory chain dysfunction modulates metalloproteases-1, -3 and -13 in human normal chondrocytes in culture, *BMC Musculoskeletal Disord.*, 2013, **14**, 235.
130 C. M. Thomas, C. J. Fuller, C. E. Whittles and M. Sharif, Chondrocyte death by apoptosis is associated with cartilage matrix degradation, *Osteoarthritis Cartilage*, 2006, **15**, 27–34.
131 T. Aigner and H. A. Kim, Apoptosis and cellular vitality: issues in osteoarthritic cartilage degeneration, *Arthritis Rheum.*, 2002, **46**, 1986–1996.
132 M. L. Circu and T. Y. Aw, Reactive oxygen species, cellular redox systems, and apoptosis, *Free Radicals Biol. Med.*, 2010, **48**, 749–762.
133 Y. E. Henrotin, P. Bruckner and J. P. Pujol, The role of reactive oxygen species in homeostasis and degradation of cartilage, *Osteoarthritis Cartilage*, 2003, **11**, 747–755.
134 A. J. Schuerwegh, E. J. Dombrecht, W. J. Stevens, J. F. Van Offel, C. H. Bridts and L. S. De Clerck, Influence of pro-inflammatory (IL-1 alpha, IL-6, TNF-alpha, IFN-gamma) and anti-inflammatory (IL-4) cytokines on chondrocyte function, *Osteoarthritis Cartilage*, 2003, **11**, 681–687.
135 M. J. López-Armada, B. Caramés, M. Lires-Deán, B. Cillero-Pastor, C. Ruiz-Romero, F. Galdo and F. J. Blanco, Cytokines, tumor necrosis factor-alpha and interleukin-1beta, differentially regulate apoptosis in osteoarthritis cultured human chondrocytes, *Osteoarthritis Cartilage*, 2006, **14**, 660–669.
136 J. C. Fernandes, J. Martel-Pelletier and J. P. Pelletier, The role of cytokines in osteoarthritis pathophysiology, *Biorheology*, 2002, **39**, 237–246.
137 Y. S. Li, Z. H. Wang, R. C. Beier, J. Z. Shen, D. De Smet, S. De Saeger and S. X. Zhang, T-2 toxin, a trichothecene mycotoxin: review of toxicity, metabolism, and analytical methods, *J. Agric. Food Chem.*, 2011, **59**, 3441–3453.
138 D. X. Mo, T-2 toxin: the pathogen of two endemic disease, *Chin. J. Control Endem. Dis.*, 1995, **10**, 294–298 (in Chinese).