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

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Running title: T-2 toxin involved in KBD

Abstract

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 damages and KBD pathological changes, seven electronic databases were searched to obtain epidemiological and experimental studies. For epidemiological studies, subgroup analysis of positive detection rate (PDR) of T-2 toxin and PDR of T-2 toxin with concentrations (PDRC of T-2) > 100 ng/g were carried out, together with histogram of the T-2 toxin concentrations among different food types in KBD and non-KBD areas. For experimental studies, systematic review of variety of chondrocytes and cartilage changes and damages induced by T-2 toxin were performed. As a result, in epidemiological studies, meta-analysis demonstrated that T-2 toxin PDR and the overall PDRC of T-2 toxin > 100 ng/g 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, T-2 toxin could induce the 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, detection amount of T-2 toxin has a more significant influence on KBD prevalence and development as compared to T-2 toxin detection rate. Besides, T-2 toxin induces chondrocytes and cartilage damages through apoptosis, catabolism promotion and intracellular impairments, which are similar to KBD changes.

Key words: T-2 toxin; Kashin-Beck Disease; Detection; Chondrocytes; Cartilage; Damage

Highlights (Main results)

1. The overall PDR of T-2 toxin and PDRC of T-2 toxin > 100 ng/g in food samples was higher in endemic areas than those in non-endemic areas, especially in wheat powder.
2. T-2 toxin contamination in food samples, especially in wheat flour was more serious in endemic areas than that in non-endemic areas.
3. The effects of T-2 toxin in both *in vitro* and *in vivo* studies included the damage of chondrocytes morphology, nucleus, cytoplasm, organelle, and membrane.
4. T-2 toxin showed a restriction effect on the viability and proliferation of chondrocytes as well as antioxidant capacity related to mitochondrial damage.
5. T-2 toxin induced apoptosis in chondrocytes, mainly through Fas and p53 up-regulation following Bcl-2 family and caspases alteration.
6. T-2 toxin perturbed the synthesis of proteoglycan and collagens, leading to metabolic disturbance in the ECM.

1 Introduction

T-2 toxin, a kind of trichothecene mycotoxin, is produced by Fusarium Fungus.[1] In 1968, T-2 toxin was separated and purified for the first time by Bamburg *et al*.[2] With a wide range of distribution in many parts of the world,[3] T-2 toxin can be detected in approximately 20% of food samples from 12 European Union countries.[4] Meanwhile, it has been reported that 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 T-2 toxin. Moreover, T-2 toxin contamination shows no specificity to food samples, which can occur in a number of field crops (wheat, maize, barley and oats) and processed grains (malt, beer and bread).[1] T-2 toxin is demonstrated with a variety of toxic effects on both experimental animals and humans, including dermal toxicity, lethal effect with disruption of central nervous system, inhibition of protein, DNA and RNA synthesis[6] as well as the damage of chondrocytes and cartilage.

Kashin-Beck disease (KBD), an endemic, chronic and deformed osteoarthropathy disease, is firstly 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 the endemic areas 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 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 T-2 toxin in endemic food samples remains at a high level (2.0-1549.4 ng/g, with an average of 468.7 ng/g).[8] In addition, it is also reported that the pathologic changes of the cartilage from chicks fed with food containing T-2 toxin are quite similar to KBD patients in animal studies.[8] However, it is still difficult to confirm that T-2 toxin is one of the important etiological factors for KBD, because the discrepancies existed in the detection rate and detection amount of T-2 toxin 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 area 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 the KBD prevalence and development, as well as the role of T-2 toxin on chondrocyte or cartilage damage in human or animal subjects and its mechanisms.

2 Materials and methods

2.1 Search strategy

According to the search strings: for epidemiological studies, searching items of "KBD" or "Kashin-Beck disease", "T-2 toxin" and "Endemic detection" were used; and for experimental studies, searching items 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 Literature Database), and Wan Fang database were used independently for the search process together with other relevant published studies. There were no restrictions to the languages, dates, designs and publications of the study. 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 T-2 toxin, the specimens should be food samples, positive detection rates (PDRs) or average contents 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 chondrocytes

130 or cartilage damage, and the researches of T-2 toxin plus other interventions would be
131 excluded. Any studies would be excluded if they were failure to one of the criteria.
132
133 **2.3 Study selection**
134 Firstly, all included titles were screened by three reviewers (LDY, HJ and YFF) in
135 order to remove duplicate studies. Then the abstracts of the selected studies were
136 reviewed if they met the selection criteria. Any articles that did not match the
137 standards were excluded. And after full-text articles assessed for eligibility, some of
138 them were eliminated because of data duplication or inconformity to the criteria.
139
140 **2.4 Methodical evaluation**
141 For the epidemiological studies, after carefully reviewed, all the included studies were
142 found to be cross-sectional studies. Thus AHRQ (Agency for Healthcare Research and
143 Quality) standard[12] was applied for assessing the studies. According to the standard,
144 11 items (Table 1) were evaluated by answering with "Yes", "No" or "Unclear"
145 respectively, including the source of information, the character of subjects, and the
146 quality assessment of the articles and so on.
147 Experimental studies were divided into *in vitro* studies and *in vivo* studies. Due
148 to a lack of an agreed evaluation standard at present, the "Evidence Pyramid"[13] and
149 the grading system by the previous studies[14, 15] were used. For the *in vitro* studies, the
150 articles were evaluated according to the following standards: A. systematic reviews
151 (including meta-analyses) of studies *in vitro*; B. with comparable baseline; C. baseline

unknown; D. no comparable baseline. For the *in vivo* studies, the evaluation standards were used as following: A. systematic reviews (including meta-analyses) of study in animals; B. randomized controlled study, or inbred animal study; C. controlled study; D. non-controlled study.

2.5 Data extraction and collection

For the epidemiological studies, data were extracted from the 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 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 PDR of T-2 toxin and PDR of T-2 toxin with concentrations (PDRC of T-2 toxin) > 100 ng/g in KBD and non-KBD areas was performed according to food types by 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. A "Fixed-effect" model was used when heterogeneity was statistical insignificant, otherwise a "Random-effect" model was used (when $P < 0.05$) to pool RRs. Low, moderate and high heterogeneity was considered when I^2 = 25%, 50%, 75% separately. In addition, histogram of the T-2 toxin concentrations in various food types from endemic and non-endemic regions was shown by Microsoft Excel 2003.

In the experimental studies, we reviewed the effects of T-2 toxin on chondrocytes and cartilage from human 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 extracellular matrix in cartilage were investigated as well. The supposed toxic mechanism of T-2 toxin on the prevalence and development of KBD, including chondrocytes and cartilage damages through apoptosis, catabolism promotion and intracellular impairments, were proposed by drawing a conclusion from the extracted data.

3 Results

3.1 Search results and study quality

Total of 1999 citations were initially included in this article. After the titles or

196 abstracts were reviewed, 82 articles were enrolled for full text reviewing. Finally, 72
197 articles were selected and assessed against the exclusion criteria, including seven
198 epidemiological articles and 65 experimental articles [33 *in vitro* studies and 33 *in*
199 *vivo* studies (one article covers both *in vitro* and *in vivo* study)[16]] (Figure 1).

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

207

208 **3.2 Accumulation of T-2 toxin in food samples of epidemiological studies**

209 **Characteristics of epidemiological studies**

210 The characteristics of all included 15 epidemiological studies in seven articles[17-23]
211 were shown in Table 2. Most of the investigations were performed from 1990 to 2010
212 in Northwest and Northeast of China. Four kinds of food including wheat flour (six
213 studies), wheat (two studies), corn flour (five studies) and rice (two studies), were
214 investigated in these studies. Ten food studies showed the results of PDR of T-2 toxin
215 with a maximum rate of 100% in five KBD and one non-KBD areas.[19, 22] The highest
216 contents of T-2 toxin in the average of wheat flour samples were 468.7 ng/g in
217 endemic regions[23] and 152.1 ng/g in control regions,[19] respectively.

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 T-2 toxin between endemic and normal areas (Figure 2). The heterogeneity of the studies was examined with "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 overall PDR of 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, T-2 toxin detection rate in wheat flour was a bit higher in KBD areas than that in control areas, but no obvious difference were observed on T-2 toxin detection rate in 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 on 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, $P = 0.459$).

Meta-analysis of PDRC of T-2 toxin > 100 ng/g in epidemiological studies

Total of four studies in three articles[20, 21, 23] were included for assessing the PDRC of T-2 toxin > 100 ng/g in different subgroups for meta-analysis (Figure 3). Since the heterogeneity of studies was insignificant 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), "Fixed-effect model" was applied. The overall PDRC of T-2 toxin > 100 ng/g 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, PDRC of T-2 toxin > 100 ng/g 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).

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 nine studies in six articles (Figure 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 Food and Agriculture Organization (FAO) standard related to food contamination with T-2 toxin (the maximum detection of T-2 toxin < 100 ng/g),[24] the average contents of T-2 toxin in five studies were above 100 ng/g (three wheat flour samples and two corn flour samples in endemic areas, and one corn flour sample in non-endemic area) among all nine studies. More seriously, the average contents of 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,[18, 19] which exceeded 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 chondrocytes damage and cell proliferation

Total of 12 *in vitro* studies[26-37] were involved in the assessment of damage effects of T-2 toxin on chondrocytes morphology. As shown in Table 3, T-2 toxin at different doses could induce the damages of cell structure in human fetus, Wistar rat and rabbit with a decrease in cell density and increase of cell separation, and incomplete cytomembrane when observed by inverted/light microscope. Scanning electron microscopy (SEM) images showed that collagen microfibrils and cytoskeleton were decreased in chondrocytes from chicken embryo treated with T-2 toxin. Furthermore, the results of transmission electron microscope showed that nucleus, cytoplasm and endoplasmic reticulum damage could be found in most chondrocytes of 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 activity of chondrocyte could be visible from 14 *in vitro* studies (Table 4).[27, 28, 38-49] This effect was independent from the concentration of 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 human, human fetus and broiler chicken was significantly increased with 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 treated with T-2 toxin. In Bcl-2 family, Bax mRNA and protein expression were up-regulated, whereas Bcl-xL expression was down-regulated after treatment with T-2 toxin. The ratio of Bcl-2/Bcl-xL at protein level was consistent in different studies. Moreover, both caspase-9 and caspase-3 at protein and mRNA level increased after T-2 toxin treatment. In addition, JNK, p38 and mitochondria pathways were involved in mediating apoptosis by T-2 toxin.

Metabolism of chondrocytes

The metabolic inhibition of T-2 toxin-treated chondrocytes was found in 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 gene and protein levels, aggrecanase-1, 2 mRNAs and a disintegrin and metalloproteinase with thrombospondin motifs 4, 5 (ADAMTS 4, 5) proteins, pro-inflammatory factors such as IL-1β, IL-6 and TNF-α were increased. Meanwhile, tissue inhibitors of metalloproteinase 1-3 (TIMP 1-3) and 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 were still controversial. Additionally, other factors such as CD44, hyaluronan synthetase 2 (HAS2) and integrins at the mRNA and protein level were also changed.

Other intracellular changes in chondrocytes (Table 7)

Alteration of DNA and proteins

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

Mitochondria damage

All four *in vitro* studies[35-37, 40] referred to the damage of mitochondria showed that T-2 toxin destroyed the antioxidant defense system, including the inhibition of glutathione peroxidase (GPx) activity and intracellular glutathione (GSH) content. 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 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 T-2 toxin in chondrocytes from human and chick embryo.

Oxidative stress

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

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 of T-2 toxin. The expression of inducible nitric oxide synthase (iNOS) also had a significant promotion when treated with 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: damages of epiphyseal growth plate, articular cartilage and chondrocyte necrosis in cartilage after 7-day exposure to T-2 toxin, which could be classified as short term toxic effect of T-2 toxin; and the injury of epiphyseal growth plate, articular cartilage and chondrocyte in 1-6 months, which would be the consequences of subchronic toxicity effect of T-2 toxin. However, no

effects of T-2 toxin treatment have been found in two studies, which showed no damage on epiphyseal growth plate after T-2 toxin treatment.[73, 78] In addition, a study by Pang *et al* [72] reported a reduction of bone mineralization rate after 4 week exposure to 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 damages of epiphyseal growth plate in cartilage of Wistar rats after eight weeks exposure.

Intracellular changes in cartilage (Table 9)

Cell growth and metabolism

The inhibition effects of 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/kg BW/d T-2 toxin exposure for 5 or 8 weeks. During the exposure of 1.0 mg/kg BW/d T-2 toxin for 1 week in 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/kg BW/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 substances (TBARS) content in the cartilage of SD rats fed with 100, 200 ng/g BW/d T-2 toxin in four weeks. Glutathione peroxidase (GSH-Px), glutathione peroxidases (GPX), SOD and CAT at protein and mRNA

levels were decreased.

Apoptosis

With 200 ng/g BW/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 were increased in costal cartilage of SD rats after T-2 toxin treatment.

Metabolism of extracellular matrix in cartilage

Changes in cartilage matrix metabolism[16, 59-64, 68, 73, 75, 76, 81, 88, 89] induced by T-2 toxin were listed in Table 10. In SD rat's cartilage, T-2 toxin at concentration of 100-200 ng/g BW/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 T-2 toxin significantly decreased 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 cartilage from Wistar rats after 6 months, but fibrils appeared at 3 months from 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/g BW/d T-2 toxin. Proteoglycan and its composition (sulfate groups, hexosamine and glucuronic acid) were decreased in cartilage of Wistar rats after 3-6 months of T-2 toxin intervention. Similarly in cartilage from SD rats and chicks fed with T-2 toxin, total PG, sulfated glycosaminoglycan (sGAG), keratan sulfate and chondroitin sulfate were

394 also decreased in 4-9 weeks.

395

396 **4. Discussion**

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

398 In generally, subgroup meta-analysis showed that the overall PDR of T-2 toxin
399 and PDRC of T-2 toxin > 100 ng/g in food samples was higher in endemic areas,
400 especially in wheat powder. Moreover, T-2 toxin contamination in wheat flour was
401 more serious in KBD endemic areas as compared to non-endemic areas.

402 A recent study by meta-analysis of community-based trials of changing grains
403 has demonstrated its benefits for the prevention and treatment of KBD in China,[90]
404 which verified that local food might be one of the factors for KBD incidence. As T-2
405 toxin contamination was the most investigated food contamination in KBD regions,
406 more attention should be paid to the causes of accumulating T-2 toxin as well as the
407 methods of controlling and reducing T-2 toxin in staple food. First of all, because of
408 the climate and soil situation in KBD areas, local residents preferred to cultivate
409 wheat and corn,[8, 91] and use wheat flour as their main staple food. However, these
410 areas were marked with cold temperature and humid environment,[8, 91, 92] which
411 provided suitable condition for T-2 toxin synthesis.[93-95] Thus, it would be better for
412 local people to use rice for their staple food, which was also proposed in the study by
413 Sun *et al*.[96] Secondly, in local endemic areas, inadequate food farming, harvesting and
414 processing procedures also increased the opportunity of T-2 toxin propagation.[97, 98, 99]
415 When most of the cereals and foodstuffs were placed in moist storage environment

and bad sanitary conditions, it might induce more production of poisoned T-2 toxin.[92, 97-101] Therefore, the environment for grain processing and storage should be improved such as improving hygiene 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 PDR of T-2 toxin was up to 60% in most non-endemic survey sites, and PDRC above 100 ng/g T-2 toxin was found in food samples from three non-endemic regions. This phenomenon suggested that 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, PDRC of T-2 toxin at 100 ng/g in food was claimed as a heavy T-2 toxin pollution. While according to World Health Organization (WHO) standard, a maximum tolerable daily intake of T-2 toxin was less than 60 ng/kg of body weight per day (which equaled to a daily consumption of 500 g staple food containing 7.2 ng/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.

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

Comparison of morphological and ultrastructure damages

The effects of T-2 toxin in both *in vitro* and *in vivo* studies including the damage of

chondrocytes morphology, nucleus, cytoplasm, organelle, and membrane were investigated. T-2 toxin caused a short term and subchronic toxicity to chondrocytes and induced damages at subcellular, cellular and tissue levels without species specificity. When compared with the characteristics of KBD patients, some changes of chondrocytes and cartilages induced by T-2 toxin were quite similar such as focal chondronecrosis in the hypertrophic zone of growth plate and in the deep zone of articular cartilage,[103, 104] suggesting that T-2 toxin-induced chondrocytes 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 T-2 toxin could be associated with apoptosis, metabolism alteration and intracellular changes.

Comparison of proliferation and alterations of antioxidant capacity

The results from MTT and cell counting showed a restriction effect of 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 chondrocytes proliferation and metabolism. Besides, the increase of superoxide with decreased antioxidant ability might be responsible for oxidative stress. ROS, MDA, TBARS were the factors mediating lipid peroxidation activated by T-2 toxin. In contrast, antioxidants such as GSH, 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 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 T-2 toxin-intervened chondrocyte or cartilage. Meanwhile, ROS was increased as one of the mitochondrial apoptotic factors by T-2 toxin treatment. T-2 toxin restrained the activities of complexes, H^+-ATP enzyme and cytochrome C oxidase, a manifest 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 these consequences mentioned above indicated a connection of chondrocytes changes between T-2 toxin exposure and KBD.

Comparison of apoptosis changes

As mentioned above, T-2 toxin induced apoptosis in chondrocytes from human and animals. 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 important regulator of apoptosis was altered,[109, 110] especially the expression of Bax in mRNA and protein levels as well as the ratio of Bax/Bcl-2 and Bax/Bcl-xL at protein level. A previous study has shown that the ratio of pro-apoptotic and anti-apoptotic proteins in Bcl-2 family might be the core factor of apoptosis process,[111] so the increase of heterodimerization of Bcl-2 family indicated chondrocytes apoptosis induced by 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, T-2 toxin might induce Fas and p53 up-regulation following Bcl-2 family and caspases alteration, which resulted in chondrocytes apoptosis. In KBD patients, previous studies have demonstrated that the expression of Fas, Bax, Bcl-2 and caspases in chondrocytes was also rised,[114-117] thus, the mechanism of chondrocytes apoptosis induced by T-2 toxin is linked to KBD pathogenesis. Besides, T-2 toxin also caused other mechanisms related to apoptosis such as NO and mitochondrial-related pathways which needed more experiments to confirm. Furthermore, NO content and iNOS expression were elevated in the serum of KBD patients as well as in the chondrocytes after exposed to T-2 toxin.[118]

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, T-2 toxin perturb 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 cartilage, collagen changed after exposure to 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 T-2 toxin.[119] Simultaneously, 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, T-2 toxin enhanced pro-inflammatory factors including TNF-α, IL-1β and IL-6. All of them act as a kind of catabolic cytokines resulting in matrix degradation. Some other molecules such as CD44 and integrins related to chondrocytes metabolism were also influenced by T-2 toxin, as certified in chondrocytes catabolism promotion. In summary, after cartilage or chondrocytes exposed to 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 low type II collagen expression[120, 121] and decreased PG.[10, 122] MMP-13 was elevated in 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 chondrocytes and cartilage between KBD and T-2 toxin intervention.

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 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

ago in Northeast of China. Therefore, high-quality and well-designed experiments are required. It is suggested that survey locations could expand in more KBD regions and focus more on T-2 toxin concentration in different food types with a unified measurement control condition. The studies should also reveal informations, such as the effect of evaluators of subjective components of study, the handling of missing data from analysis, but few of them referred to the evaluation of confounding factors so that the results may be limited by potential bias and confounding factors. As 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 contents in food. In addition, the relationship between T-2 toxin and other factors still needs to be investigated in future studies.

For experimental studies included in this article, they were almost B level as high quality but the evaluation standard is insufficient. Further standard needs to be improved to assess relevant experimental studies accurately. According to our results, T-2 toxin could destroy the chondrocytes and cartilage through a variety of pathways including apoptosis, changes of metabolism, DNA and protein, oxidative stress, mitochondria damage and NO synthesis. Some of these pathways are linked with 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 chondrocytes apoptosis as well.[135, 136] However, whether T-2 toxin has direct interventions or indirect effects on these connected pathways and the involved factors are not completely confirmed yet. Moreover, since 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 effect of T-2 toxin and HT-2 toxin in *in vitro* experiments as well. Finally, cartilage is not the only targeted organ of T-2 toxin, some articles[83, 137] reported that T-2 toxin could result in damages in other organs such as 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 T-2 toxin on these organs and diseases are also needed to investigate in further studies.

In order to confirm the etiology of KBD, the most convincing evidence is in accordance with the results from cohort study and case-control study in epidemiology. But no studies have directly shown the causality of T-2 toxin and KBD at present. Further confirmation of the etiologic relationship is needed in the subsequent epidemiological investigation. Moreover, with further investigations resulting in the definition of clear clinical signs of T-2 toxin detection rate in KBD patients, we may draw a more reasonable conclusion about the effects of T-2 toxin on KBD prevalence. However, no data on the T-2 toxin concentration in human body has been obtained in any of the studies yet. This review indicates a high-degree of similarity in the

570 pathology and mechanism of T-2 toxin and KBD. Combining with the summarized
571 results of cross-sectional studies and experimental studies, T-2 toxin is a likely cause
572 for KBD prevalence. But to some extent, the conclusion is still preliminary. Current
573 experimental studies have only provided a possible explanation for T-2 toxin on the
574 pathogenesis of KBD based on similar comparison results, and a correlation between
575 KBD and T-2 toxin is simply presented in cross-sectional, *in vitro* and *in vivo* studies,
576 which lack of population-based studies due to ethics. Our present results may provide
577 a new insight for better understanding the effect of T-2 toxin on the etiology and
578 pathogenesis of KBD.
579

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587 **Conflict of interests**

588 The authors declare no conflicts of interest. The author's affiliation is as shown on the
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591

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Figure Legends

Figure 1 Flow chart of study selection process

Figure 2 Subgroup analysis of positive detection rate of T-2 toxin in endemic and non-endemic areas

Figure 3 Subgroup analysis of positive detection rate of T-2 toxin with concentrations > 100 ng/g in endemic and non-endemic areas

Figure 4 Histogram of T-2 toxin contents in endemic and non-endemic areas (EA: endemic areas; NEA: non-endemic areas; *: T-2 toxin average contents > 100 ng/g; **: T-2 toxin average contents > 200 ng/g)

Table Legends

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

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

Table 3 Morphological damages in chondrocytes

Table 4 Cell viability and proliferative activity of chondrocytes

Table 5 Apoptosis in chondrocytes

Table 6 Metabolism of chondrocytes

Table 7 Other intracellular changes in chondrocytes

Table 8 Morphological and radiological changes in cartilage

Table 9 Intracellular damages in cartilage

Table 10 Metabolism of cellular matrix in cartilage

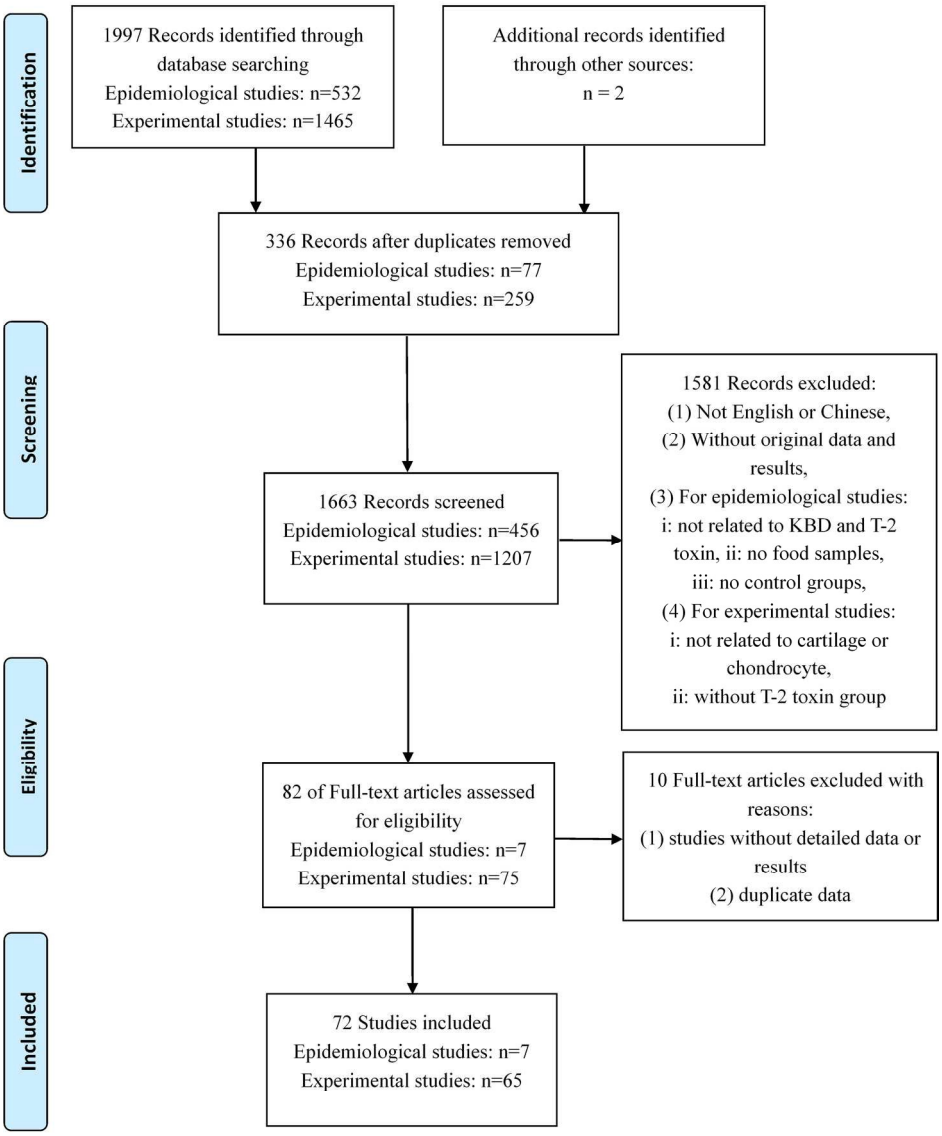

Figure 1 Flow chart of study selection process
192x247mm (300 x 300 DPI)

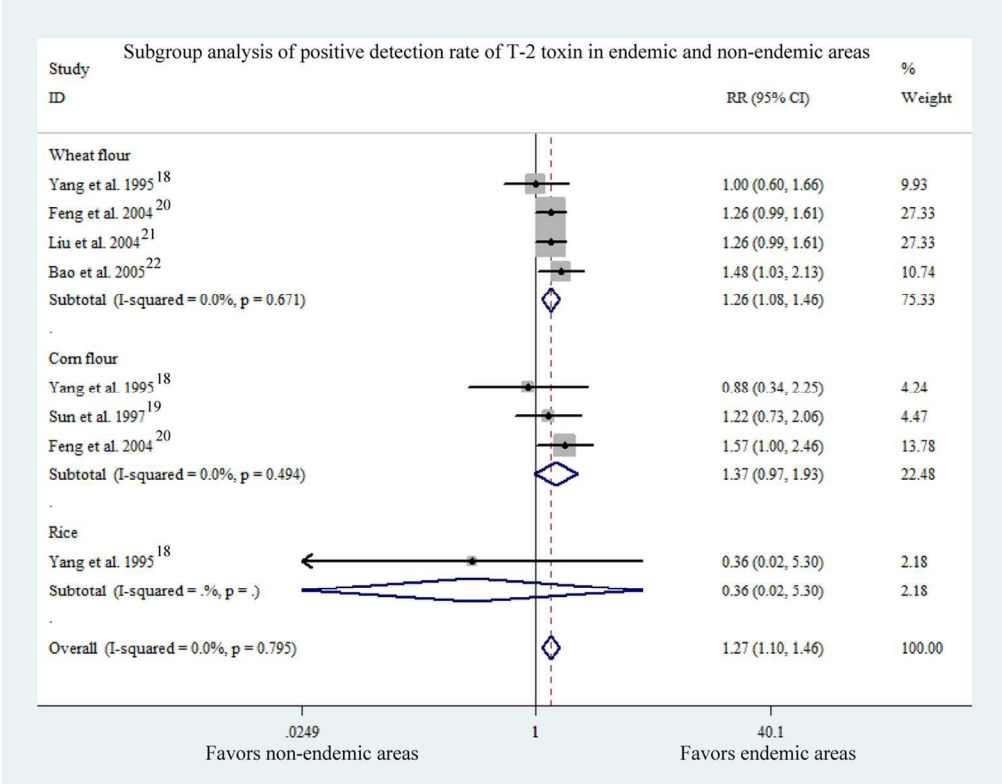

Figure 2 Subgroup analysis of positive detection rate of T-2 toxin in endemic and non-endemic areas
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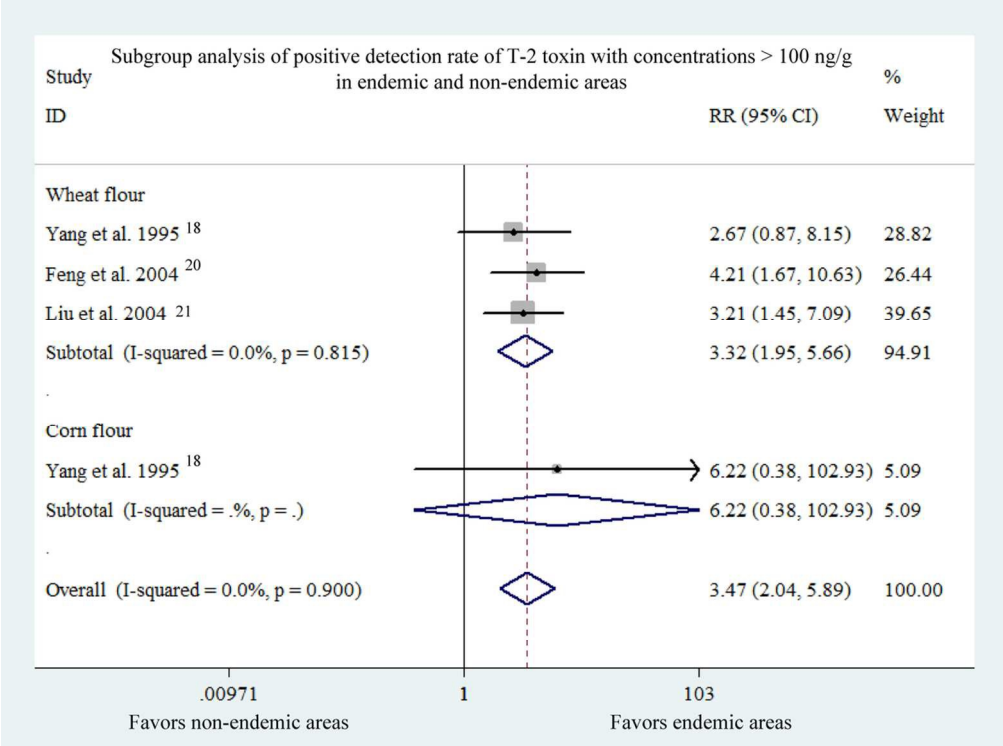

Figure 3 Subgroup analysis of positive detection rate of T-2 toxin with concentrations > 100 ng/g in endemic and non-endemic areas
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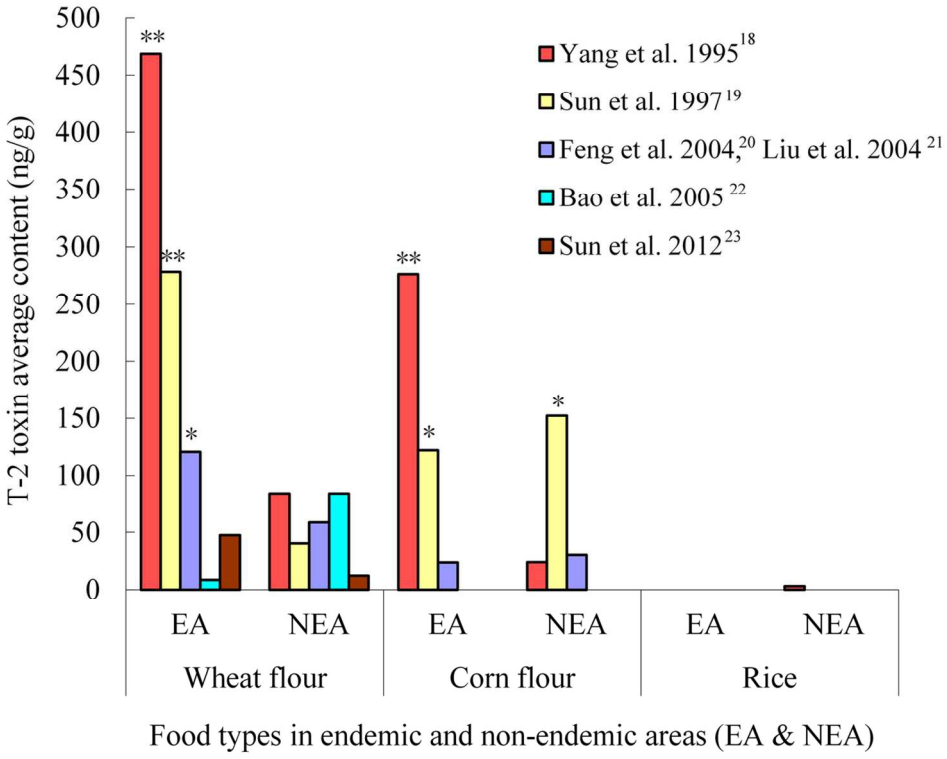

Figure 4 Histogram of T-2 toxin contents in endemic and non-endemic areas (EA: endemic areas; NEA: non-endemic areas; *: T-2 toxin average contents > 100 ng/g; **: T-2 toxin average contents > 200 ng/g)
121x97mm (300 x 300 DPI)

Table 1 Methodological quality of cross-sectional studies according to 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.

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

Ref	Sites	Food type	Endemic areas					Non-endemic areas				
			Number of Samples			T-2 toxin (ng/g)		Number of Samples			T-2 Toxin (ng/g)	
			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 couty 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.

Table 3 Morphological damages in chondrocytes

Ref	Sources	Interventions		Outcomes						
		T-2 toxin	Time	LM	SEM	TEM (Damages of)				
						Membrane	Mitrochondria	Endoplasmic reticulum	Nucleus	Cytoplasm
Chen et al.2005[26]	Human fetus	1, 10, 20 μg/L	5 d				Y	Y	Y	Y
Chen et al.2006[27]	Human fetus	10 ng/mL	5 d						Y	Y
Chen et al.2006[28]	Human fetus	1, 10, 20 ng/mL	5 d				Y	Y	Y	Y
Li et al.2008[29]	Human fetus	0.01 μg/mL	18 d	Nucleus fragmentation↑, Integral cytomembrane↓, Cell ghosts↑						
Huo et al.1998[30]	Wistar rat	0.0005, 0.001, 0.005 mg/L	2 d, 4 d	Cell density↓				Y	Y	Y
Wang et al.2005[31]	Wistar rat	0.5, 1.0 μg/L	1 d	Cell falls off↑			Y	Y	Y	Y
Cao et al.1994[32]	Rabbit	0.005, 0.01, 0.02 μg/mL	2 d, 4 d	Cell density↓, Cell falls off↑						
Cao et al.1995[33]	Rabbit	0.005, 0.01, 0.02 μg/mL	4 d	Cell proliferation↓, Cell density↓		Y	Y	Y	Y	Y
Cao et al.1995[34]	Rabbit	0.005, 0.0l, 0.02 μg/mL	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 particles reduction.
Mitrochondria: vacuolar degeneration, medullary change, and cristae fractured.
Endoplasmic reticulum: cystic dilatation.
Nucleus: nuclear condensation, nuclear membrane thickening, defect, and uneven distribution of chromatin.
Cytoplasm: the number of organelles reduction and fuzzy, the number of cytoplasmic lysosomes, vacuoles, medullary structure increase, some constituent in the cytoplasm dissolution.

Table 4 Cell viability and proliferative activity of chondrocytes

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

↓: decreased.

Table 5 Apoptosis in chondrocytes

Ref	Sources	Interventions		Outcomes				
		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	16 h	Y				Apoptosis according to TUNEL staining↑
Chen et al.2005[26]	Human fetus	1,10,20 μg /l /10 μg /l	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	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	5 d	Y	Fas (P)↑			NO↑, iNOS↑
Chen et al.2008[44]	Human fetus	1, 10, 20 ng/ml	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	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	5 d		P53 (P, R)↑	Bcl-xL (P)↓, Bcl-xL (R) (-)	Caspase-3 (P, R)↑	
Han et al.2013[39]	Human	20 ng/ml	3 d/ 24 h	Y				AFT2，JNK and p38↑
Liu et al.2014[40]	Human	1,10,20 ng/ml	5 d	Y			Caspase-3, 9 (P)↑	Cytochrome c release↑
He et al.2011[46]	Broiler chicken	5,50,500 nmol/l	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

Ref	Sources	Interventions		Outcomes					
		T-2 toxin	Time	MMPs, Aggrecanase	TIMPs,α2M	ILs, TNFs	Collagens	PG, Aggrecan	Others
Yang et al.2001[41]	Human fetus	8 μg /l	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	5 d				Type II (P, R)↓	Aggrecan (P, R)↓	
Li et al.2008[29]	Human fetus	0.01 μg/ml	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/ 10 ng/ml	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	5 d	Aggrecanase-1, 2 (R)↑				Aggrecan (P)↓	
Lu et al.2012[54]	Human fetus	0.01 μg/ml	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	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	24 h	MMP-13 promoter↑					
Cao et al.2007[55]	Wistar rat	0.4, 0.8, 1.6, 3.2 μg /l	24 h	MMP-13 (P)↑					
Tian et al.2012[49]	Murine (ATDC5)	20 μg/l/ 10-80 μg /l	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	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	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		Outcomes
		T-2 toxin	Time	
Alteration of DNA and proteins				
Li et al.2008[29]	Human fetus	0.01 μg/ml	5 d	DNA content↓
Cao et al.1994[32]	Rabbit	0.005,0.01,0.02 μg/ml	4 d	DNA content↓, GLcUA content in matrix↓
Huo et al.1998[30]	Rabbit	0.0005,0.001,0.005 mg/l	4 d	DNA content↓, Protein content ↓
Wang et al.2006[56]	Wistar rat	1,10,100 μg/l	24 h	DNA damage↑
Mitochondria damage				
Liu et al.2014[40]	Human	1,10,20 ng/ml	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	48 h	ROS↑, MDA↑, CAT↑, SOD↑, ALP↓, GSH↓
Tian et al.2012[49]	Murine (ATDC5)	10, 20,40 μg/l	1- 24 h	ROS↑
NO synthesis				
Chen et al.2006[28]	Human fetus	1, 10, 20 ng/ml	2 d,5 d	NO↑, iNOS↑
Yang et al.2008[57]	Human fetus	1, 10, 20 μg/l	2 d,5 d	NO↑, iNOS↑

↑: increased; ↓: decreased; (-): unchanged.

Table 8 Morphological and radiological changes in cartilage

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

Wang et al.2006[81]	Chicks	100, 600 μg/kgBW/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/kgBW/d	8, 12 w	Y		
Kang et al.2013[68]	Wistar rats	0.1 mg/kgBW/d	8, 12 w	Y		
Yan et al.2014[69]	Wistar rats	0.04 mg/kgBW/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, cells 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, focally 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 damages in cartilage

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

↑: increased; ↓: decreased; (-): unchanged; BW: body weight.

Table 10 Metabolism of 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 /kgBW/2d	100 d			Total collagen↓ (SP)	Sulfate groups↓ (SP) , Hexosamine↓ (SP) , Glucuronic acid↓ (SP)
Kang et al.2009[59]	Wistar rats	1 mg/kgBW/d	2, 4 w			Total collagen↓(MS)	
Wang et al.2009[60]	Wistar rats	100 ng/g	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/d	1, 2, 4 w			Total collagen↓ (MS)	
Yao et al.2010[61]	Wistar rats	1 mg/kgBW/d	2, 4 w			Total collagen↓ (MS)	
Yao et al.2010[62]	Wistar rats	10 mg/kgBW/d	4 w			Total collagen↓ (MS)	
Meng et al.2011[64]	Wistar rats	100, 200, 300 μg/kg	6 m			Collagen fibers breakage↑ (SEM)	PG↓ (SEM)
Kang et al.2013[68]	Wistar rats	0.1 mg/kgBW/d	8, 12 w			Total collagen↓ (MS)	
Chen et al.2010[73]	SD rats	100, 200 ng/g BW/d	12 w			Fibrils appear↑ (HE)	
Guan et al.2013[75]	SD rats	100, 200 ng/g BW/d	4 w				sGAG↓ (TB)
Chen et al.2014[16]	SD rats	100 μg/kgBW/d	30 d	MMP-13↑ (IH)			
Zhou et al.2014[76]	SD rats	100, 200 ng/gBW/d	4 w		IL-6↑,IL-lβ↑,TNF-α↑, IL-6 mRNA↑, IL-lβ mRNA↑ TNF-α mRNA↑		sGAG↓ (TB)
Hu et al.1996[89]	Chicks	0.4 mg/kgBW	9 w			Type I↑, type II↓ (IH)	Keratan sulfate↓, Chondroitin sulfate↓ (HC)
Wang et al.2006[81]	Chicks	100,600 μg/kgBW/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: inmmunohistochemistry; SEM: scanning electron microscope.

The interpretations of high T-2 toxin detection rate and amount in endemic areas on Kashin-Beck diseas prevalence and development.