

1 **Strong negative association between intake of Tofu and anemia among Chinese**
2 **adults in Jiangsu China**

3
4 Zumin Shi, MD, PhD^{1,2*}, Xiaoshu Hu, MD¹, Baojun Yuan, MD¹, Xiaoqun Pan, MD¹,
5 Yue Dai, MD¹, Gerd Holmboe-Ottesen, PhD³, Julie E Byles, MD, PhD²
6
7

8 ¹ Jiangsu Provincial Center for Disease Control and Prevention, Nanjing, China.
9

10 ² Research Center for Gender, Health and Ageing, Hunter Medical Research Institute,
11 The University of Newcastle, Australia.
12

13 ³ Section of Preventive Medicine and Epidemiology, Institute of General Practice and
14 Community Medicine, University of Oslo, P.O Box 1130, N- 0318 Oslo, Norway.
15
16

17 Z. Shi is an associate professor of Jiangsu Provincial Center for Disease Control and
18 Prevention, China, and research fellow of Research Center for Gender, Health, and
19 Ageing, The University of Newcastle, Newcastle, Australia.

20 X. Hu is a professor of Jiangsu Provincial Center for Disease Control and Prevention,
21 Nanjing, China.

22 B. Yuan is a professor of Jiangsu Provincial Center for Disease Control and
23 Prevention, Nanjing, China.

24 X. Pan is an associate professor of Jiangsu Provincial Center for Disease Control and
25 Prevention, Nanjing, China.

26 Y. Dai is an associate professor of Jiangsu Provincial Center for Disease Control and
27 Prevention, Nanjing, China.

28 G. Holmboe-Ottesen is a professor at the Institute of General Practice and Community
29 Medicine of University of Oslo, Oslo, Norway

30 J.E. Byles is a professor at the Research Center for Gender, Health and Ageing,
31 Hunter Medical Research Institute, The University of Newcastle, Newcastle,
32 Australia.
33

34 **Address of corresponding author:**
35 Zumin Shi, MD, PhD
36 Department of Nutrition and Food Hygiene,
37 Jiangsu Provincial Center for Disease Control and Prevention.
38 172 Jiangsu Road, Nanjing 210009, P. R. China.
39 Telephone number:+86-25-83759341
40 Fax number: +86-25-83759341
41 E-mail: zumins@vip.sina.com
42

43 **Running head:** Tofu intake and anemia of Chinese adults.
44 Word count: Text 2741 Abstract 250
45

46 **Abstract**

47 **Background:** Anemia is prevalent in China. Tofu made from soybean is a popular
48 food. Soybean is shown to have an effect on iron status. No study has examined the
49 relation between tofu and iron status.

50 **Objective:** To investigate the association between tofu intake and anemia among
51 Chinese adults.

52 **Design:** A cross-sectional household survey of 2849 men and women aged 20 years
53 and over (mean 47.0, standard deviation [SD] 14.5), from a nationally representative
54 random sample in Jiangsu province undertaken in 2002 (response rate 89.0%). Tofu
55 intake was assessed by food frequency questionnaire. Nutrient intake was measured
56 by three day weighed food records. Serum ferritin (SF), and hemoglobin (Hb) were
57 measured.

58 **Results:** The prevalence of anemia was 18.3% in men and 31.5% in women. Mean
59 Hb increased by quartiles of tofu intake (men: 14.1, 14.0, 14.5 and 14.8; women: 12.4,
60 12.5, 12.6 and 13.3 g/dl), while the prevalence of anemia decreased concomitantly.
61 Comparing first and fourth quartiles of tofu intake, the prevalence of anemia was
62 23.9% vs 10.7% in men, and 38.1% vs 16.8% in women. Tofu intake was inversely
63 associated with SF levels in women. In multivariate analyses, the odds ratio (OR) of
64 anemia for men in fourth compared to first quartile of tofu intake was 0.30 (95%
65 confidence interval [CI] 0.17-0.50), and the corresponding OR for women was 0.31
66 (95%CI 0.20-0.47). The association between tofu intake and anemia was independent
67 of iron intake.

68 **Conclusion:** Tofu intake was associated with lower risk of anemia among Chinese
69 adults in both genders.

70 **Key words:** Hemoglobin, anemia, serum ferritin, tofu intake, China

71 **Introduction**

72 Iron deficiency affects about 2 billion people worldwide, being one of the leading risk
73 factors for disability and death (1). In developing countries, 42.3% women and 30.0%
74 men aged 15-59 years are anemic, with half having iron deficiency anemia (IDA) (2).
75 Even in developed countries, 10.3% of women are anemic (2). Iron deficiency in
76 developing countries is about twice as frequent as IDA (2). Although anemia can be
77 caused by different factors, the main cause is iron deficiency. Low iron content in the
78 diet and low bioavailability from plant-based diets are main causes of IDA. Other
79 causes of IDA include pregnancy, gastrointestinal blood loss, menstruation, growth
80 and development, intestinal parasites such as hookworm, and Helicobacter pylori
81 infection (1). Iron fortified wheat flour and soy sauce are used in population
82 approaches to prevent IDA (1-3).

83

84 Intake of soybeans may prevent anemia through two different mechanisms. First,
85 soybean has a high iron and ferritin content (4, 5). Iron from soybean ferritin is highly
86 absorbed and may provide a model for novel, utilizable, plant-based forms of iron for
87 populations with a low iron status (6). Shoyu polysaccharides from soy sauce enhance
88 iron absorption in animals and humans (7). Second, soy intake can lower
89 inflammation levels and thereby reduce metabolic causes of anemia (1, 8-10).

90

91 At present, few population studies report the association between intake of tofu and
92 anemia. Most other studies on the association between soy and anemia focus on either
93 soy protein or phytoestrogens. Tofu differs from soy protein in that it has a very high
94 calcium content (due to the calcium coagulant used in the curding process). Present
95 knowledge on the association between calcium and iron absorption is controversial
96 (11-14). In this study, it is hypothesized that there would be an association between
97 intake of tofu and anemia and that such an association would be independent of iron
98 intake. This hypothesis was tested among adults in a cross-sectional population survey.

99 **Subjects and methods**

100 **Population and Sample**

101 China has a long history of consuming tofu, but there is a large variation in the intake
102 of tofu and other soy products in the population (15, 16). There is also a high
103 prevalence of anemia in the Chinese population (around 25%) (17). Thus, China
104 provides a good population to study the association of tofu intake with anemia.

105 In 2002, China launched a national study in nutrition and health under the approval of
106 the Chinese Ministry of Health. A multistage cluster sampling method was used to
107 select the participants. The data presented in this article are based on a subsample
108 from Jiangsu (population 73.6 million), one of the provinces in China experiencing
109 rapid economic growth. The rural sample was selected from 6 counties (Jiangyin,
110 Taicang, Suining, Jurong, Sihong and Haimen). From each of the six counties, three
111 towns were randomly selected. The urban sample was selected from two prefecture
112 capital cities (Nanjing and Xuzhou). From each prefecture city, three streets were
113 randomly selected. The six counties and two prefectures cities represented a
114 geographically and economically diverse population with gross domestic product
115 (GDP) ranging from 3221Yuan/capita/year (403 USD) to 35169 Yuan/capita/year
116 (4396 USD) (mean 1993 USD) (18). Nanjing, Jurong, Taichang and Jiangyin are in
117 the South. The south has higher GDP than the north (24702 vs 7183 Yuan). In each
118 town/street, two villages/neighborhoods were further randomly selected. In each
119 village/neighborhood, 30 households were randomly selected. All members in the
120 households including children were invited to take part in the study. The study was

121 approved by the ethical committee in Chinese National Center for Disease Control
122 (CDC) and Prevention. Written consents were obtained from all the participants. In
123 this study, only data for adults aged 20 years and over were analyzed.

124

125 **Measurement and interview**

126 Participants were interviewed in their homes by trained health workers using a
127 pre-coded questionnaire. Interviews took approximately two hours to complete and
128 included questions on diet, socio-demographic information, medical history, drug
129 treatment, health habits such as cigarette smoking and physical activity, and other
130 lifestyle factors. Height was measured without shoes and weight with light clothing.
131 Body mass index (BMI) was calculated as weight in kilograms divided by the square
132 of the height in metres.

133 *Hemoglobin (Hb) and serum ferritin (SF)*

134 All participants were invited to provide a fasting blood sample. All the blood
135 samples were analyzed for plasma glucose and Hb in the local centers for disease
136 control and prevention with quality control set by the National CDC and Prevention.
137 Cyanmethemoglobin method was used to measure Hb (19). A commercially available
138 radioimmunoassay kit (Radioimmunoassay kit for SF, Beijing North Institute of
139 Biological Technology, Beijing) was used to analyze SF by the National CDC in
140 Beijing.

141 Anemia was defined as a Hb level below 13 g/dl for men and 12 g/dl for women (2).

142 The definition of IDA was based on the presence of both anemia and a SF level

143 <15µg/l (1).

144 *Dietary measurements.* Diet in the past year was investigated by a series of detailed
145 questions regarding usual frequency and quantity of intake of 33 foods and beverages.
146 Portion size for each food was established by reference to food models. Subjects were
147 asked to recall the frequency of consumption of individual food items (number of
148 times/day, /week, /month, /year) and the estimated portion size, using local weight
149 units (i.e., a liang which is equivalent to 50 g) or cups. Intakes of foods were
150 converted into grams per week and were used in further analysis. The questionnaire
151 includes both wet tofu and dried tofu. Tofu intake was calculated by summing up the
152 intakes of both types. Fresh and dried soybeans were not included in the analysis.

153 The food frequency questionnaire has been validated (20, 21) and reported to be a
154 useful method for the collection of individual food consumption information in a
155 face-to-face interview, but not in self-administered surveys due to the current
156 educational level of the majority of the Chinese population.

157 Nutrient intake was also measured by three day weighed food records. Food
158 consumption data were analysed using the Chinese Food Composition Table (4).

159 *Socio-economic status (SES)* SES was assessed by the question ‘What was your
160 family’s income per person in 2001?’. The response categories for the question were
161 less than 800, 800-1999, 2000-4999, 5000-9999, 10000-19999 and >20000Yuan. SES
162 was constructed from income: ‘low’: less than 1999Yuan; ‘medium’: 2000-4999Yuan;
163 ‘high’: more than 5000Yuan.

164 **Statistical analyses**

165 Tofu intake was divided into gender specific quartiles, implying increased intake from
166 quartile 1 to quartile 4. Chi square test was used to compare difference between
167 categorical variables. Logistic regression was used to determine the association
168 between tofu intake and anemia adjusted for socio-demographic factors including
169 residence, age, socioeconomic status (SES), region and education, intake of fruits,
170 vegetables, and animal foods. Estimates were presented in either mean and standard
171 deviation (SD) or odds ratio (OR) and 95% confidence interval (CI). All the analyses

172 were performed by using STATA software (version 9, 2005, Stata Corp, College
173 Station, TX).

174 **Results**

175 The total sample included 1308 men and 1541 women, of them 711 participants were
176 from the urban area. The response rate of the study was 89.0%. The mean age of the
177 sample was 47.0 years (SD 14.5). Table 1 shows the demographic characteristics of
178 the study population by gender. The prevalence of anemia was 18.3% in men and
179 31.5% in women. The distribution of SF was not normal, and skewed to the lower
180 values. Only 2.1% men and 15.1% women had SF < 15µg/l. The prevalence of IDA
181 was thus low, 6.3% among women and 0.7% among men. Tofu intake was not
182 normally distributed and was skewed to the lower values. The mean tofu intake was
183 50.9 g/day for men and 44.5g/day for women.

184

185 Table 2 shows the sample characteristics across tofu intake quartiles. The mean daily
186 intake of tofu in the lowest and highest quartile ranged from 9.1 to 119.8 g in men and
187 7.0 to 110.4 g in women. Daily intake of calcium, iron, magnesium, and fruits was
188 positively associated with increasing tofu intake in both genders, while in regard to
189 intake of meat this association was only significant for women. The intake of vitamin
190 C varied according to quartiles of tofu intake only in women, but there was no
191 consistent trend. No significant association between tofu intake and vegetable intake
192 was found. Participants with low SES tended to have higher tofu intake than those
193 with higher SES.

194

195 Figure 1 shows the association between tofu intake and Hb. Tofu intake was positively
196 associated with Hb in men and women (Table 3). The probability of having a Hb level
197 in the highest quartile increased across the quartiles of tofu intake in men and women.
198 A clear decreasing trend of prevalence of anemia was seen across quartiles of tofu
199 intake from low to high in men and women. The prevalence of anemia in the first and
200 fourth quartiles of tofu intake was 23.9% vs 10.7% in men; the corresponding figure
201 was 38.1% vs 16.8% in women (p<0.0001). There was no statistically significant
202 difference in mean SF across quartiles of tofu intake. However, the probability of
203 being in the highest quartile of SF decreased across tofu intake quartiles in women (p

204 for trend is 0.030). Women in the lowest tofu intake group had the highest probability
205 of being in the highest SF quartile (Q1 vs Q4: 31.0% vs 20.3%). No such association
206 was found in men.

207

208 In multivariate analysis, after adjusting for age, BMI, residence, SES, education,
209 intake of fruits, vegetables, and animal foods, people in the highest quartile of tofu
210 intake had a lower risk of anemia compared with the lowest quartile (Q1) (men: OR
211 0.30 (95%CI 0.17-0.50); women 0.31 (95%CI 0.20-0.47) (Table 4). Further
212 adjustment of iron intake did not change the estimates. Adding calcium intake
213 quartiles into the multivariate model did not change the association. Calcium intake
214 was not associated with anemia in the sample while iron intake was inversely
215 associated with anemia in women (data not shown).

216 **Discussion**

217 In this cross-sectional study, high intake of tofu was associated with lower risk of
218 anemia in both men and women. The association was still remained highly significant
219 after adjusting for sociodemographic characteristics, BMI, intake of foods known to
220 affect iron status, and iron. Tofu intake was negatively associated with having higher
221 SF in women but not in men.

222 The total iron intake in the sample was found to be higher than in Western populations,
223 but similar with findings from another study in China (22). The iron intake increased
224 somewhat across the quartiles of tofu intake. This probably due to the fact that tofu
225 has a high content of iron, much higher than in rice and wheat flour (4). However,
226 tofu is consumed in much lower quantities thus contributing a relatively low
227 proportion of total iron intake except in the highest quartiles (110-120 g tofu
228 consumed in the fourth quartile means less than 2 mg iron per day). Although soybean
229 contains phytate, which can decrease the bioavailability of iron, this compound is
230 degraded in the production of tofu, which implies soaking, grinding, and boiling (23).
231 The present results are consistent with present evidence of high bioavailability of iron
232 from soybean or soybean in population studies (5, 6, 24), despite the fact that tofu in
233 China has a high molar ratio of phytate to iron, ranging from 16 - 61 (23).

234 Intake of meat and fruits increased across the quartiles of tofu intake from low to high.
235 Meat intake is a known enhancing factor of non-heme iron absorption (24). Also,
236 fruits and vegetables may enhance absorption of non-heme iron in the diet due to the
237 high content of vitamin C (25). The intake of this vitamin did not vary by tofu intake
238 quartiles in men, and even though there was a change in women, this trend was not
239 consistent and could not explain the variation in anemia.

240 Although the mechanism is not clear, tofu intake is negatively associated with blood
241 lead levels in the Chinese population (26). Whether decreased blood lead level is one
242 of the pathways in the association between tofu intake and anemia needs further
243 research.

244 The association between calcium intake and anemia is ambiguous (11-14). In this
245 study, no effect of calcium intake on the association between tofu intake and anemia

246 was found. This finding may be due to the fact that the intake of calcium was low in
247 all groups even if there were differences according to different tofu intake groups.
248 The association between tofu intake and anemia was independent of iron intake. After
249 adjusting for intake of fruits and vegetables and animal foods, the association between
250 intake of Tofu and anemia still remained.

251 Another potential cause of anemia could be high levels of inflammation and infection
252 in the subjects, as shown in a recent review (1). Soy intake was found to be negatively
253 associated with inflammation. In a study of postmenopausal women with metabolic
254 syndrome, short-term soy nut consumption was related to a reduction of some markers
255 of inflammation (8). Soy contains fiber, polyunsaturated fat, and phytoestrogens,
256 which are all negatively associated with levels of inflammatory markers (8).

257 In the present study, intake of tofu was also positively associated with magnesium
258 intake. Magnesium has been found to be inversely associated with markers of
259 systematic inflammation (27-29). Magnesium supplementation increases erythrocytes
260 and hemoglobin levels of athletes (30).

261 It is common to use SF as a marker of stored body iron (31), but SF is also increased
262 by inflammation and infection (32, 33). The negative association between intake of
263 tofu and high SF in women suggests an anti-inflammatory effect of tofu. The highest
264 prevalence of anemia combined with high SF was observed in the low tofu intake
265 group. Furthermore, due to the very small percentage having low SF the prevalence of
266 IDA was low, despite the high prevalence of anemia. Since Thalassemia is not a
267 problem in this area with a prevalence of 0.09% (34), it is reasonable to assume that a
268 considerable proportion of the anemia in the population is caused by inflammation. In
269 the study, markers of inflammation such as c-reactive protein, were not measured, but
270 it has been reported that the prevalence of the hepatitis B virus marker is 42.6% and
271 chronic hepatitis B surface antigen carriage is 10.2% in various parts of China (35).

272 In the Shanghai Women's Health Study, the intake of soy foods included 11 items. In
273 this study, the food frequency questionnaire included 4 food items related to soy
274 intake. For simplicity in the analysis, the wet and dry tofu were summed up by giving
275 the same weight to each item. This may underestimate the association between intake

276 of tofu and iron status as the wet tofu contains a lot of water. Different mechanisms
277 may be involved, and the effect of soy ferritin, isoflavonoids, and soy protein on the
278 risk of anemia was not assessed.

279 There are several limitations to this study. The cross-sectional study design and the
280 lack of measurement of some potential confounding factors including inflammation
281 markers imply that no conclusions on the etiological association between tofu
282 consumption and Hb concentration can be drawn. It was also not possible to control
283 for the potential confounding effects of these infections in the analysis. Furthermore,
284 the potentially complex interactions of multiple nutrients with iron cannot be assessed,
285 and therefore cannot encourage tofu intake for the purpose of anemia prevention
286 without further investigation.

287

288 **Conclusions**

289 This is the first study to report the association between tofu intake and anemia at the
290 population level. It supports the idea of adding soy in the healthy eating index as
291 suggested by the US Department of Agriculture. The findings indicate that the anemia
292 in the region could be related to high rates of infection and inflammatory processes.
293 Although the region is one of the richest in China, the prevalence of anemia is the
294 highest in China as observed by three national nutrition surveys (36). The study may
295 have public health significance. Except for the iron fortified soysauce intervention
296 project in this region, there is no other intervention project yet. Taking into account
297 the long tradition of consuming tofu in the country, its low economic burden, as well
298 as the health benefits, it may be promising to promote tofu as part of a healthy food
299 choice in the prevention of anemia. The results may provide useful knowledge to
300 other countries with the tradition of eating tofu that have a high prevalence of anemia.

301

302 In conclusion, a high intake of tofu was associated with a lower prevalence of anemia.

303

304

305 **Reference**

- 306 1. Zimmermann MB, Hurrell RF. Nutritional iron deficiency. *Lancet*.
307 2007;370:511-20.
- 308 2. WHO. Iron Deficiency Anaemia. Assessment, Prevention and Control: A
309 Guide for Programme Managers: WHO/NHD/01.3, 2001.
- 310 3. Mannar V, Gallego EB. Iron fortification: country level experiences and
311 lessons learned. *J Nutr*. 2002;132:856S-8S.
- 312 4. Yang Y. Chinese Food Composition Table 2004. Beijing: Peking University
313 Medical Press, 2005.
- 314 5. Murray-Kolb LE, Welch R, Theil EC, Beard JL. Women with low iron stores
315 absorb iron from soybeans. *Am J Clin Nutr*. 2003;77:180-4.
- 316 6. Lonnerdal B, Bryant A, Liu X, Theil EC. Iron absorption from soybean ferritin
317 in nonanemic women. *Am J Clin Nutr*. 2006;83:103-7.
- 318 7. Kobayashi M, Nagatani Y, Magishi N, Tokuriki N, Nakata Y, Tsukiyama R,
319 Imai H, Suzuki M, Saito M, Tsuji K. Promotive effect of Shoyu
320 polysaccharides from soy sauce on iron absorption in animals and humans. *Int*
321 *J Mol Med*. 2006;18:1159-63.
- 322 8. Azadbakht L, Kimiagar M, Mehrabi Y, Esmailzadeh A, Hu FB, Willett WC.
323 Soy consumption, markers of Inflammation, and endothelial function: a
324 cross-over study in postmenopausal women with the metabolic syndrome.
325 *Diabetes Care*. 2007;30:967-73.
- 326 9. Jenkins DJ, Kendall CW, Connelly PW, Jackson CJ, Parker T, Faulkner D,
327 Vidgen E. Effects of high- and low-isoflavone (phytoestrogen) soy foods on
328 inflammatory biomarkers and proinflammatory cytokines in middle-aged men
329 and women. *Metabolism*. 2002;51:919-24.
- 330 10. Blum A, Lang N, Peleg A, Vigder F, Israeli P, Gumanovsky M, Lupovitz S,
331 Elgazi A, Ben-Ami M. Effects of oral soy protein on markers of inflammation
332 in postmenopausal women with mild hypercholesterolemia. *Am Heart J*.
333 2003;145:e7.
- 334 11. Cook JD, Dassenko SA, Whittaker P. Calcium supplementation: effect on iron

- 335 absorption. *Am J Clin Nutr.* 1991;53:106-11.
- 336 12. Grindler-Pedersen L, Bukhave K, Jensen M, Hojgaard L, Hansen M. Calcium
337 from milk or calcium-fortified foods does not inhibit nonheme-iron absorption
338 from a whole diet consumed over a 4-d period. *Am J Clin Nutr.*
339 2004;80:404-9.
- 340 13. Reddy MB, Cook JD. Effect of calcium intake on nonheme-iron absorption
341 from a complete diet. *Am J Clin Nutr.* 1997;65:1820-5.
- 342 14. Roughead ZK, Zito CA, Hunt JR. Inhibitory effects of dietary calcium on the
343 initial uptake and subsequent retention of heme and nonheme iron in humans:
344 comparisons using an intestinal lavage method. *Am J Clin Nutr.*
345 2005;82:589-97.
- 346 15. Liu Z, Li W, Sun J, Liu C, Zeng Q, Huang J, Yu B, Huo J. Intake of soy foods
347 and soy isoflavones by rural adult women in China. *Asia Pac J Clin Nutr.*
348 2004;13:204-9.
- 349 16. Yang G, Shu XO, Jin F, Zhang X, Li HL, Li Q, Gao YT, Zheng W.
350 Longitudinal study of soy food intake and blood pressure among middle-aged
351 and elderly Chinese women. *Am J Clin Nutr.* 2005;81:1012-7.
- 352 17. Li L, Rao K, Kong L, Yao C, Xiang H, Zhai F, Ma G, Yang X. A description on
353 the Chinese national nutrition and health survey in 2002. *Zhonghua Liu Xing*
354 *Bing Xue Za Zhi.* 2005;26:474-84.
- 355 18. Jiangsu Bureau of statistics. Statistical yearbook of Jiangsu. Beijing: China
356 Statistics Press, 2002.
- 357 19. Dallman PR. Diagnosis of anemia and iron deficiency: analytic and biological
358 variations of laboratory tests. *Am J Clin Nutr.* 1984;39:937-41.
- 359 20. Zhang G, Zhu ZM, Zhao ZG, Ni GX, Yan ZC, Li Q, Liu HY. Comparative
360 study of different diagnostic criteria of metabolic syndrome. *Zhonghua Yi Xue*
361 *Za Zhi.* 2005;85:490-1.
- 362 21. Li YP, He YN, Zhai FY, Yang XG, Hu XQ, Zhao WH, Ma GS. Comparison of
363 assessment of food intakes by using 3 dietary survey methods. *Zhonghua Yu*
364 *Fang Yi Xue Za Zhi.* 2006;40:273-80.

- 365 22. Chen Z, Shu XO, Yang G, Li H, Li Q, Gao YT, Zheng W. Nutrient intake
366 among Chinese women living in Shanghai, China. *Br J Nutr.* 2006;96:393-9.
- 367 23. Ma G, Jin Y, Piao J, Kok F, Guusje B, Jacobsen E. Phytate, calcium, iron, and
368 zinc contents and their molar ratios in foods commonly consumed in China. *J*
369 *Agric Food Chem.* 2005;53:10285-90.
- 370 24. Hallberg L, Rossander L. Improvement of iron nutrition in developing
371 countries: comparison of adding meat, soy protein, ascorbic acid, citric acid,
372 and ferrous sulphate on iron absorption from a simple Latin American-type of
373 meal. *Am J Clin Nutr.* 1984;39:577-83.
- 374 25. Lopez MA, Martos FC. Iron availability: An updated review. *Int J Food Sci*
375 *Nutr.* 2004;55:597-606.
- 376 26. Chen C, Wang X, Chen D, Li G, Ronnenberg A, Watanabe H, Wang X, Ryan L,
377 Christiani DC, Xu X. Tofu consumption and blood lead levels in young
378 Chinese adults. *Am J Epidemiol.* 2001;153:1206-12.
- 379 27. Song Y, Li TY, van Dam RM, Manson JE, Hu FB. Magnesium intake and
380 plasma concentrations of markers of systemic inflammation and endothelial
381 dysfunction in women. *Am J Clin Nutr.* 2007;85:1068-74.
- 382 28. Bo S, Durazzo M, Guidi S, Carello M, Sacerdote C, Silli B, Rosato R,
383 Cassader M, Gentile L, Pagano G. Dietary magnesium and fiber intakes and
384 inflammatory and metabolic indicators in middle-aged subjects from a
385 population-based cohort. *Am J Clin Nutr.* 2006;84:1062-9.
- 386 29. King DE, Mainous AG, 3rd, Geesey ME, Ellis T. Magnesium intake and serum
387 C-reactive protein levels in children. *Magnes Res.* 2007;20:32-6.
- 388 30. Cinar V, Nizamlioglu M, Mogulkoc R, Baltaci AK. Effects of magnesium
389 supplementation on blood parameters of athletes at rest and after exercise. *Biol*
390 *Trace Elem Res.* 2007;115:205-12.
- 391 31. Cook JD, Lipschitz DA, Miles LE, Finch CA. Serum ferritin as a measure of
392 iron stores in normal subjects. *Am J Clin Nutr.* 1974;27:681-7.
- 393 32. Pan Y, Jackson RT. Ethnic difference in the relationship between acute
394 inflammation and serum ferritin in US adult males. *Epidemiol Infect.*

- 395 2007:1-11.
- 396 33. Baynes RD. Assessment of Iron Status. *Clinical Biochemistry*.
397 1996;29:209-215.
- 398 34. Zeng YT, Huang SZ. Disorders of haemoglobin in China. *J Med Genet*.
399 1987;24:578-83.
- 400 35. Yao GB. Importance of perinatal versus horizontal transmission of hepatitis B
401 virus infection in China. *Gut*. 1996;38 Suppl 2:S39-42.
- 402 36. Chang S, Ge K, Zhai F, Jia F, Xu X. The analysis of nutritional factors on
403 anemia in Chinese adults. *Acta Nutrimenta Sinica*. 1998;20:132-137.
- 404

405 Table 1 Characteristic of the sample by genders among adults in Jiangsu China 2002

	Men (n=1308)	Women (n=1541)
Age, yrs (mean, SD ^a)	47.2(14.6)	46.8(14.4)
Hb ^b , g/dl (mean, SD)	14.3(1.7)	12.7(1.5)
Anemia (%) ^c	18.3 (16.3-20.6)	31.5 (29.2-33.9)
SF ^d , µg/l (mean, SD)	131.9(90.1)	71.1(71.5)
<15 µg/l (%)	2.1	15.1
≥200 µg/l (%)	18.6	5.8
IDA (%) ^e	0.7	6.3
Nutrients and foods intake (mean, SD)		
Iron, mg/day	28.2(12.0)	23.4(9.5)
Magnesium, mg/day	363.9(151.5)	305.8(129.9)
Calcium, mg/day	455.0(224.3)	388.8(190.9)
Vitamin C, mg/day	65.6(38.7)	60.4(38.0)
Tofu, g/day	50.9(51.9)	44.5(28.6)
Fruits, g/day	61.7(85.9)	66.9(89.0)
Vegetables, g/day	260.7(166.0)	247.3(161.9)
Meat, g/day	69.0(65.6)	52.1(54.5)
BMI ^f , kg/m ² (mean, SD)	23.4(3.2)	23.6(3.7)
Overweight (%) ^g	29.2	30.7
Urban dwellers (%)	25.2	24.7
South dwellers (%)	53.7	52.0
Education (%)		
Primary	36.0	57.5
Junior school	43.5	30.1
High school	16.3	10.8
University	4.2	1.6

406 ^a SD-Standard deviation.407 ^b Hb-hemoglobin408 ^c Anemia was defined as Hb level below 13 g/dl for men and 12 g/dl for women.409 ^d SF-Serum ferritin.410 ^e IDA-iron deficiency anemia. Defined as Hb level below 13 g/dl for men and 12 g/dl for women and

411 SF<15 µg/l.

412 ^f BMI-Body mass index.413 ^g Defined as BMI≥25 kg/m²

414

Table 2 Sample characteristics across tofu intake quartiles among adults in Jiangsu China 2002 (n=2849)

	Men					Women				
	Q1 ^a (n=330)	Q2(n=341)	Q3(n=320)	Q4(n=317)	P ^b	Q1(n=394)	Q2(n=392)	Q3(n=416)	Q4(n=339)	P
Intake of tofu (g/day)										
Mean	9.1	28	50.1	119.8	<0.001	7.0	24.2	45.5	110.4	<0.001
SD ^c	6.1	4.9	7.6	62.2		5.0	4.4	8.6	54.5	
Min	0	20.7	36.4	65.7		0.0	15.0	29.3	58.6	
Max	20	35.7	64.3	600		14.3	28.6	57.1	452.9	
Daily foods and nutrients intake ^d (Mean, SE ^e)										
Calcium (mg/day)	445(11)	441(11)	460(12)	471(12)	0.117	387(9)	369(9)	384(9)	419(9)	0.001
Iron (mg/day)	27(1)	28(1)	28(1)	30(1)	0.001	23.2(0.4)	22.6(0.4)	23.2(0.3)	24.9(0.4)	<0.001
Vitamin C (mg/day)	67(2)	63(2)	63(2)	65(2)	0.625	62(2)	59(2)	57(2)	63(2)	0.041
Magnesium (mg/day)	352(6)	353(6)	359(6)	392(6)	<0.001	305(5)	286(5)	301(5)	335(5)	<0.001
Meat (g/day)	67(4)	63(4)	73(4)	72(4)	0.141	45(3)	51(3)	56(3)	57(4)	0.006
Fruits (g/day)	51(5)	60(5)	63(5)	72(5)	0.012	56(4)	61(4)	73(4)	77(4)	0.002
Vegetables (g/day)	261(9)	244(9)	268(9)	272(9)	0.135	256(8)	233(8)	246(8)	255(9)	0.178
Age (yrs)										
Mean	48.4	48.5	46.1	46.4	0.058	48.3	45.5	46.2	47	0.046
SD	14.8	13.9	15	14.4		15.3	13.3	14.4	14.3	
Body mass index (kg/m ²)										
Mean	23.5	23.3	23.6	23.2	0.369	23.4	23.5	23.6	24.1	0.059
SD	3.3	3.2	3.2	3.3		3.7	3.6	3.6	3.8	
Socio-economic status										
Low	28.3	26.9	27.1	47.5		31.6	24.6	30.3	44.8	

Medium	32.6	35.5	33.4	25.6	<0.001	32.9	39.7	30.8	26.8	<0.001
High	39.1	37.6	39.4	26.9		35.5	35.6	38.8	28.3	
Education (%)										
Primary	38.5	38.4	26.9	40.1		60.6	57.1	51.9	61.4	
Junior school	44.9	37.5	49.7	42.3	0.006	28.5	29.9	33.9	27.7	0.200
High school	13.6	19.1	18.1	14.2		9.2	11.0	13.2	9.4	
University	3.0	5.0	5.3	3.5		1.8	2.0	1.0	1.5	
Residence (%)										
Urban	20.0	24.9	32.2	24.0	0.004	20.3	25.3	28.6	24.5	0.056
Rural	80.0	75.1	67.8	76.0		79.7	74.7	71.4	75.5	
Region (%)										
South	55.8	57.8	56.9	43.9	0.001	48.2	59.4	54.3	45.1	<0.001
North	44.2	42.2	43.1	56.2		51.8	40.6	45.7	54.9	

^a Q1 Stands for the lowest quartile

^b Chi-square test for categorical variables and ANOVA for continuous variables

^c SD-Standard deviation

^d Energy adjusted

^e SE-Standard error

Table 3 Association between quartiles of tofu intake and hemoglobin, serum ferritin and anemia among men and women in Jiangsu China

	Men					Women				
	Q1 ^a (n=330)	Q2(n=341)	Q3(n=320)	Q4(n=317)	P ^b	Q1(n=394)	Q2(n=392)	Q3(n=416)	Q4(n=339)	p
Hb ^c										
Mean (g/dl)	14.1	14.0	14.5	14.8	<0.001	12.4	12.5	12.6	13.3	<0.001
SD ^d	1.7	1.6	1.6	1.7		1.6	1.5	1.5	1.4	
Hb>cut-off, ^e fourth										
quartile (%)	21.1	17.0	26.3	35.6	<0.001	21.3	21.3	23.6	33.9	<0.001
Anemia (%) ^f	23.9	23.5	14.7	10.7	<0.001	38.1	37.2	31.7	16.8	<0.001
SF ^g										
Mean (µg/l)	135.6	129.5	134.5	128	0.569	75.1	74.0	66	69.5	0.540
SD	93.1	91.3	88.4	87.4		71.4	73.3	66.6	75.1	
SF>cut-off ^h , fourth										
quartile (%)	25.5	24.5	25.8	24.2	0.858	31.0	25.3	23.4	20.3	0.030

^a Q1 stands for the lowest quartile.

^b Chi-square test for categorical variables and ANOVA for continuous variables, in the test for mean SF difference, the value of SF was log transformed

^c Hb-Hemoglobin

^d SD-Standard deviation

^e Hb \geq 15.5 g/dl in men and Hb \geq 13.7 g/dl in women

^fAnemia was defined as Hb level below 13 g/dl for men and 12 g/dl for women.

^g SF-Serum ferritin

^h SF \geq 175.6 μ g/l in men and SF \geq 95.6 μ g/l in women

Table 4 Odds ratio (OR 95%CI ^a) for anemia according to tofu intake quartiles in Chinese adults (n=2849)

	Tofu intake quartiles				P for trend
	Q1 ^b (low)	Q2	Q3	Q4(high)	
Men					
Model 1 ^c	1	0.77(0.55-1.09)	0.71(0.46-1.09)	0.27(0.16-0.45)	<0.001
Model 2 ^d	1	0.77(0.55-1.10)	0.70(0.45-1.07)	0.27(0.16-0.45)	<0.001
Model 3 ^e	1	0.85(0.59-1.21)	0.76(0.49-1.18)	0.31(0.19-0.52)	<0.001
Model 4 ^f	1	0.81(0.57-1.16)	0.76(0.48-1.19)	0.30(0.17-0.50)	<0.001
Model 5 ^g	1	0.83(0.58-1.19)	0.78(0.50-1.22)	0.31(0.18-0.52)	<0.001
Women					
Model 1 ^c	1	0.88(0.67-1.17)	0.67(0.50-0.89)	0.26(0.17-0.40)	<0.001
Model 2 ^d	1	0.89(0.67-1.18)	0.68(0.51-0.91)	0.27(0.18-0.41)	<0.001
Model 3 ^e	1	0.93(0.70-1.24)	0.70(0.52-0.94)	0.30(0.20-0.46)	<0.001
Model 4 ^f	1	0.94(0.70-1.26)	0.70(0.52-0.95)	0.31(0.20-0.47)	<0.001
Model 5 ^g	1	0.95(0.71-1.27)	0.70(0.52-0.94)	0.32(0.21-0.49)	<0.001

^a CI-Confidence interval.

^b Q1 stands for the lowest quartile.

^c Adjusted for age

^d Adjusted for age, body mass index (continuous)

^e Adjusted for age, body mass index, urban/rural, household socio-economic status, education, south/north

^f Additional adjusted for intake of fruits and vegetables, intake of pork, beef and lamb (continuous).

^g Additional adjusted for intake of energy and iron

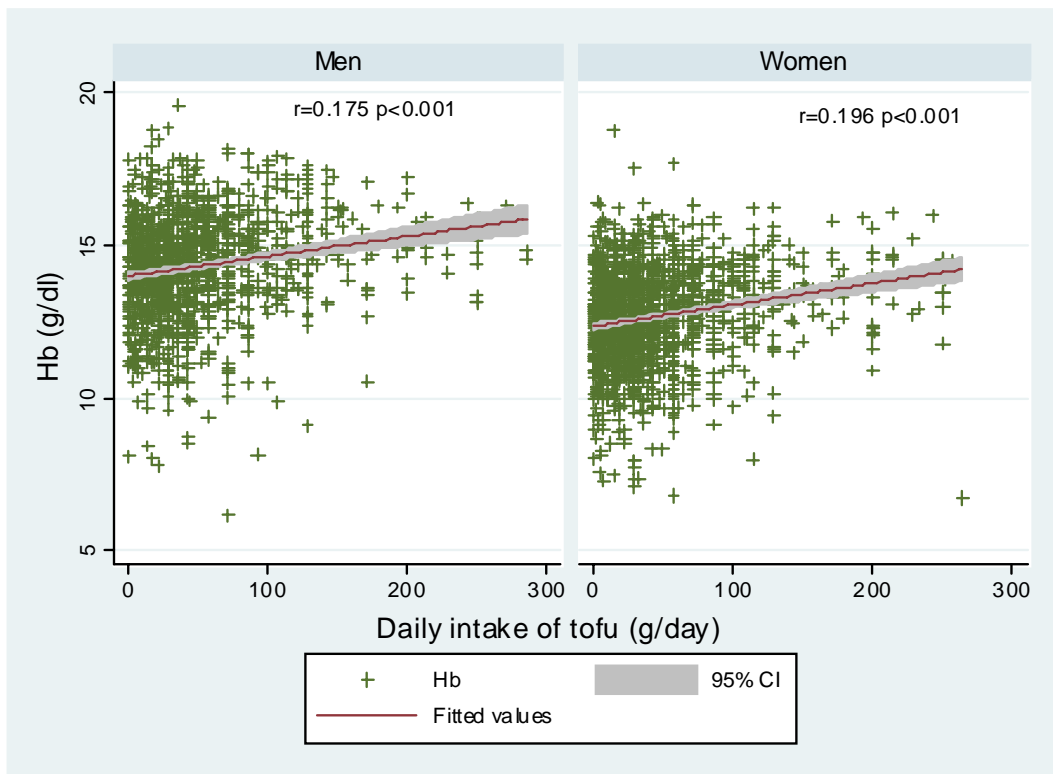


Fig 1 Association between daily intake of tofu and hemoglobin (Hb) (excluded 9 participants with tofu intake>300g/day, n=2840)