Abstract
Dietary meats play a crucial role in human health. The objective of this survey was to determine the fatty acid content and omega-6/omega-3 polyunsaturated fatty acids (n-6/n-3 PUFA) ratio of fresh red meat (beef and pork) from four cities (Shanghai, Nanjing, Yinchuan and Hohhot) in China. The results showed that the n-6/n-3 PUFA ratio from all the samples ranged from 6 to 23. The total n-6 PUFA concentrations ranged from 290.54 mg/100 g in beef from Nanjing to 1601.48 mg/100 g in pork from Hohhot, whereas the total concentrations of n-3 PUFA ranged from 46.34 mg/100 g in beef from Nanjing to 96.03 mg/100 g in pork from Nanjing. The results indicated that the n-6/n-3 ratio in the red meat from all four regions is unbalanced and is much higher than that (< 5:1) recommended by the WHO/FAO. The total amount of n-3 PUFA was far lower than the required daily dose. Therefore, potential solutions to increase the n-3 PUFA content in meat products or to provide alternative source of n-3 PUFA should be explored.

Keywords: lipid, polyunsaturated fatty acid, red meat

INTRODUCTION
Both omega-3 and omega-6 polyunsaturated fatty acids (n-3 and n-6 PUFAs) are essential fatty acids for humans because they cannot be synthesized by the human body. PUFAs are not only the key components of cell membranes, participating in and regulating receptor activities and signal transduction on cell membranes, but they are also the substrates of many important autocrine or paracrine lipid mediators such as prostaglandin, leukotriene, prostacyclins, thromboxanes, resolvins and protectins[1-4]. It is thought that human beings evolved consuming a diet that contained about an equal amount of n-3 and n-6 EFAs. However, over the past 100-150 years there has been an enormous increase in the consumption of n-6 PUFAs due to increased intake of vegetable oils from corn, sunflower seeds, cottonseed and soybeans, which contain high amounts of n-6 PUFAs but none of n-3 PUFAs. Furthermore, industrial production of animal feed made from grains containing large amounts of n-6 PUFAs led to the production of meat rich in n-6 but poor in n-3 PUFAs.

Many studies in the last 20 years have shown that
the high n-6/n-3 PUFA ratio may contribute to the high prevalence of many modern diseases (e.g. heart disease, autoimmune disorders and depression)\[^{5,6}\]. Studies have also shown that n-3 PUFAs are essential for normal growth and the development of the retina and brain. They may also play an important role in the prevention and treatment of coronary artery disease, hypertension, diabetes, arthritis, other inflammatory or autoimmune disorders, depression and cancer. Extensive epidemiological studies have shown that increased n-3 PUFA reduces the risk for coronary heart diseases\[^{7}\], improves inflammatory conditions\[^{8}\], as well as possibly improving symptoms of depression\[^{9}\].

Meat is a significant dietary source of PUFAs. Recent reports have shown that there is a high n-6/ n-3 ratio in meat products in Western countries\[^{10,11}\]. However, there are very few quantitative studies assessing the n-6/n-3 ratio and fatty acid composition of meat products in China\[^{12,13}\]. Pork and beef are the most popular red meats consumed in China. The objective of this study was to investigate the n-3 and n-6 PUFA content and ratio in beef and pork available at retail markets from four representative regions in China. The findings may provide some evidence to draw correlations between an unbalanced n-6/n-3 ratio in the diet and people’s health conditions across different regions in China.

**MATERIALS AND METHODS**

**Sample preparation**

Four representative cities in China were selected: Nanjing for inland east, Shanghai for coastal city in the east, Yinchuan for the inland northwest, and Hohhot for inland north of China (Fig. 1). A total of 12 samples each of local major brand fresh pork and beef round chop were obtained from each representative city and snap-frozen on dry ice in small pieces and stored at -80°C until further analysis. Results were reported for all PUFAs that could be identified and quantified by gas chromatograph (GC) analysis.

**Fatty acid analysis**

Fatty acids were extracted by preparation of methyl esters as described by Metcalfe et al.\[^{14}\] with some modifications. Briefly, meat samples were thawed at room temperature (25°C) and approximately 0.2 g meat was extracted by adding methanol (CH\(_3\)OH) 4 mL, chloroform (CHCl\(_3\)) 2 mL, and water (H\(_2\)O) 1.5 mL. As an internal standard to quantify total meat fat, 25 mg nonadecanoic acid methyl ester was added. The sample was fully oscillated and then allowed to stand at room temperature for 15 minutes. Then, additional 2 mL of CHCl\(_3\) and H\(_2\)O were added, thoroughly mixed and allowed to stand for at least 5 minutes. The lower phase was transferred after being centrifuged at 1500 g and 16°C for 30 minutes and then completely dried at 35°C water bath in nitrogen flow. The meat lipids were converted to fatty acid methyl esters using a mixture of 14% BF3/MeOH reagent (Sigma, St. Louis, MO) at 90°C for 30 minutes under nitrogen. After cooling to room temperature, 4 mL of pentane (C\(_5\)H\(_{12}\)) and 1.5 mL of H\(_2\)O were added and mixed by vortex for 10 seconds and then centrifuged at 2000 g for 2 minutes at 16°C. The supernatant was then transferred and dried under nitrogen in a water bath around 35°C. The fatty acid methyl esters were separated by gas chromatography equipped with an autosampler and a flame ionization detector (Model 6890 N, Agilent Technologies, Shanghai, China). The initial temperature of the program was 45°C held for 4 minutes, and then increased at a rate of 13°C/minutes to 175°C, and held for 27 minutes. The oven temperature was then further increased to 215°C at a rate of 4°C/minutes and held for 35 minutes. Each fatty acid was identified by comparison of its retention time with that of external standards. Results were presented as the weight percentage of each fatty acid.

**Statistical analysis**

Each sample was analyzed in triplicate. Results were expressed as mean±standard deviation for all samples. Analysis of variance followed by Student’s t test (two-tailed), where applicable, was used for statistical evaluation of significant differences between groups.

**RESULTS**

Total fat content, n-3 and n-6 PUFAs and n-6/n-3...
The analysis of n6/n3 ratio of beef and pork in 4 cities. The n6/n3 ratios of beef and pork in Nanjing, Shanghai, Yinchuan and Hohhot were (6.28 ± 0.12)%, (5.96 ± 0.15)%, (11.86 ± 0.20)% and (10.34 ± 0.43)%, respectively. And the n6/n3 ratios of pork in Nanjing, Shanghai, Yinchuan and Hohhot were (10.78 ± 0.37)%, (17.52 ± 0.20)%, (16.52 ± 0.20)% and (23.0 ± 3.34)%, respectively. The n6/n3 ratio in beef in Yinchuan and Hohhot were much higher than that in Nanjing and Shanghai. The n6/n3 ratio in pork from Nanjing was significantly lower than that from the other cities investigated in this study. The n6/n3 ratios both in pork and beef from Hohhot represented the highest value among four cities. *P < 0.01, beef compared with pork from same area; #P < 0.01, beef in Yinchuan and Hohhot compared with beef in Nanjing; $P < 0.01, pork in other three regions compared with pork in Nanjing.

Table 1 n-3 polyunsaturated fatty acid concentration of pork and beef from four regions in China

| Meat | Region  | 18:3       | 20:5       | 22:6       | Total       |
|------|---------|------------|------------|------------|-------------|
| Pork | Nanjing | 38.92 ± 0.51 | 28.73 ± 1.31 | 28.37 ± 0.99 | ND | 96.03 ± 1.07 |
|      | Shanghai | 13.40 ± 2.48 | 6.94 ± 2.01 | 27.69 ± 9.95 | 12.43 ± 4.48 | 60.46 ± 18.91 |
|      | Yinchuan | 33.74 ± 0.64 | 8.14 ± 0.22 | 50.94 ± 1.89 | ND | 92.82 ± 1.50 |
|      | Hohhot   | 24.04 ± 2.38 | ND          | 25.06 ± 1.84 | 18.31 ± 1.60 | 67.40 ± 4.66 |
| Beef | Nanjing | 10.20 ± 1.36 | 2.40 ± 2.12 | 23.74 ± 3.42 | ND | 46.34 ± 6.86 |
|      | Shanghai | 18.89 ± 1.37 | 15.95 ± 2.82 | 37.23 ± 5.52 | 3.27 ± 0.65 | 75.43 ± 10.36 |
|      | Yinchuan | 21.83 ± 0.58 | 9.62 ± 0.71 | 26.24 ± 0.56 | 11.30 ± 0.76 | 68.98 ± 1.46 |
|      | Hohhot   | 14.52 ± 0.80 | 14.28 ± 3.24 | 34.93 ± 7.81 | ND | 63.73 ± 10.94 |

Values are mean ± SD, n = 3. ND: not detectable.
DISCUSSION

In this study, the total fat, n-3 and n-6 PUFAs and n-6/n-3 ratio were tested with pork and beef from four representative regions in China. Nanjing and Shanghai represent Eastern coastal and inland region, Yinchuan and Hohhot represent Northwestern and Northern inland regions. As we expected, the content of n-3 PUFAs was substantially lower than that of n-6 PUFAs in both beef and pork from all four regions. The data also showed that the n6/n3 ratio in beef from Yinchuan and Hohhot is almost twice of that from Nanjing and Shanghai. The n6/n3 ratios in both pork and beef from Hohhot showed the highest value among four cities. The results indicate that n6/n3 ratio in the feed for livestock in northern China is much higher than that in southeastern part of China, which may result from the fact that grains with higher n-6 PUFAs are used to produce animal feed in northern China. It is also possible that feed for pigs in the coastal region may contain some fish products. Our results confirmed that the n-6/n-3 ratio in red meat in China is unbalanced and it is consistent with the other reports, which showed high n6/n3 ratios in red meat from other countries and regions.\textsuperscript{15-17}

Although the n-6/n-3 ratio is a critical indicator for the nutritional value of the meat, it is also very important to determine the total amount of n-3 and n-6 PUFAs in the meat samples from the four regions. Our results indicated that, not only is the n-6/n-3 ratio unbalanced and unhealthy in all the red meat samples tested, the total amount of n-3 PUFAs is extremely low which makes it impossible to meet the dose of daily intake of 1-2 g n-3 PUFAs suggested by the American Heart Association (AHA) or the World Health Organization (WHO).\textsuperscript{18}

The fatty acid contents of the pork and beef samples from the four regions were diverse and differed

Table 2 n-3 polyunsaturated fatty acid composition of pork and beef from four regions in China (% of total fatty acid)

| Meat | Region | 18:3       | 20:5       | 22:5       | 22:6       | Total     |
|------|--------|------------|------------|------------|------------|-----------|
| Pork | Nanjing| 0.94±0.01  | 0.69±0.03  | 0.68±0.02  | ND         | 2.33±0.04 |
|      | Shanghai| 0.32±0.06  | 0.16±0.05  | 0.65±0.23  | 0.29±0.10  | 1.42±0.44 |
|      | Yinchuan| 0.322±0.01 | 0.08±0.01  | 0.49±0.02  | ND         | 0.89±0.01 |
|      | Hohhot  | 0.33±0.03  | ND         | 0.34±0.02  | 0.25±0.02  | 0.91±0.06 |
| Beef | Nanjing| 0.47±0.06  | 0.58±0.10  | 1.10±0.16  | ND         | 2.16±0.32 |
|      | Shanghai| 0.70±0.05  | 0.58±0.10  | 1.36±0.20  | 0.12±0.02  | 2.76±0.38 |
|      | Yinchuan| 0.63±0.02  | 0.28±0.02  | 0.75±0.02  | 0.32±0.02  | 1.98±0.04 |
|      | Hohhot  | 0.48±0.03  | 0.47±0.11  | 1.15±0.26  | ND         | 2.10±0.36 |

Values are mean±SD, n = 3. ND: not detectable.

Table 3 n-6 polyunsaturated fatty acid concentration of pork and beef from four regions in China

| Meat | Region | 18:2         | 18:3       | 20:4       | 22:4       | Total     |
|------|--------|--------------|------------|------------|------------|-----------|
| Pork | Nanjing| 874.53±12.28 | ND         | 160.28±31.56 | ND         | 1034.80±43.81 |
|      | Shanghai| 693.26±145.53 | 4.78±1.57  | 323.96±78.66 | 34.94±18.62 | 1056.84±242.8 |
|      | Yinchuan| 1 133.88±31.36 | ND         | 337.72±8.62  | 62.16±1.45  | 1 533.76±41.42 |
|      | Hohhot  | 1 261.2±242.58 | 14.75±6.96 | 292.09±64.04 | 33.43±4.61  | 1 601.48±31.13 |
| Beef | Nanjing| 209.68±27.64 | ND         | 72.60±10.50  | 8.26±1.32   | 290.54±39.42 |
|      | Shanghai| 312.75±34.76 | 3.79±0.36  | 118.59±14.98 | 14.11±1.34  | 449.23±50.73 |
|      | Yinchuan| 617.33±20.94 | 4.99±0.13  | 178.39±9.47  | 17.58±0.21  | 818.29±30.75 |
|      | Hohhot  | 294.85±12.86 | ND         | 300.02±75.04 | 32.37±4.94  | 649.74±82.72 |

Values are mean±SD, n = 3. ND: not detectable.
Table 4 n-6 polyunsaturated fatty acid composition of pork and beef from four regions in China (% of total fatty acid)

| Meat  | Region | 18:2  | 18:3  | 20:4  | 22:4  | Total  |
|-------|--------|-------|-------|-------|-------|--------|
| Pork  | Nanjing| 21.17 | ND    | 3.88  | ND    | 25.06  |
|       | Shanghai| 16.23 | 0.11  | 7.59  | 0.82  | 24.75  |
|       | Yinchuan| 10.85 | ND    | 3.23  | 0.59  | 14.68  |
|       | Hohhot  | 17.07 | 0.20  | 3.95  | 0.45  | 21.67  |
| Beef  | Nanjing| 9.75  | ND    | 3.38  | 0.38  | 13.51  |
|       | Shanghai| 11.46 | 0.14  | 4.34  | 0.52  | 16.61  |
|       | Yinchuan| 17.74 | 0.14  | 5.13  | 0.51  | 23.51  |
|       | Hohhot  | 9.70  | ND    | 10.17 | 1.06  | 21.37  |

Values are mean ± SD, n = 3. ND: not detectable.

Table 5 Fatty acid content in pork and beef from four regions in China

| Fatty acids | City       | Beef (g/100 g) | Pork (g/100 g) |
|------------|------------|---------------|---------------|
|            | Nanjing    | 2.15 ±0.10    | 4.13 ±0.45    |
|            | Shanghai   | 2.73 ±0.10    | 4.27 ±0.20    |
|            | Yinchuan   | 3.48 ±0.11    | 10.45 ±2.41   |
|            | Hohhot     | 3.04 ±0.11    | 7.39 ±0.16    |

*P < 0.05, compared with pork from same area; **P < 0.01, compared with pork from same area; *P < 0.05, compared with beef from Nanjing; #P < 0.01, compared with pork from Nanjing.

It is well recognized, biologically, that it is very important to maintain the relative balanced n-6/n-3 PUFA ratio in the human body. A high n-6/n-3 ratio results in a high inflammation status in the body, which may be the cause of many diseases such as cardiovascular disease, autoimmune diseases, and cancers. In this study, the n-6/n-3 PUFA ratio in four cities was in the range of 6 to 23, which was much higher than (≤ 5:1) recommended by the WHO/FAO.[19] Therefore, an increase of n-3 PUFA as well as reduction of n-6 intake in the diet should be taken into major consideration in all regions of China. Recent production of transgenic pigs and cows[20,21], rich in n-3 PUFA, provides a potential alternative source to provide more n-3 PUFA with daily meat or milk consumption.

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