

Available online at www.jbr-pub.org Open Access at PubMed Central

The Journal of Biomedical Research, 2013, 27(5):366–371

Research Paper

Unbalanced omega-6/omega-3 ratio in red meat products in China

Ming Yu[△], Qianqian Gao[△], Yan Wang, Wei Zhang, Lin Li, Ying Wang[⊠], Yifan Dai[⊠]

State Key Laboratory of Reproductive Medicine, Jiangsu Key Laboratory of Xenotransplantation, Nanjing Medical University, Nanjing, Jiangsu 210029, China. Received 02 May 2013, Revised 23 May 2013, Accepted 29 May 2013, Epub 10 June 2013

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. How– ever, 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

Ph.D., State Key Laboratory of Reproductive Medicine, Jiangsu Key Laboratory of Xenotransplan-tation, Nanjing Medical University, 140 Hanzhong Road, Nanjing, Jiangsu 210029, China. Tel: +86-13851745229, E-mail: wangying_yc@hotmail.com.

 $^{\Delta}$ These authors contributed equally to this work.

The authors reported no conflict of interests.

This work was supported by the National Natural Science Foundation of China (No. 81070671) and the National Basic Research Program (973 Program, No. 2009CB918904).

^{III} Corresponding authors: Yifan Dai, M.D., State Key Laboratory of Reproductive Medicine, Jiangsu Key Laboratory of Xenotransplantation, Nanjing Medical University, 140 Hanzhong Road, Nanjing, Jiangsu 210029, China. E-mail: daiyifan@njmu.edu.cn; Ying Wang,

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 (CH₃OH) 4 mL, chloroform (CHCl₃) 2 mL, and water (H₂O) 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 CHCl₃ and H₂O 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_5H_{12}) and 1.5 mL of H₂O 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 \pm 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



Fig. 1 A map of the four cities in China surveyed in this study.



Fig. **2** 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. The n6/n3 ratios of beef in Nanjing, Shanghai, Yinchuan and Hohhot were $(6.28\pm0.12)\%$, $(5.96\pm0.15)\%$, $(11.86\pm0.20)\%$ and $(10.34\pm0.43)\%$, respectively. And the n6/n3 ratios of pork in Nanjing, Shanghai, Yinchuan and Hohhot were $(10.78\pm0.37)\%$, $(17.52\pm0.20)\%$, $(16.52\pm0.20)\%$ and $(23.0\pm3.34)\%$, respectively. The n6/ n3 ratios 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.

ratio data were obtained from pork and beef samples from the four representative regions in China. 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 n6/n3 ratios of beef in Nanjing, Shanghai, Yinchuan and Hohhot were $(6.28\pm0.12)\%$, $(5.96\pm0.15)\%$, $(11.86\pm0.20)\%$ and $(10.34\pm0.43)\%$, respectively (*Fig. 2*). On the other hand, the n6/n3 ratios of pork in Nanjing, Shanghai, Yinchuan and Hohhot were $(10.78\pm0.37)\%$, $(17.52\pm0.20)\%$, $(16.52\pm0.20)\%$ and $(23.0\pm3.34)\%$, respectively, which is much higher than the ratios in beef (*P* < 0.01)

(*Fig. 2*). 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.

It was also very important to determine the total amount of n-3 and n-6 PUFAs in the meat samples from those four regions. Eight PUFAs were identified in the pork and beef samples. There was a significant difference among the total PUFA content in pork and beef from all four regions in China. The total amount of n-3 PUFA in all the samples was very low (from 46-96 mg/100 g) (Table 1). The relative proportion of total n-3 PUFA to total fatty acids ranged from about 0.89% to 2.76% in eight samples (Table 2). On the other hand, 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 (Table 3). The relative proportion of total n-6 PUFA to total fatty acids ranged from 14.68% to 25.06% (Table 4).

The pork and beef samples from the four regions were diverse and differed substantially in their fatty acid content. Table 4 showed that the total fatty acid content ranged from 4.13 g/100 g (Nanjing, China) to 10.45 g/100 g (Yinchuan, China) in pork and from 2.15 g/100 g (Nanjing, China) to 3.48 g/100 g (Yinchuan, China) in beef, respectively. Total fatty acid concentration in pork from Yinchuan and Hohhot was nearly twice as high as that from Shanghai and Nanjing, whereas the fatty acid content in beef was at a comparable level among these four regions. Within the same city, total fatty acid concentration of pork meat was higher than that of beef (Table 5). The pork and beef from Nanjing and Yinchuan contained fatty acid at level comparable with those from Shanghai and Hohhot, respectively.

Meat	Dagian		n-3 series (mg/100 g)					
	Region	18:3	20:5	22:5	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 \pm SD, n = 3. ND: not detectable.

Meat	Region	n-3 series				
		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

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

Values are mean \pm 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 the 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 southeast 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^[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)^[18].

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

Maat	Region	n-6 series (mg/100 g)				
Meat		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	34.84 ± 18.62	1056.84 ± 242.8
	Yinchuan	1133.88 ± 31.36	ND	337.72 ± 8.62	62.16 ± 1.45	1533.76 ± 41.42
	Hohhot	1261.2 ± 242.58	14.75 ± 6.96	292.09 ± 64.04	33.43 ± 4.61	1601.48 ± 311.3
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	309.02 ± 75.04	32.37 ± 4.94	649.74±82.72

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

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

Meat	Region	n-6 series				
		18:2	18:3	20:4	22:4	Total
Pork	Nanjing	21.17 ± 0.30	ND	3.88 ± 0.76	ND	25.06 ± 1.06
	Shanghai	16.23 ± 3.41	0.11 ± 0.04	7.59 ± 1.84	0.82 ± 0.44	24.75 ± 5.69
	Yinchuan	10.85 ± 0.30	ND	3.23 ± 0.08	0.59 ± 0.01	14.68 ± 0.40
	Hohhot	17.07 ± 3.28	0.20 ± 0.09	3.95 ± 0.87	0.45 ± 0.06	21.67 ± 4.21
Beef	Nanjing	9.75 ± 1.29	ND	3.38 ± 0.49	0.38 ± 0.06	13.51 ± 1.83
	Shanghai	11.46 ± 1.27	0.14 ± 0.01	4.34 ± 0.55	0.52 ± 0.05	16.46 ± 1.86
	Yinchuan	17.74 ± 0.60	0.14 ± 0.01	5.13 ± 0.27	0.51 ± 0.01	23.51 ± 0.88
	Hohhot	9.70 ± 0.42	ND	10.17 ± 2.47	1.06 ± 0.16	21.37 ± 2.72

Table 4 n-6 polyunsaturated fatty acid composition of pork and beef from four regions in China (% of total fatty acid)

Values are mean \pm 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	$2.15 \pm 0.10^{*}$	4.13 ± 0.45
Shanghai	$2.73 \pm 0.10^{**}$	4.27 ± 0.20
Yinchuan	$3.48 \pm 0.11^{* \ \text{\#}}$	$10.45 \pm 2.47^{\&}$
Hohhot	$3.04 \pm 0.11^{* \ \text{\#}}$	$7.39 \pm 0.16^{\&}$

^{**}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.

substantially. Total fatty acid concentration in pork from Yinchuan and Hohhot is nearly twice as high as that from Shanghai and Nanjing, whereas the fatty acid content in beef is at a comparable level among these four regions. Within the same city, the total fatty acid concentration of pork meat was higher than that of beef. The pork and beef from Nanjing and Yinchuan contained fatty acid at levels comparable with those from Shanghai and Hohhot, respectively, which may reflect that there is a regional difference of fatty acid content in pork and beef between north and south China.

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 PUFAs, provide a potential alternative source to provide more n-3 PUFA with daily meat or milk consumption.

Acknowledgments

We thank Haiyuan Yang for critical comments and assistance in the preparation of the manuscript.

References

- [1] Keller H, Dreyer C, Medin J, Mahfoudi A, Ozato K, Wahli W. Fatty acids and retinoids control lipid metabolism through activation of peroxisome proliferatoractivated receptor-retinoid X receptor heterodimers. *Proc Natl Acad Sci U S A* 1993; 90: 2160-4.
- [2] Tapiero H, Nguyen Ba G, Couvreur P, Tew KD. Poly– unsaturated fatty acids (PUFA) and eicosanoids in hu– man health and pathologies. *Biomed Pharmacother* 2002; 56: 215-22.
- [3] Motter AL, Ahern GP. Trpal is a polyunsaturated fatty acid sensor in mammals. *PLoS ONE* 2012; 7: e38439.
- [4] Basiouni S, Stöckel K, Fuhrmann H, Schumann J. Poly– unsaturated fatty acid supplements modulate mast cell membrane microdomain composition. *Cell Immunol* 2012; 275(1-2): 42-6.
- [5] Simopoulos AP. Omega-6/Omega-3 essential fatty acid ratio and chronic diseases. *Food Rev Int* 2004; 20: 77-90.
- [6] Simopoulos AP. The importance of the omega-6/omega-3 fatty acid ratio in cardiovascular disease and other chronic diseases. *Exp Biol Med* 2008; 233: 674-88.
- [7] Mozaffarian D, Ascherio A, Hu FB, Stampfer MJ, Willett WC, Siscovick DS, et al. Interplay between different polyunsaturated fatty acids and risk of coronary heart disease in men. Circulation 2005; 111: 157-64.

- [8] Calder PC. Polyunsaturated fatty acids and inflammatory processes: New twists in an old tale. *Biochimie* 2009; 91: 791-5.
- [9] Kiecolt-Glaser JK, Belury MA, Porter K, Beversdorf DQ, Lemeshow S, Glaser R. Depressive symptoms, omega-6:omega-3 fatty acids, and inflammation in older adults. *Psychosom Med* 2007; 69: 217-24.
- [10] Wood JD, Richardson RI, Nute GR, Fisher AV, Campo MM, Kasapidou E, et al. Effects of fatty acids on meat quality: a review. *Meat Sci* 2004; 66: 21-32.
- [11] Wood JD, Enser M, Fisher AV, Nute GR, Sheard PR, Richardson RI, et al. Fat deposition, fatty acid composition and meat quality: A review. *Meat Sci* 2008; 78: 343-58.
- [12] Enser M, Hallett K, Hewitt B, Fursey GAJ, Wood JD. Fatty acid content and composition of english beef, lamb and pork at retail. *Meat Sci* 1996; 42: 443-56.
- [13] Mahgoub O, Khan AJ, Al-Maqbaly RS, Al-Sabahi JN, Annamalai K, Al-Sakry NM. Fatty acid composition of muscle and fat tissues of Omani Jebel Akhdar goats of different sexes and weights. *Meat Sci* 2002; 61: 381-7.
- [14] Metcalfe LD, Schmitz AA, Pelka JR. Rapid preparation of fatty acid esters from lipids for gas chromatographic analysis. *Anal Chem* 1966; 38: 514-5.
- [15] KarakÖK SG, Ozogul Y, Saler M, Ozogul F. Proxi-

mate analysis. fatty acid profiles and mineral contents of meats: a comparative study. *J Muscle Foods* 2010; 21: 210-23.

- [16] Sousa RV, Fialho ET, Lima JAF, Alvarez-Leite JI, Cortez WC, Ferreira MSS: Effect of different oils in diets for finishing pigs. performance, carcass traits and fatty acid profile of the meat. *Anim Prod Sci* 2010; 50: 863-8.
- [17] Yakan A, Ünal N. Meat production traits of a new sheep breed called Bafra in Turkey 2. Meat quality characteristics of lambs. *Trop Anim Health Prod* 2010; 42: 743-50.
- [18] Interim Summary of Conclusions and Dietary Recommendations on Total Fat & Fatty Acids-From the Joint FAO/WHO Expert Consultation on Fats and Fatty Acids in Human Nutrition, WHO HQ, Geneva, Switzerland, 2008.
- [19] FAO/WHO. Lipids in early development. In: Fats and oils in human nutrition. 1994; 57: 49-55.
- [20] Lai L, Kang JX, Li R, Wang J, Witt WT, Yong HY, et al. Generation of cloned transgenic pigs rich in omega-3 fatty acids. *Nat Biotechnol* 2006; 24: 435-6.
- [21] Wu X, Ouyang H, Duan B, Pang D, Zhang L, Yuan T, et al. Production of cloned transgenic cow expressing omega-3 fatty acids. *Transgenic Res* 2012; 21: 537-43.