

LARGE VARIATIONS IN K VITAMERS IN MEAT FROM NORWEGIAN RED CATTLE

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Abstract – K vitamers (VK) are important for human health, but their intake has reduced significantly over the last decades. Meat may contribute to the intake of VKs, especially MK4. This study investigated VK concentrations, variation in the concentrations, and correlations between different VKs and fat content in 70 representative samples from male and female Norwegian Red Cattle. The concentrations of vitamin K1 and MK4 were on average 4.1 µg/100 g and 6.0 µg/100 g minced meat sample, respectively, with total VK of 10.2 µg/100 g. Variation between samples was high, with total VK varying from 2.8 – 22 µg/100 g, and gender of the animals had an impact on VK levels. MK4 was correlated with MK7 and fat percentage. Due to the large variation, it is desirable to further study how VK levels in meat can be stabilized, in order to be a good source of VK in the human diet.

Key Words – Vitamin K, beef meat, fat content

I. INTRODUCTION

Compounds with vitamin K activity, *i.e.* K vitamers (VK), have been linked to human health, and insufficient intake has been associated with a number of health conditions, such as atherosclerosis, osteoporosis and insulin resistance [1,2,3]. The two main VK in the diet are vitamin K1 and MK4. Vitamin K1 is derived from plants, and is the main dietary form. The main source of MK4 are animal products.

Vitamin K1 has been associated with reduced risk of coronary heart disease [4,5], although an association was not observed in the Rotterdam study [6] and the EPIC study [7]. MK4 has been shown to possess anticancer properties and by stimulating apoptosis of tumor cells, and by inhibiting growth of hepatocarcinoma cells [8,9].

During the last fifty years, VK intake has decreased gradually with possibly as much as 40% [10], which in part may be due to changes in consumption patterns, and reduced VK in processed food due to changes in manufacturing processes.

Vitamin K1 is converted to MK4 in the tissues, with menadione as an intermediate [11]. The enzyme UBIAD1 is responsible for the MK4 biosynthesis [12]. MK6 and MK7 are of bacterial origin and are not formed in the tissues.

The main VKs in meat are vitamin K1 and MK4, with concentrations of about 1-5 µg/100 g for each VK. Due to its bacterial origin, meat contains only low concentrations of MK6 and MK7 [13,14].

Since meat can be a source of VK in the diet, and VK intake is essential for good health, our main purpose was to study: 1) the concentration of VKs in 70 representative samples of Norwegian Red cattle meat; 2) study the variation in concentrations, and 3) how the variation relates to gender of the animals. In addition, we investigated the correlations between VKs and between VKs and fat concentration of the meat.

II. MATERIALS AND METHODS

Animals were Norwegian Red Cattle, 36 young bulls (average age 18 months) and 34 cows (average age 65 months), that were slaughtered in all seasons throughout the year, from late 2013 to early 2015. The animals were fed conventionally with feed concentrate and local roughage. In the present study, no analysis of the vitamin K concentrations in feed were available.

The animals were selected randomly from different areas in Norway to cover the variability of beef production conditions existing in different parts of the country. The beef samples obtained in the present study are therefore representative for the beef that is available to the consumers.

Animals were slaughtered at local slaughterhouses, and the carcasses were sent to Animalia (Oslo)'s pilot plant for cutting and deboning, and subjected to the most common cutting pattern and macronutrient standardization using NIR. Muscle and fat tissue from each of the animals were minced, to give a product containing about 13% fat. Minced meat trimming makes up about 40% of the muscle part of the carcass; therefore, it gives a representative value of the entire animal. Meat samples were kept frozen at -80°C from day 11 until analyzed.

Vitamin K1 and MK4 were analyzed in all samples using a HPLC method. MK7 was only determined for half of the samples. In addition, fat and cholesterol concentrations were determined in all samples.

III. RESULTS AND DISCUSSION

The concentrations of vitamin K1 and MK4 were on average 4.1 µg/100 g and 6.0 µg/100 g minced meat sample, respectively, and the concentration of MK7 was 0.1 µg/100 g minced meat (Table 1). The variation between the animals was high; for vitamin K1, the concentration varied from 1.7 to 13.9 µg/100 g minced meat, for MK4 from 0.9 to 13.6 µg/100 g, and for MK7 from not detectable to 0.2 µg/100 g. The sum of Vitamin K1 + MK4 + MK7 was on average 10.2 µg/100, and varied from 2.8 to 22.0 µg/100 g minced meat. As shown by Rødbotten et al. [14], the concentration of VK in meat (in *M. biceps femoris*, *M. longissimus dorsi* and *M. psoas major*, containing about 6-8 % fat), from Norwegian Red Cattle and Jersey was about 4 µg/100 g and 6 µg/100 g meat from the two breeds, respectively. This is lower than in the present study. However, the vitamin K1 concentration in US beef (ground, 70% beef 30% fat, NDB 13497) was 3.0 µg/100 g, and was even

lower in lean beef steak; 1.6 µg/100 g (NDB 13413, USDA National Nutrient Database).

One possible reason for this may be that, in the present study, minced meat from Norwegian Red Cattle with about 13 % fat was analyzed. VK are fat-soluble and may be higher in some fat tissue; however, in the USDA National Nutrient Database (NDB 04001), the concentration of vitamin K1 in tallow is 0.0. Part of the fat in the minced meat samples in the present study originated from adipose tissue and tallow, and about half was intermuscular and intramuscular fat [15].

Table 1. Average concentration of vitamin K1, MK4, MK7 and fat in minced beef meat from Norwegian Red Cattle

Nutrient	Mean (st.dev)	Bull mean	Cow mean
K1 (µg/100 g)	4.17 (2.01)	3.15	5.26**
MK4 (µg/100 g)	6.01 (2.88)	6.53	5.46
MK7 (µg/100 g)	0.11 (0.03)	0.11	0.12
VK (µg/100 g)	10.24 (3.73)	9.73	10.77
MK4/K1	1.70 (1.19)	2.29	1.09**
MK4/VK	0.57 (0.14)	0.64	0.49**
Total fat (%)	13.18 (2.37)	13.86	12.51*
Cholesterol (mg/100g)	63.0 (6.69)	59.7	66.6**

* indicates $P < 0.05$ in *t*-test on gender effects, ** $P < 0.01$

The high concentration of VK in some of the individuals in the present study is interesting, and shows that meat from these animals would give a significant portion of the reference value for vitamin K, which is 75 ug [16]. However, beef from some of the animals would not contribute enough to cover the recommended intake of VK. The reason for the the large differences in VK concentration in Norwegian Red Cattle is unclear from this study, and should be studied further.

Vitamin K1 is converted to MK4; therefore, the ratio between these K vitamers may give an indication of conversion of K1 to MK4 in the animal. The ratio between MK4 and total VK was on average 0.6, and varied from 0.3 to 0.8 (Table 1).

Vitamin K metabolism may be dependent on gender (due to effects of estrogen on the intestinal absorption of K1 [17]). In this study, K1

concentration in minced meat from young bulls was lower than in meat from cows (3.2 µg/100 g and 5.3 µg/100 g, respectively, $P < 0.001$, Table 1), and the MK4/K1 ratio was twice as high in meat from bulls compared to meat from cows. However, MK4, MK7, and the sum of the VKs was not affected by gender. Whether these results are really due to gender, or rather due to differences in age (bulls were typically younger than cows) or feeding strategy (cows often receive more nutrient-dense feed during lactation), cannot be concluded from this study. Currently, the ratio of meat from cows and bulls in Norway is 50:50.

Vitamin K1 did not correlate with MK4, but the ratio MK4/VK was negatively correlated with vitamin K1 (Table 2). This may indicate that a diet rich in K1 leads to lower synthesis of MK4, possibly via a feedback regulation of UBIAD1 [18]. Because MK7 is a product of bacterial synthesis [19], there was, as could be expected, no significant correlation between K1 and MK7. However, there was a positive correlation between MK4 and MK7, between MK7 and VK, and between MK7 and the ratio MK7/MK4 and MK7/VK, indicating a strong link between MK7 and MK4 (Table 2). If this is specific to ruminants with their special microbial digestion in the rumen is not known.

There was a positive correlation between MK4 and fat (g/100 g minced meat), but no correlation between the concentration of K1 and fat (Table 2). The reason for this may be speculated to be that adipogenesis involves storage of MK4, or increased conversion of K1 to MK4 in the adipose tissue. However, in contrast to this, Rødbotten et al. [14] found that the content of K1 was highly correlated to the amount of intramuscular fat, while MK4 was not associated with intramuscular fat. That study used whole cuts of meat with relatively low intramuscular fat levels, while this study used minced meat with a standardized fat level.

The significant positive correlation between K1 and cholesterol may possibly be rooted in multiple feedback regulation mechanisms in the biosynthesis of sterols and MK4, as suggested by Schumacher et al. [18].

The higher concentrations of both cholesterol and K1 in cows compared to bulls might indicate possible hormonal regulations.

Table 2. Correlations between K vitamers (VK) and fat and cholesterol concentrations in minced meat from Norwegian Red Cattle (R-values)

	K1	MK4	MK7	VK	MK4/VK	MK7/VK	MK7/MK4
K1	1						
MK4	0.130	1					
MK7	0.235	0.327	1				
VK	0.642	0.844	0.387	1			
MK4/VK	-0.465	0.762	0.104	0.338	1		
MK7/VK	-0.068	-0.140	0.476	-0.130	-0.048	1	
MK7/MK4	0.064	-0.260	0.303	-0.153	-0.256	0.936	1
Fat	0.052	0.301	-0.236	0.265	0.310	0.185	0.106
Cholesterol	0.439	0.021	-0.037	0.258	-0.292	0.195	0.298

IV. CONCLUSION

The present study is a survey of the vitamin K concentrations in representative Norwegian minced beef meat. The large variation in VK in meat from 70 Norwegian Red Cattle from 3 µg/100g to 22 µg/100 g minced meat is very interesting in relation to human vitamin K requirements, and should be studied further in order to understand how to stabilize the level above 15% of the reference value (current average value is 13.7). If this can be established through for example feeding or management strategies, then Norwegian minced beef may in the future be labeled as 'a source of vitamin K', which might be of interest to a selection of consumer segments and may therefore have some marketing advantages.

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REFERENCES

1. Brandenburg, V. M., Schurgers, L. J., Kaesler, N., Püsche, K., van Gorp, R. H., Leftheriotis, G., Reinartz, S., Koos, R. & Krüger, T. (2015). Prevention of vasculopathy by vitamin K supplementation: Can we turn fiction into fact?. *Atherosclerosis* 240: 10-16.
2. Pilkey, R. M., Morton, A. R., Boffa, M. B., Noordhof, C., Day, A. G., Su, Y., Miller, L.M. Koschinsky, M. L., & Booth, S. L. (2007). Subclinical vitamin K deficiency in hemodialysis patients. *American Journal of Kidney Diseases* 49: 432-439.
3. Nicoll, R., McLaren Howard J., & Henein, M. (2015) Cardiovascular calcification and bone: A comparison of the effects of dietary and serum vitamin K and its dependent proteins. *International Cardiovascular Forum Journal* 4: 6-13.
4. Erkkilä, A. T., Booth, S. L., Hu, F. B., Jacques, P. F., Manson, J. E., Rexrode, K. M., Stampfer, M. J., & Lichtenstein, A. H. (2005) Phylloquinone intake as a marker for coronary heart disease risk but not stroke in women. *European journal of clinical nutrition* 59: 196-204.
5. Erkkilä, A. T., Booth, S. L., Hu, F. B., Jacques, P. F., & Lichtenstein, A. H. (2007). Phylloquinone intake and risk of cardiovascular diseases in men. *Nutrition, metabolism and cardiovascular diseases* 17: 58-62.
6. Geleijnse, J. M., Vermeer, C., Grobbee, D. E., Schurgers, L. J., Knapen, M. H. J., Van Der Meer, I. M., Hofman, A., & Witteman, J. C. M. (2004). Dietary intake of menaquinone is associated with a reduced risk of coronary heart disease: the Rotterdam Study. *The Journal of nutrition* 134: 3100-3105.
7. Gast, G. C. M., de Roos, N. M., Sluijs, I., Bots, M. L., Beulens, J. W., Geleijnse, J. M., ... & van der Schouw, Y. T. (2009). A high menaquinone intake reduces the incidence of coronary heart disease. *Nutrition, Metabolism and Cardiovascular Diseases* 19: 504-510.
8. Shibayama-Imazu, T., Aiuchi, T., & Nakaya, K. (2008). Vitamin K 2-Mediated Apoptosis in Cancer Cells: Role of Mitochondrial Transmembrane Potential. *Vitamins & Hormones* 78: 211-226.
9. Ozaki, I., Zhang, H., Mizuta, T., Ide, Y., Eguchi, Y., Yasutake, T., Sakamaki, T., Pestell, R.G. & Yamamoto, K. (2007). Menatetrenone, a vitamin K2 analogue, inhibits hepatocellular carcinoma cell growth by suppressing cyclin D1 expression through inhibition of nuclear factor κ B activation. *Clinical cancer research* 13: 2236-2245.
10. Prynne, C. J., Thane, C. W., Prentice, A., & Wadsworth, M. E. J. (2005). Intake and sources of phylloquinone (vitamin K 1) in 4-year-old British children: comparison between 1950 and the 1990s. *Public health nutrition* 8: 171-180.
11. Thijssen, H. H. W., & Drittij-Reijnders, M. J. (1994). Vitamin K distribution in rat tissues: dietary phylloquinone is a source of tissue menaquinone-4. *British Journal of nutrition* 72: 415-425.
12. Nakagawa, K., Sawada, N., Hirota, Y., Uchino, Y., Suhara, Y., Hasegawa, T., Amizuka, N., Okamoto, T., Tsugawa, N., Kamao, M. and Funahashi, N., (2014). Vitamin K 2 Biosynthetic Enzyme, UBIAD1 Is Essential for Embryonic Development of Mice. *PloS one* 9: e104078.
13. Elder, S. J., Haytowitz, D. B., Howe, J., Peterson, J. W., & Booth, S. L. (2006). Vitamin K contents of meat, dairy, and fast food in the US diet. *Journal of agricultural and food chemistry* 54: 463-467.
14. Rødbotten, R., Gundersen, T., Vermeer, C., & Kirkhus, B. (2014). Vitamin K2 in different bovine muscles and breeds. *Meat science* 97: 49-53.
15. <http://www.matvaretabellen.no>
16. European Food Safety Authority (2011). Regulation (EU) No 1169/2011 on the provision of food information to consumers, annex XIII 1A. Available at: <http://eur-lex.europa.eu/legal-content/EN/TXT/HTML/?uri=CELEX:32011R1169&from=EN>
17. Ferland, G., Doucet, I., & Mainville, D. (2016). Phylloquinone and Menaquinone-4 Tissue Distribution at Different Life Stages in Male and Female Sprague–Dawley Rats Fed Different VK Levels Since Weaning or Subjected to a 40% Calorie Restriction since Adulthood. *Nutrients* 8: 141.
18. Schumacher, M. M., Elsabrouty, R., Seemann, J., Jo, Y., DeBose-Boyd, R. A. (2015). The prenyltransferase UBIAD1 is the target of geranylgeraniol in degradation of HMG CoA reductase. 5: 4.
19. Walther, B., Karl, J. P., Booth, S. L., Boyaval, P. (2013). Menaquinones, bacteria, and the food supply: the relevance of dairy and fermented food products to vitamin K requirements. *Advances in Nutrition* 1: 463-73