Contribution of Vitamin D_2 and D_3 and Their Respective 25-Hydroxy Metabolites to the Total Vitamin D Content of Beef and Lamb

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ABSTRACT

Background: Red meat and meat products can contribute meaningfully to the mean daily intake of vitamin D. Beef and lamb can contain vitamin D_3 and 25-hydroxyvitamin D_3 [25(OH)D_3] but also potentially vitamin D_2 and 25-hydroxyvitamin D_2 [25(OH)D_2], all of which contribute to meat's vitamin D activity.

Objectives: We aimed to measure the vitamin D_3, vitamin D_2, 25(OH)D_3, and 25(OH)D_2 content of Irish beef and lamb.

Methods: Full striploin steaks (longissimus dorsi) ($n = 39$) from beef cattle slaughtered in winter, spring, summer, and autumn as well as lamb steaks (hind leg) from sheep slaughtered in autumn ($n = 8$) were sourced and homogenized. The contents of all 4 vitamin D–related compounds were analyzed using an LC-tandem MS method in conjunction with the National Institute of Standards and Technology's standard reference material no. 1546a-Meat Homogenate. The total vitamin D activity of meat was defined as: {vitamin D_3 + [25(OH)D_3 × 5] + vitamin D_2 + [25(OH)D_2 × 5]}.

Results: The median (IQR) total vitamin D activity of striploin beef steak ($n = 39$, irrespective of season) was 0.56 (0.37–0.91) μg/100 g. The content of all 4 vitamin D compounds in beef steak varied significantly ($P < 0.0001$) with season ($n = 8$–11/season group). Median total vitamin D activity of beef steak increased in a stepwise manner ($P < 0.0001$) from winter to the following autumn (increasing from 0.31 to 1.07 μg/100 g). The mean total vitamin D activity of lamb samples ($n = 8$) from autumn was 0.47 μg/100 g.

Conclusions: About one-third of the total vitamin D activity of Irish beef was attributable to its combined vitamin D_2 and 25(OH)D_2 content, estimates of which are largely or completely missed in food composition tables. There was significant seasonal variation in all 4 vitamin D compounds as well as in total vitamin D activity, which has implications for vitamin D nutrient claims for beef. *Curr Dev Nutr* 2020;4:nzaa112.

Keywords: vitamin D, vitamin D_3, vitamin D_2, 25-hydroxyvitamin D_3, 25-hydroxyvitamin D_2, red meat, beef, lamb

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Abbreviations used: FDC, FoodData Central; LC-MS/MS, LC-tandem MS; LOD, limit of detection; NIST, National Institute of Standards and Technology; SRM, standard reference material; 25(OH)D, 25-hydroxyvitamin D; 25(OH)D_2, 25-hydroxyvitamin D_2; 25(OH)D_3, 25-hydroxyvitamin D_3.

Introduction

Vitamin D deficiency is prevalent in the population (1–4), which has led to calls to action for its prevention (1, 5). Although the action of UVB radiation on skin is highly effective in terms of the biosynthesis of vitamin D, there are several environmental factors and personal characteristics which can limit this supply route (6). Dietary supply of vitamin D is an important additional source throughout the year (7), but especially in winter when UVB availability is limited (6). Data from national nutrition surveys in Europe, the United States, and Canada suggest that mean intakes of vitamin D within the adult population are in the range of 3–8 μg/d (7–9) and that the prevalence of inadequacy of vitamin D intake in European and North American populations is very high at 47%–100% (8–10). The *2015–2020 Dietary Guidelines for Americans* included vitamin D among the 4 shortfall nutrients of public health concern (11). Thus, there is a need to address inadequacy of vitamin D intake in the population with a view toward the goal of preventing vitamin D deficiency.

The use of food-based approaches to address micronutrient malnutrition, such as increasing dietary diversity (including recognition of the importance of animal source foods) as well as food fortification, has been stressed by the WHO-FAO (12). However, these are not without their challenges and limitations. With regard to increasing dietary diversity, although the most desirable and sustainable option, the WHO-FAO highlight the need for education on the nutritional value of specific foods and also a change in behavior in terms of food selection (12). In relation to the former point, there is an underappreciation of the contribution that meat and meat products make to the mean daily intakes of vitamin D in some countries. For example, national nutrition survey data in Ireland and the United Kingdom show that 16%–30% and

25%–35% of the mean daily intake of vitamin D in adults and children, respectively, is attributable to meat and meat products (13, 14). It should be emphasized, however, that differences exist in the extent of coverage of the vitamin D content, including 25-hydroxyvitamin D_3 [25(OH)D_3], of foods, as well as in whether an activity factor of 5 for 25(OH)D_3 is applied or not, across different food composition tables (15, 16). For example, the UK food compositional tables incorporate content data on vitamin D_3 plus 25(OH)D_3 multiplied by 5 in their total vitamin D values for meat and other foods (17). The USDA food compositional database [FoodData Central (FDC)] has recently reported 25(OH)D_3 data for beef and a limited number of other foods (18), but does not include these data in total vitamin D values for these foods. The inclusion or omission of activity-adjusted 25(OH)D_3 compositional data for meat and other select animal source foods has been shown to predict meaningful increases and decreases in vitamin D intake estimates, respectively (19).

Beyond vitamin D_3-related compounds, we have recently shown that beef steaks from animals in a 30-d preslaughter vitamin D dietary feeding trial in Ireland had vitamin D_2 and 25(OH)D_2 contents as well as vitamin D_3 and 25(OH)D_3 (20). The source of the vitamin D_2 is not clear. Jäpelt et al. (21) reported that vitamin D_2 is present in ryegrass, and peaked in September, then dropped by a factor of 5 by the second week of November. Thus, beef cattle on grass pasture at different times of the year may be consuming different amounts of vitamin D_2. Meat from grazing sheep may also contain vitamin D_2 and 25(OH)D_2, but analytical data are lacking. The UK food compositional database does not include data on vitamin D_2 or 25(OH)D_2 in meat (17). Likewise, the recently updated USDA FDC does not include data on 25(OH)D_2 in beef, but does report vitamin D_2 data, and reports data on vitamin D_3 only in lamb (18). Because the total vitamin D activity of meat can include all 4 vitamin D compounds [i.e., vitamin D_3 and D_2 as well as 25(OH)D_3 and 25(OH)D_2], analytical data on their content at different times of the year are a key data requirement. This information is also of importance in terms of nutritional labeling of meat, which is a key educational component on how certain foods provide essential micronutrients, as highlighted by the WHO-FAO (12). For example, for meats to be labeled as "a source of" vitamin D in Europe, a minimum content of 0.8 μg/100 g must be substantiated (22).

Thus, the primary aims of the present work were to measure vitamin D_3, vitamin D_2, 25(OH)D_3, and 25(OH)D_2 content of Irish beef sampled from 4 different seasons as well as Irish lamb from autumn, and to calculate the associated total vitamin D activity content values. As a secondary aim, we wished to explore whether vitamin D_2 and 25(OH)D_2 were evident in Irish meats other than beef and lamb.

Methods

Meat samples, including mincing and preparation

Full striploin steaks (longissimus dorsi) taken from beef cattle slaughtered in December 2017 ("winter"), February 2018 ("spring"), July 2018 ("summer"), and September/October 2018 ("autumn") were received from Slaney Foods International, Bunclody, Ireland, a major processor of beef in Ireland with >3000 farm suppliers. The steak samples arrived frozen and were immediately transferred into a −20°C freezer within the Cork Centre for Vitamin D and Nutrition Research for storage until analysis. The breed make-up of the beef cattle from which the striploin steaks were taken consisted of 46%, 18%, and 15% Aberdeen Angus, Charolais, and Limousin, respectively, with the remaining 21% being 6 additional breeds (3%–5% each).

At the time of preparation of the beef samples for vitamin D analysis, full steaks (including the subcutaneous fat layer) were firstly thawed, after which they went through a coarse meat mincer. This was then followed by further homogenization with a Buchi blender to ensure a more even distribution of fat. The fat and moisture contents of the finely minced beef samples were measured using a CEM SMART Trac Rapid Fat and Moisture Analyser (CEM Microwave Technology Ltd.). The samples were stored at −20°C until analysis.

In addition, 8 lamb steaks from the hind leg of sheep slaughtered in October 2018 were supplied by Irish Country Meats, Camolin, Ireland. Convenience samples of pork and turkey (both as mince) as well as chicken (as fillet) were each purchased at 6 different local retail outlets in the greater Cork city area during June 2018. The chicken fillets were minced. All the minced samples were stored at −20°C until required for analysis.

Measurement of the vitamin D content of meat

The vitamin D_3, 25(OH)D_3, vitamin D_2, and 25(OH)D_2 contents of the meat samples were analyzed using a modification of a sensitive LC-tandem MS (LC-MS/MS) method, as described in detail elsewhere (20, 23). The extraction procedure for the analysis was a modification from a previously published method (23), which included an overnight saponification and solid phase extraction followed by 4-phenyl-1,2, 4-triazoline-3.5-dione derivatization. The method's limit of detection (LOD) for vitamin D_3, vitamin D_2, 25(OH)D_3, and 25(OH)D_2 was 0.007, 0.009, 0.003, and 0.007 μg/100 g, respectively. The National Institute of Standards and Technology (NIST) standard reference material (SRM) no. 1546a: Meat Homogenate (24) was analyzed for the 4 vitamin D compounds so that a comparison could be made with its reference vitamin D_3 and certified 25(OH)D_3 values.

The total vitamin D activity of meat was defined as {vitamin D_3 + [25(OH)D_3 × 5] + vitamin D_2 + [25(OH)D_2 × 5]}. The conversion factor of 5 is applied to the 25(OH)D_3 content on the basis of efficacy data from a randomized controlled trial with oral vitamin D_3 and 25(OH)D_3 in healthy adults (25), and is a factor commonly used in a number of food composition tables (26–28). It should be noted, however, that equivalent data do not exist for vitamin D_2 and 25(OH)D_2, so we assumed a conversion factor of 5 in our calculation of the total vitamin D activity of meat (20).

Statistical analysis of the beef data

Statistical analysis of the data was conducted using SPSS for Windows, version 20.0 (SPSS Inc.). Distributions of all variables were tested via Kolmogorov–Smirnov tests. Descriptive statistics (means ± SDs or medians and IQRs, when appropriate) were determined for all variables. The beef fat content was normally distributed. The vitamin D_3, vitamin D_2, 25(OH)D_3, 25(OH)D_2, and total vitamin D activity of beef were nonnormally distributed. A log transformation of total vitamin D activity achieved a near-normal distribution, whereas various transformations of vitamin D_3, vitamin D_2, 25(OH)D_3, and 25(OH)D_2 did not improve the normality. A 1-factor ANOVA followed by Tukey's test were used to compare the mean total vitamin D activity and fat content

TABLE 1 The measured vitamin D, 25-hydroxyvitamin D, and total vitamin D activity of Irish striploin beef steak, sampled over 4 seasonal time points throughout 2017–2018[1]

	December 2017	February 2018	July 2018	Sept/Oct 2018
n	10	10	11	8
Vitamin D_3, μg/100 g	0.028 (0.025–0.038)^a	0.026 (0.022–0.032)^a	0.057 (0.049–0.074)^b	0.023 (0.022–0.041)^a
Vitamin D_2, μg/100 g	0.012 (0.011–0.012)^a	0.011 (0.010–0.011)^a	0.036 (0.034–0.040)^b	0.034 (0.032–0.035)^b
25-Hydroxyvitamin D_3, μg/100 g	0.039 (0.036–0.047)^a	0.065 (0.061–0.068)^b	0.109 (0.091–0.123)^c	0.136 (0.128–0.150)^d
25-Hydroxyvitamin D_2, μg/100 g	0.012 (0.011–0.012)^a	0.033 (0.032–0.034)^b	0.032 (0.023–0.037)^b	0.061 (0.048–0.073)^c
Total vitamin D activity, μg/100 g	0.314 (0.271–0.351)^a	0.525 (0.513–0.550)^b	0.774 (0.680–0.878)^c	1.074 (0.990–1.242)^d

[1]Values are medians (IQRs) unless otherwise indicated. Values in a row without a common letter are significantly different, $P < 0.05$–0.001 via Kruskal–Wallis or 1-factor ANOVA followed by Mann–Whitney test or Tukey's test, respectively.

of beef steaks from the 4 different sampling points, whereas Kruskal–Wallis tests followed by Mann–Whitney tests were used for comparison of vitamin D_3, vitamin D_2, 25(OH)D_3, and 25(OH)D_2 contents of the meat.

Beef fat has been shown to have a higher content of vitamin D_3, and possibly 25(OH)D_3, than lean beef meat (29); therefore, in an additional analysis, differences in fat content of the striploin steaks sampled from different seasons were accounted for in a regression model. The exploration of whether vitamin D_2 and/or 25(OH)D_2 was evident in Irish meats other than beef was guided by the presence of contents above the LOD for these compounds, and not subjected to statistical testing.

Results

Our analysis of the NIST SRM 1546a Meat Homogenate showed that our analytical method allowed for 84.0% and 83.4% recovery of vitamin D_3 and 25(OH)D_3, respectively, in the meat homogenate, based on its respective reference mass and certified mass values (24).

Taking the complete collection of Irish striploin beef steak samples ($n = 39$), and irrespective of season of sampling, the median (IQR) total vitamin D activity was 0.56 (0.37–0.91) μg/100 g. The median (IQR) for vitamin D_2, vitamin D_3, 25(OH)D_2, and 25(OH)D_3 content was 0.024 (0.011–0.035), 0.034 (0.023–0.049), 0.032 (0.022–0.037), and 0.070 (0.050–0.123) μg/100 g, respectively.

Table 1 shows the content of the 4 vitamin D compounds in the Irish striploin beef steak, as sampled over 4 seasons. The content of all 4 vitamin D compounds in the beef steak varied significantly ($P < 0.0001$) with sampling period; median vitamin D_3 content of meat samples from summer was approximately twice that of meat samples from the other 3 seasons, whereas the median vitamin D_2 content of meat samples from summer and autumn was ∼3 times that of meat samples from either winter or spring. There were stepwise increases in the median 25(OH)D_3 content of meat samples on going from winter through autumn, such that the content in meat from September/October was ∼3.5-fold higher than that in meat from December. The median 25(OH)D_2 content of meat samples from spring and summer was significantly ($P < 0.001$) higher than that of meat samples from winter, whereas median 25(OH)D_2 was significantly ($P < 0.001$) higher in meat samples from autumn than the other 3 seasonal sampling points (1.8- to 5-fold).

There were also stepwise increases in the median total vitamin D activity of beef steaks on going from winter through autumn, such that the content in meat from September/October was ∼3.5-fold higher than that in meat from December (at 1.07 and 0.31 μg/100 g, respectively).

Table 2 shows the mean percentage fat of the Irish steak samples from the 4 seasonal sampling points. The mean fat content from meat samples from winter was significantly lower than that from spring or summer but similar to that from autumn, with no significant differences in the mean fat content of meat from these latter 3 seasonal sampling points.

The significant differences in estimated median total vitamin D activity of Irish beef steak among the 4 sampling points were still evident even after controlling for differences in percentage fat content of meat within a regression model ($P < 0.0001$) (data not shown).

The mean ± SD total vitamin D activity of the collection of lamb steaks was 0.47 ± 0.17 μg/100 g. The 25(OH)D_2, adjusted for an activity factor of 5, contributed 21% to the total vitamin D activity of lamb steaks (this value was 28% in beef steaks for the equivalent sampling point). None of the 8 lamb steaks had vitamin D_2 content at concentrations above the LOD.

Vitamin D_2 and 25(OH)D_2 in Irish meats other than beef and lamb?

Minced pork, turkey, and chicken had vitamin D_3 (mean range: 0.018–0.026 μg/100 g) and 25(OH)D_3 contents (mean range: 0.022–0.041 μg/100 g), but none had vitamin D_2 or 25(OH)D_2 contents at concentrations above the LOD of our LC-MS/MS method (data not shown).

TABLE 2 The mean measured percentage fat content of the raw Irish striploin beef steak samples sampled over 4 time points throughout 2017–2018[1]

	December 2017	February 2018	July 2018	Sept/Oct 2018
n	10	10	11	8
Fat, g/100 g	7.1 ± 1.9^a	13.1 ± 2.9^b	12.1 ± 2.4^b	10.0 ± 2.9^{ab}

[1]Values are means ± SDs unless otherwise indicated. Values in a row without a common letter are significantly different ($P < 0.05$) via 1-factor ANOVA followed by Tukey's test.

Discussion

The present work highlighted how, in addition to vitamin D_3 and $25(OH)D_3$, Irish beef contained vitamin D_2 and $25(OH)D_2$, whereas other Irish meats had essentially none, or very little, of either compound, with the exception of $25(OH)D_2$ in Irish lamb. About one-third of the total vitamin D activity of Irish beef was attributable to its combined vitamin D_2 and $25(OH)D_2$ content. When the 4 vitamin D–related compounds were included in the calculation, Irish striploin beef steak (∼11% fat) had a median total vitamin D activity of 0.56 μg/100 g, which is similar to the 0.4 and 0.5 μg/100 g reported for sirloin beef steak (lean and 18% fat, respectively) in the UK food composition database (17). These Irish and UK estimates, however, are much higher than that reported in the USDA FDC for top loin steak trimmed to 1/8” (3.2 mm) fat {0.1 μg vitamin D plus 0.26 μg 25-hydroxyvitamin D [25(OH)D]/100 g meat} (18). Neither the US nor the UK food composition database reports data on striploin steaks, but there is little variation in the vitamin D content of different steak cuts within the US database (18). It should be noted that the total vitamin D content for Irish/UK beef includes estimates of meat 25(OH)D content and applies an efficacy factor of 5 to the same (17), whereas the US database individually reports vitamin D (as vitamin $D_3 + D_2$) and 25(OH)D estimates for beef, but does not multiply the 25(OH)D by 5 and add it to the vitamin D (18). If the Irish/UK mode of calculation was applied to the US data, the total vitamin D content of the top loin steak would be 1.4 μg/100 g. Taylor et al. (19) estimated that incorporation of this efficacy-adjusted 25(OH)D data into total vitamin D content values for beef would increase the mean daily vitamin D intake of men and women in the United States by an extra 0.5 and 1 μg/d, respectively.

Neither the UK nor US food composition tables provide information on the time of year the steaks were sampled. This is of relevance because the present work highlighted significant seasonal variation in all 4 vitamin D compounds as well as in the associated estimates of total vitamin D activity, which could be, on average, as high as 1.1 μg/100 g in autumn and as low as 0.3 μg/100 g in winter. This is, to our knowledge, the first study to directly show this seasonal variation in vitamin D content of beef. Liu et al. (29) measured the vitamin D content of beef rump steak sampled from 2 different latitudes within Australia (17°S and 41°S) at the end of their winter, as a surrogate of differences in UV availability across seasons (higher compared with low, respectively). Using their reported data on vitamin D_3 and $25(OH)D_3$ content of the lean beef (and assuming 12% fat), crude estimates of total vitamin D activity can be generated and it appears to be higher in meat from the sunnier region (∼0.8 μg/100 g) than in meat from the less sunny region (∼0.2 μg/100 g). Casas et al. (30) clearly showed a seasonal variation in serum 25(OH)D concentration in beef cattle reared in the central United States, such that the mean concentrations were 28, 65, 120, and 137 nmol/L during February/March, June, August, and September/October, respectively (meat vitamin D analysis was not performed within the study). This trend in serum 25(OH)D concentrations aligns with the beef total vitamin D activity in the present study.

This seasonal variation in total vitamin D activity has implications for potential nutritional labeling of steaks in relation to vitamin D in Ireland and the United Kingdom. In the present work, steaks from beef cattle slaughtered in summer and autumn had median total vitamin D activity estimates >0.8 μg/100 g, which would allow for a “source of” European nutrient content claim (22), whereas the steaks from cattle slaughtered in winter and spring had median total vitamin D activity estimates less than the 0.8 μg/100 g benchmark. Of note, recent data from a limited number of vitamin D dietary feeding trials in beef cattle (20, 31, 32) have shown how a vitamin D supplementation strategy during winter and spring could help protect against the seasonal decline evident in beef in the present study, and also facilitate a year-round labeling claim. For example, beef from animals whose finishing diet was supplemented with 4000 IU vitamin D_3/kg feed (the maximum allowable level of addition in Europe) had a total vitamin D activity of 1.4–1.5 μg/100 g (31, 32). It should be noted that the US FDA–mandated labeling for vitamin D is based on measured content and expressing this as a percentage of the Daily Value (20 μg/d) (33).

Although $25(OH)D_3$ was the compound in beef most variable by season, $25(OH)D_2$ also varied considerably. This might also help explain, at least in part, the difference between the present Irish estimates and that in the UK food composition data for beef steaks (0.56 compared with ∼0.45 μg/100 g, respectively). For example, using the median estimates of vitamin D_2 and $25(OH)D_2$ from our analysis of meat from all 4 time points combined, and adding these to the UK estimate which is solely based on vitamin D_3 and $25(OH)D_3$, the recalculated total vitamin D activity of UK beef would be 0.60 μg/100 g, very comparable with our estimate for Irish beef (0.56 μg/100 g). The vitamin D_2 entry for top loin steak trimmed to 1/8” (3.2 mm) fat in the USDA FDC (Foundation data) is 0 μg/100 g meat (18), but it is not clear if this is a measured value. For example, within the FDC, the Foundation data also report 0 μg/100 g top loin steak for vitamin D_3, whereas its Standard Reference legacy data suggest 0.1 μg/100 g (18).

The source of the vitamin D_2 in the meat has to be dietary derived, unlike vitamin D_3, which is synthesized in the skin of vertebrates on exposure to UVB-containing sunlight of sufficient strength. When ergosterol in grassland, present owing to fungal growth, is exposed to UVB of sufficient strength it is converted to vitamin D_2, which is then consumed by grazing cattle. Jäpelt et al. (21) have shown that the vitamin D_2 content of perennial ryegrass, the most important agricultural grass seed sold in Ireland (34), is much higher in July and September and considerably lower during June and November. This might explain the higher vitamin D_2 content of beef in the present study during July and September/October, and also the peak in $25(OH)D_2$ in September/October, after circulating vitamin D_2 is hydroxylated in the liver of the cattle and enters the muscle. The lack of vitamin D_2 or $25(OH)D_2$ in our pork, turkey, or chicken meat samples in the present work is likely due to the fact that pigs and poultry do not eat grass.

Owing to the time-consuming and expensive nature of food vitamin D analysis (35), we had to limit our analysis to just 1 cut each for beef and lamb, which is a limitation of the present work. Although there is little variation in the vitamin D_3 content of different steak cuts within the US database (18), future work will seek to confirm whether this is the case for $25(OH)D_3$ and the 2 vitamin D_2–related compounds in different steak cuts as well as other types of Irish beef and lamb.

In conclusion, the findings of the present work are of importance in relation to beef’s potential contribution to vitamin D intake, and also as a means of educating the public of the same. Unprocessed beef is consumed by a majority (≥80%) of Irish individuals and the Irish food portion database shows that the median portion weight of fried/grilled beef steak is 147 g/d and 160 g/d (equivalent to ∼184 g/d and ∼200 g/d, respectively, raw weights based on a 25% moisture loss during cooking) in Irish teenagers and adults, respectively (36). Using the overall median and autumn-based median total vitamin D content of striploin steak in the present analyses, these beef intakes would contribute ∼1 and ∼2 μg vitamin D/d, respectively. A daily vitamin D intake of ∼6 μg can maintain serum 25(OH)D ≥25 nmol/L (the threshold for vitamin D deficiency in the United Kingdom) in 97.5% of individuals (37). It should be noted, however, that the ∼1–2 μg vitamin D from steak would only contribute between 5% and 13% to the US RDA for vitamin D based on a serum 25(OH)D threshold of 50 nmol/L [at 15 and 20 μg/d for those aged 1–70 and >70 y, respectively (38)]. Consideration of the contribution of lean red meat to vitamin D intake would also need to be cognizant of the number of servings and/or amounts recommended in various dietary guidelines internationally (39). The findings are also of relevance in terms of nutritional labeling of meat in Europe, which is a key educational component on how certain foods provide essential micronutrients, as highlighted by the WHO-FAO (12). The present work shows how, depending on the season in question, up to about half the year's supply of beef may contain concentrations of vitamin D compounds such that a "source of vitamin D" nutrition claim could be made in Europe (i.e., once content ≥ 0.8 μg/100 g). This may be missed completely by the use of values taken from food composition tables (which is allowable for health claims), because these have reported estimates of 0.1–0.5 μg/100 g, depending on the tables used (17, 18).

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