Original Article

New data for vitamin D in Australian foods of animal origin: impact on estimates of national adult vitamin D intakes in 1995 and 2011-13

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Objectives: To assess the potential dietary supply of vitamin D to Australian adults by application of new data for Australian primary foods of animal origin. **Methods:** New published analytical data on the vitamin D contents of Australian primary foods from animal products were obtained and assessed for reliability. Using food consumption data from Australian population dietary surveys for 1995 and 2011-2013, estimates were made of the likely average daily intakes of vitamin D equivalents from these sources by Australian adults. **Results:** Meats, chicken, fish, eggs and dairy produce may alone have contributed about 4.2 μg vitamin D equivalents per day to average Australian diets of adults >18 years in 1995 and 4.3 μg in 2011-2013. **Conclusions:** Dietary vitamin D intake in Australia is likely to be higher than previously estimated because new data from improved analytical methods reveal the contributions to vitamin D supply from foods of animal origin. Absence of reliable vitamin D data for milk and milk products, and the gaps in vitamin D data for many commonly consumed seafood, poultry, eggs and processed animal products greatly limit estimation of dietary vitamin D intakes by Australians.

Key Words: vitamin D, animal origin foods, Australia, adult diets, dietary survey

INTRODUCTION

Considerable concern has been expressed about the prevalence of low vitamin D status among Australians. A recent major population-based study of over 11,000 adults aged >18 years, using serum concentrations of 25-hydroxy-vitamin D, estimated nearly one-quarter were vitamin D deficient at <50 nmol/L, with adults aged 18-34 years, those born outside Australia, northern Europe or America and those who work indoors being most at risk.[1] Earlier clinical studies had already identified particular subgroups at high risk in the general population, notably the elderly, women with deeply pigmented skin or veiled, and those living in residential care.[2] The 2012-2013 Australian Aboriginal and Torres Strait Islander Health Survey stated that 26.5% of Aboriginal and Torres Strait Islanders had vitamin D deficiency (<50 nmol/L).[3] National strategies to improve vitamin D status in Australia have been suggested,[2] including additional fortification of the food supply.[4] The Australian Adequate Intake (AI) is 5 μg per day for adults aged 19-50 years and 10 μg for older adults.[5]

Vitamin D_3 (cholecalciferol) can be obtained from exposure of skin to solar UV-B light. Vitamin D_3 is then transported to the liver where it is converted to 25-hydroxy-vitamin D_3 ($25OHD_3$), the form present in the circulation.[6] The kidney converts $25OHD_3$ to 1,25-dihydroxy vitamin D_3, the major biologically active form responsible for calcium homeostasis.[7] Edible mammals, poultry and aquatic species also contain, to varying degrees, vitamin D_3 and $25OHD_3$, particularly when fed on fortified feeds.[8] Furthermore, since vitamin D activity is specifically required for embryonic development, egg yolks may contain high levels of vitamin D.[9]

Vitamin D activity is expressed mainly by four compounds: vitamin D_3, vitamin D_2 (ergocalciferol) and their metabolites, $25OHD_3$ and 25-hydroxy-vitamin D_2. It is known that the bioactivity of oral $25OHD_3$ is higher than that of vitamin D_3. However, the exact relative bioactivity of these two forms of vitamin D_3 in humans is still debated. The factor most commonly quoted is that $25OHD_3$ is five times more active than vitamin D_3, based on a study performed on rats, published in 1973.[10] A more recently

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published randomised, controlled intervention study comparing the relative effectiveness of oral D_3 and $25OHD_3$ in raising serum $25OHD_3$ in adults aged >50 years has also found a biopotency for $25OHD_3$ of five times that of vitamin D_3.[11] For the purposes of this article the term "vitamin D equivalents" ("VitDE") will be used, where appropriate, to denote vitamin D activity as: ug of VitDE = 1 x ug vitamin D_3 + 5 x ug $25(OH)D_3$.

It is frustrating that so little is known about vitamin D activity levels in the Australian diet. This lack of basic information is an impediment to development of food-based strategies for counteracting the reported population deficiency of vitamin D. Food Standards Australia and New Zealand (FSANZ) maintains Australia's national food composition database, NUTTAB, primarily in order to support food standards. The most recent edition of this database, NUTTAB 2010,[12] does not contain values for vitamin D activity in Australian foods due to the paucity of data and concerns about the reliability of available analytical methods and results. However, there is an accompanying Excel file which lists some vitamin D activity data for a small number of foods,[13] determined by analysis or estimation. The analytical method used was HPLC with UV detection, and a usual level of reporting (LOR) of 5 µg/100 g for vitamin D_3[14] which means that it cannot detect the relatively low levels of vitamin D compounds naturally present in foods.

FSANZ also prepares survey-specific databases for population surveys from NUTTAB and other sources. The most recent survey-specific database is the AUSNUT 2011-2013 database.[15] This database does not contain data for vitamin D or its related compounds, and an accompanying explanatory notes state: "There is limited Australian derived analytical data available for vitamin D levels found in foods and there are analytical difficulties associated with measuring the low levels of vitamin D found naturally occurring in food". Both NUTTAB and AUSNUT databases are available publicly and are frequently used for dietary analysis purposes in Australia via software packages. Their lack of reliable values for many foods that may contribute to vitamin D intake may well mean that estimates of intakes[16-17] based on them are of doubtful accuracy.

A contributing factor to the lack of reliable information for vitamin D active compounds in foods has been a poor capacity internationally for analysis, especially at the low levels that are expected to occur naturally in foods. In Australia this situation has now improved due to the recent development and establishment of liquid chromatography mass spectrometry methods (LC-MS) for vitamin D activity in meat[18] and in milk;[19] and of a HPLC method[20] adapted from a Danish method developed for pork.[21]

There are four objectives for the present study: First, to collate and present new preliminary analytical data for vitamin D activity in some major Australian food commodities of animal origin; secondly, to use these data to estimate and compare dietary intake from these foods, on the basis of representative population food intake data. These estimates, in turn, can be used to determine whether the average adult diet would likely meet the Australian adequate intake (AI) of 5 µg/day for adults up to 50 years of age (expressed as µg vitamin D_3 + 5 x µg $25OHD_3$[5]); thirdly, to determine if there is a difference in dietary VitDE intake between 1995 and 2011/13; and finally, to highlight current major challenges associated with completion of a more accurate representation of Australian dietary VitDE supply.

MATERIALS AND METHODS

Sampling and analysis

For this study, the majority of the data have been consolidated from recent publications. Original data were obtained for wild caught blue mackerel (*Scomber australasius*) from the Sydney Fish Market.[22] Additional seafood data were obtained from the Australian Seafood Compositional Profiles study[23] which analysed samples of farmed Atlantic salmon (*Salmo salar*), farmed barramundi (*Lates calcarifer*), farmed ocean trout (*Oncorhynchus mykiss*), farmed yellowtail kingfish (*Seriola lalandi*), and wild catch burrowing blackfish (*Actinopyga spinea*). Ninety six whole chickens (two major brands) were collected from Queensland, Western Australia, New South Wales and South Australia for two nationwide analytical surveys of the nutrient composition of conventional and free-range chicken.[24-25]

Three brands (A, B, and C) of chicken eggs were purchased from Sydney retailers in an exploratory study.[20] Analytical studies were conducted of vitamin D activity in samples of beef and lamb from supermarkets and butchers across high and low socio-economic status regions of Sydney, New South Wales, and from Queensland and Tasmania.[26] Opportunistic lean pork samples were from a Sydney butcher,[18] and unfortified milk (3.8% fat) was purchased from retailers in South Vermont, Victoria.[19] Whole egg and butter (salted), purchased in New South Wales, Western Australia, Tasmania, Australian Capital Territory and Victoria, were analysed by the National Measurement Institute of Australia for FSANZ.[27]

Mackerel (edible portion) and egg yolks were analysed for vitamin D_3 and $25OHD_3$ using HPLC.[20] Chicken, lamb, and beef, were separated into lean and fat tissue before being analysed using liquid chromatography with ion trap spectrometry (LC-IT-MS).[26] Whole egg, butter (salted), lean pork and edible portions of farmed fish were analysed using the same method. Milk was analysed using LCMS.[19]

Dietary estimation

To estimate intake of dietary VitDE from primary animal foods in 1995, data for adults over 18 years of age were drawn from the 1995 National Nutrition Survey.[28] Because the published summary results of this survey did not clearly distinguish between different species of animal product consumed, the proportion of chicken, beef, lamb, and pork consumption were instead obtained from the Apparent Consumption of Food Stuffs report[29] from the same period. More recent estimations were drawn from the 2011-2013 Australian Health Survey.[30] Again, this summary report did not distinguish between different species of animal product, in all relevant food products. Apparent consumption figures for this time period were collected from industry data[31] as there were no published government figures.

Table 1. Moisture, vitamin D_3, 25-hydroxy-vitamin D_3 ($25OHD_3$), and total vitamin D equivalent (vitamin D_3 plus 5x $25OHD_3$) content of various raw primary animal foods compared with values listed by Food Standards Australia New Zealand.

Food	n	Moisture (%)	Analysed Vitamin D_3	Analysed $25OHD_3$	Calculated VitDE †	FSANZ file 2010 VitDE
Mackerel, wild catch[22]	4	NA	4.91	<LOQ	4.91	NA
Atlantic salmon, farmed[23,40]	3	62.3	5.81	<LOQ	5.81	NA
Barramundi, farmed[23,40]	2	72.0	10.7	<LOQ	10.7	NA
Ocean trout, farmed[23,40]	2	54.3	1.63	<LOQ	1.63	NA
Yellowtail kingfish, farmed[23,40]	1	63.1	1.30	<LOQ	1.30	NA
Egg, chicken, whole[27‡]	1	76.1	1.1	0.7	4.6	0
Egg yolk (brand A)[20§]	6	NA	2.93	4.20	23.9	NA
Egg yolk (brand B)[20§]	6	NA	2.11	1.36	8.92	2.20
Egg yolk (brand C) [20§]	4	NA	0.46	<LOQ	0.46	NA
Beef (1995) [26¶]	8	71.6	0.11	0.16	0.90	0
Lamb (1995)[26¶]	8	NA	0.27	0.16	1.05	0
Beef (2011/12) [26††]	8	71.6	0.14	0.15	0.90	0
Lamb (2011/12) [26††]	8	NA	0.17	0.18	1.07	0
Conventional chicken (project 1)[24‡‡]	48	73.2	0.29	0.51	2.84	0
Conventional chicken (project 2)[25§§]	24	72.4	0.44	0.36	2.24	0
Free range chicken (project 2) [25‡‡]	24	72.8	0.41	0.49	2.86	NA
Pork, lean[18]	1	NA	0.18	0.17	1.03	NA
Milk, unfortified, 3.8% fat[19]	1	NA	0.02	NA	0.02	0.3
Butter, salted[27¶¶]	1	14.8	1.2	<LOQ	1.2	0

All values are in µg/100 g unless otherwise indicated.
n: Number of food samples, analysed in duplicate apart from mackerel, egg, butter; NA: not available; LOQ: limit of quantification, a concentration below the threshold of accurate quantification. This value ranged between 0.01 to 0.05 µg/100 g, depending on the method used (except for FSANZ file LOQ 5 ug/100 g).
†Sum of vitamin D_3 content plus 5 x $25OHD_3$ content.
‡Composite sample of 5 different brands and production methods from 5 States/Territories.
§Origin of hen eggs.
¶In 1995 beef and lamb meat were assumed to be 78.25% lean and 21.75% fat[32].
††In 2011/12 beef and lamb meat were assumed to be semi-trimmed as defined by AUSNUT[15].
‡‡Samples were 4 homogenates, each composed of 6 chickens (3M, 3F) from each of 2 processing plants in each of 2 states (QLD and WA).
§§Analytical samples were 4 homogenates, each composed of 6 chickens (3M, 3F) from each of 2 processing plants in each of 4 states (NSW, SA, QLD, WA).
¶¶Composite sample of 4 Australian and 1 New Zealand brands from 4 States/Territories.

Making the assumption that these new analytical data for food VitDE applied to the Australian food supply in both 1995 and 2011/13, and to the above mentioned survey data, it was possible to estimate the likely daily consumption, and hence potential total VitDE contribution of certain animal foods.

In 1995, VitDE from five available finfish species were averaged. For eggs, VitDE from three available brands were averaged and it was assumed the amount of egg yolk consumed was a third of the amount of whole egg consumed. Beef and lamb were assumed to be 78.25% lean and 21.75% fat.[32] The VitDE data for chicken were averaged between three national chicken projects which covered traditional and free range farming systems.[24-25] All edible components of chicken were assumed to be consumed. Pork was assumed to be 100% lean as only lean samples were available for analysis. Milk (including milk products) and dairy fats were also included. VitDE (µg) were calculated as: µg vitamin D_3 + (5 x µg $25OHD_3$).

For 2011-2013, a similar approach to the above was used to estimate VitDE consumed. Adjustments were also made to the lean to fat ratio of meat to account for possible changes in trimming practices over the years. For beef and lamb, three levels of trim were explored; untrimmed, semi-trimmed, and fully-trimmed, as defined by AUSNUT 2011-2013. For beef, full trimmed, semi trimmed, and untrimmed were defined as 2.6%, 7.2%, and 15.2% fat respectively. For lamb, full trimmed, semi trimmed, and untrimmed were defined as 4.9%, 9.4%, and 19.7% fat respectively.[15] Because level of trim had a minimal effect on resulting estimates of VitDE consumption, semi-trimmed was selected as the representative level of trim for this time period.

RESULTS

Analytical data for vitamin D in foods

Analytical data for VitDE in foods of animal origin varied greatly in quality, particularly in terms of the breadth of sample representation for each food analysed. Major issues were low numbers of samples and use of composite rather than individual samples (Table 1). The content of both vitamin D_3 and $25OHD_3$ varied considerably between foods with species and feeding methods being major determinants. Egg yolks examined varied in content from 0.46 to 23.9 µg VitDE/100 g, depending on brand and layer feed. Meat from intensively reared terrestrial animals contained between 1.03 (pork) and 2.86 (chicken) µg VitDE/100 g, while meat from pasture-raised animals contained between 0.9 and 1.07 µg/100 g. Farmed fish varied from 1.30 to 10.7 µg VitDE/100 g depending on species, while wild catch mackerel contained 4.91 µg/100

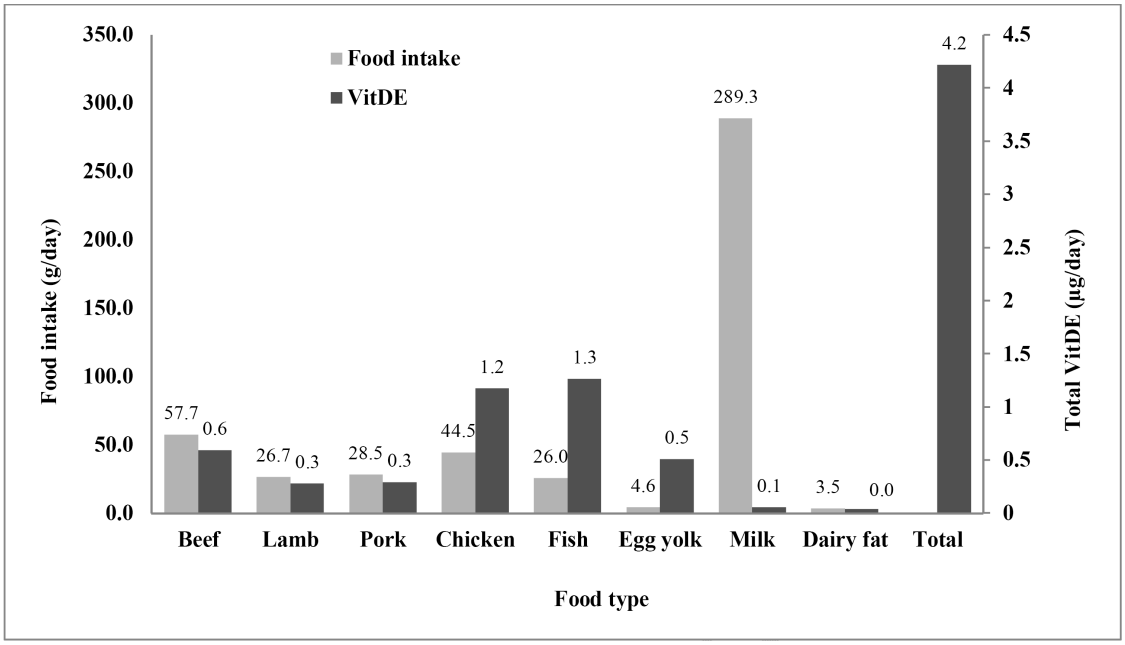

Figure 1. Average amount of food consumed (g/day) by Australians >18 years in 1995, and the corresponding vitamin D equivalents (VitDE) intake (µg/day). VitDE values were calculated by averaging analytical values from Table 1.

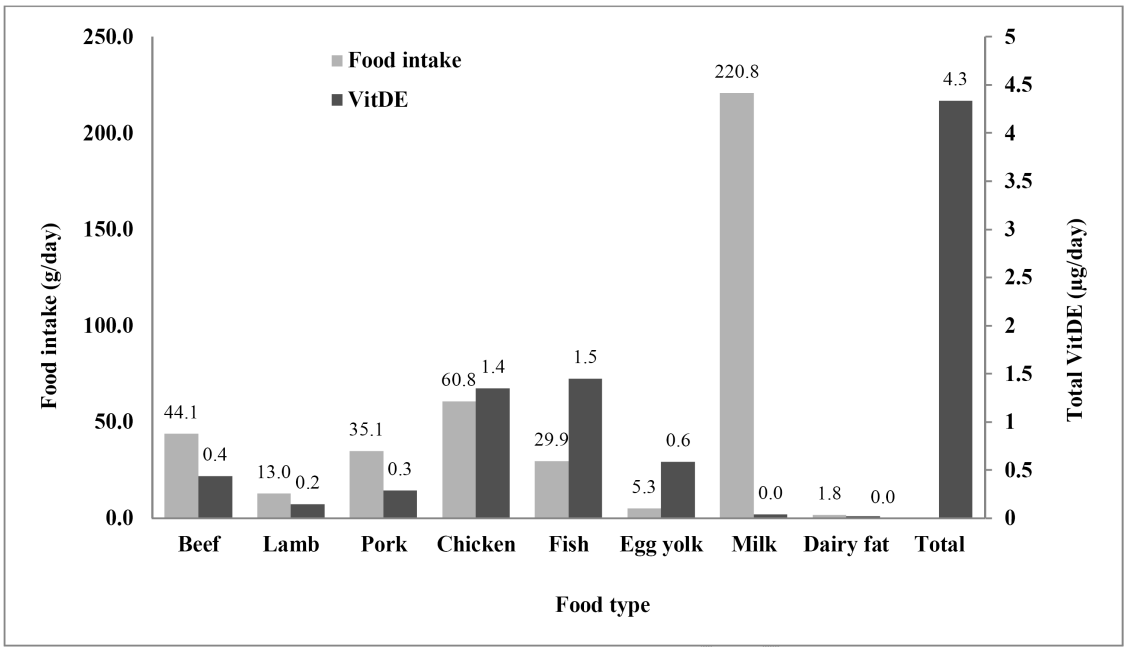

Figure 2. Average amount of food consumed (g/day) by Australians >18 years in 2011/12, and the corresponding vitamin D equivalents (VitDE) intake (µg/day). VitDE values were calculated by averaging analytical values from Table 1.

g, and wild catch burrowing blackfish contained 0.3 µg vitamin D_3/100 g. Unfortified milk (3.8% fat) contained 0.02 µg vitamin D_3/100 mL and no detectable $25OHD_3$.[19]

The FSANZ NUTTAB 2010 vitamin D Excel file did not include values for any of the species of fish mentioned in this study. Meanwhile, analytical VitDE data for Australian eggs, beef, lamb, and chicken are all much higher than those reported by the FSANZ vitamin D file (Table 1). VitDE data for unfortified milk were higher in the FSANZ file than the published value.[19]

Consumption of vitamin D containing foods

Survey data from 1995 (Figure 1) and 2011/12 (Figure 2) show similarities in the consumption pattern of relevant foods. Although the total amount of meat consumed did not differ greatly (157 g in 1995 vs 153 g in 2011/12), the types of meat consumed did change. In 1995, beef was consumed in the highest proportion, but in 2011/12, chicken became the most consumed meat. However, despite these consumer trends, there were no major changes of VitDE intake over time.

The consumption of dairy fat, excluding dairy fat consumed in mixed foods such as cakes, declined from 3.5 g/day in 1995 to 1.8 g/day in 2011/12. Because dairy fat was consumed in such small quantities in both periods, it is likely to provide a negligible amount of VitDE to adult Australians. Although milk (including milk products) was consumed in greater amounts than other VitDE contain-

ing primary foods (289 and 220 mL/day in 1995 and 2011/12, respectively), it also contributed very low amounts of dietary VitDE due to its low VitDE content. The quantity of egg yolk consumed (4.6 g/person/day in 1995 and 5.3 g in 2011/12) was low among these foods. However, due to the relatively high concentration of VitDE in egg yolk, its contribution towards dietary VitDE is measureable (0.5 µg/day in 1995 and 0.6 µg/day in 2011/12).

In both time periods, fish was the single greatest contributor to VitDE intake, providing 1.3 µg/day in 1995 and 1.5 µg in 2011/12. Chicken was also another large contributor, providing 1.2 µg/day in 1995 and in 2011/12. Chicken, together with meat from other terrestrial species studied, provided a total of 2.4 µg/day in 1995 and 2.2 µg/day in 2011/12. In 1995, the consumption of primary animal products including beef, lamb, chicken, pork, fish, eggs, milk and dairy fats may have provided adults, on average, with 4.2 µg VitDE/day. This figure remained similar at 4.3 µg/day in 2011/12.

DISCUSSION

Despite the Australian Health Survey 2011/12 being completed recently, it could not include estimates of dietary intakes of VitDE due to the lack of reliable VitDE data (R.Sobolewski, FSANZ, 2013, personal communication). Clearly, new data for food VitDE are required to enable this latest dietary survey to provide a more accurate estimate of dietary VitDE intakes and sources in Australia. This is now possible due to recent developments in analytical techniques using HPLC and LC-MS. These methods have provided more accurate data of the current VitDE of Australian foods of animal origin (Table 1).

Comparing dietary data from 1995 to 2011/12 has shown that there have been some shifts in terrestrial meat consumption among adult Australians. However, despite this change, there was little change in total VitDE consumed. This is because consumption patterns of richer sources of VitDE have not increased and the levels of VitDE in terrestrial species are relatively low.

This study has shown that the richest source of vitamin D activity in the foods analysed was egg yolk (up to 23.93 µg VitDE/100 g for type A). However, the content of VitDE in eggs varies with the supply of dietary vitamin D_3 or $25OHD_3$ to the laying hen[33] and accounted for the large variations seen in the content of VitDE of market eggs (0.46 to 23.93 µg/100 g). These data suggest that estimating VitDE intake from food surveys would need to take into account the specific brand of eggs and types of egg-containing products consumed, and information about their market share.

Meat from terrestrial species tends to contain lower concentrations of VitDE than fish or eggs. However, because large quantities are consumed, meats could still provide a substantial proportion of total dietary VitDE. Of all terrestrial animals, chicken meat provided the highest dietary VitDE. This is probably because, unlike sheep and cattle, intensively raised chickens consume feed fortified with vitamin D active compounds.[34]

It has been previously documented that fish may contain relatively high levels of VitDE and content varies between species.[35] In 1995 fish made up a relatively small part of the Australian diet (Figure 1), and this has remained unchanged since then. In order to estimate VitDE intake more accurately, it is necessary to distinguish between species of fish consumed (if known). Analyses of market seafood species are needed, bearing in mind that about one-third of local seafood may now be farmed,[36] and VitDE content would vary according to the level of VitDE in feed, and that imported seafood (that have not to our knowledge been analysed in Australia) may equal local production.[36]

Australian data for the VitDE content of lean pork come from a single sample only.[18] Australian pigs are generally raised intensively in the absence of sunlight and fed vitamin D activity fortified feeds. More analyses of VitDE in representative samples of Australian pork and pork products are needed.

Milk (and products) contributed little VitDE to estimated overall intake (<0.1 µg/day), because of its low vitamin D activity. Analysis of unfortified Australian milk (3.8% fat) showed there is 0.02 µg VitDE/100 mL present,[19] which contrasts with the value of 0.3 µg/100 mL reported in the FSANZ vitamin D file. This further highlights the need for confirmatory analytical data of Australian milk and milk products, and fortified products. In addition, dairy fat (e.g. butter) also contributed marginally (0.05 µg/day) due to low daily intake (<4 g/day in 1995 and 2011-2013).

A previous estimate[16] of food VitDE intake of adult Australians in 1995 did not clearly identify the source of food composition data used for the analysis. Nevertheless, it stated that 2 to 3 µg vitamin D were consumed per day from all foods. This value is lower than that determined by a more recent estimate for older Australians (4.4 µg/day) from all foods (including fortified foods) and dietary supplements[17], and that of the current study (4.2 to 4.3 µg/day) for primary animal foods alone. The reason for the differences is likely to be the underestimation of the VitDE of some foods, because of inaccurate data. This emphasises the need for reliable food composition data, particularly for animal-derived foods, using modern analytical techniques capable of detecting low concentrations of vitamin D and its metabolites.

The current study contains some limitations. Although modern analytical data were applied to consumption patterns from 1995, the results should be interpreted with caution. It is possible that changes in animal husbandry have occurred between 1995 and 2011/12, which would result in animal foods containing different levels of vitamin D activity beyond that captured by trim level. For example a new high potency commercial $25OHD_3$ supplement has now been in use in poultry feed over the last decade (Personal communication, L. Browning, Poultry Research Foundation, University of Sydney), which may have significantly elevated egg yolk vitamin D activity levels since 1995.

The present study did not include vitamin D activity data from sources such as fortified foods, other dairy products, mushrooms, or dietary supplements. The small amounts of vitamin D activity from these foods, together with changes in consumption patterns since the 1995 dietary survey, make it possible that total dietary supply of VitDE may be higher now than estimated here.

Despite some shifts in dietary consumption patterns, the dietary intake of VitDE by adult Australians has remained largely the same between 1995 (4.2 μg/day) and 2011/12 (4.3 μg/day). These figures are lower than the Australian AI of 5 μg/day. However, the true intake level may be closer to the AI if data for more foods had been available for inclusion in this study, including data for mixed foods that are based on primary animal foods. For example, it has been suggested that vitamin D_2 and its 25-hydroxy metabolite, which was unable to be included in this study, may provide a significant contribution to total vitamin D status.[37]

As a food group, all terrestrial meats together are likely to be the most significant source of dietary VitDE for the Australian people, estimated as 2.2 μg/day in 2011/12. Our conclusions are in accordance with those of a similar study using US data, which found that when $25OHD_3$ was taken into account, American men were consuming approximately 4.15 μg VitDE/day.[38] The importance of the inclusion of $25OHD_3$ in food intake estimates has been further emphasised elsewhere, and has been implicated as a possible cause of discrepancy between true and estimated VitDE intake values.[39] Analytical techniques developed and established in Australia are now available to generate comprehensive vitamin D activity data for updates of the Australian food tables in order to conduct dietary assessment and to develop food-based strategies for dietary improvement. With the active collaboration of all public and private stakeholders, accurate estimates of dietary VitDE intake could be finally obtained.

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Original Article

New data for vitamin D in Australian foods of animal origin: impact on estimates of national adult vitamin D intakes in 1995 and 2011-13

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澳洲動物食物維生素 D 新數據：對 1995 及 2011-13 年全國成人維生素 D 攝取量估計的影響

目的：採用澳洲主要動物來源食物的新數據，以評估澳洲成人可能的維生素 D 膳食供應。**方法：**新發表的澳洲主要動物製品其維生素 D 含量資料收集及信度評估。食物攝取資料來自於 1995 年及 2011-2013 年澳洲族群的飲食調查，從澳洲成人的這些食物來源作為近似平均每日維生素 D 當量攝取的估計值。**結果：**1995 年，>18 歲成人，僅肉類、雞、魚、雞蛋及乳製品，即可貢獻約 4.2 μg 維生素 D 當量，2011-2013 年則約 4.3 μg。**結論：**由於改善動物來源食物的維生素 D 含量分析方法，使得澳洲飲食維生素 D 的攝取量似乎比之前的估計高。牛奶及乳製品缺乏可信的維生素 D 數據，以及許多常吃的海鮮、家禽、蛋類及加工動物製品其維生素 D 數據的分歧，使得澳洲人的維生素 D 估算極其受限。

關鍵字：維生素 D、動物來源食物、澳洲、成人飲食、飲食調查