

## Original Research Article

## Cholesterol and vitamin D content of eggs in the U.S. retail market

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## ABSTRACT

Nationwide sampling in the U.S. of whole large eggs, to update values in the United States Department of Agriculture (USDA) National Nutrient Database for Standard Reference (SR) (<http://www.ars.usda.gov/nutrientdata>), was conducted in 2000–2001 and in 2010. Retail cartons of large eggs were obtained from 12 supermarket locations using statistical sampling plans based on market share and census data. Cholesterol was analyzed at three laboratories using standard methods involving gas chromatography of the saponified total lipid extract. Vitamin D<sub>3</sub> and 25-OH-vitamin D<sub>3</sub> (2010 samples only) were analyzed by HPLC and UHPLC-MS/MS. Quality control materials were included to validate the accuracy and precision of measurements. The mean cholesterol content decreased 51 mg/100 g (12%;  $p < 0.0001$ ), from 423 mg/100 g in 2000–2001 to 372 (range 344–405) in 2010. Over the same period, average vitamin D<sub>3</sub> increased by 60%, to 2.05 μg [80 IU]/100 g (range 0.97–12.1). Samples from 2010 contained 0.65 μg 25-OH-D<sub>3</sub>/100 g (range 0.43–1.32). The disparate vitamin D (and cholesterol) content of eggs sampled from different locations may reflect industry efforts to modify poultry feed or supplements to affect the nutrient profile of eggs. Cholesterol and vitamin D<sub>3</sub> data from this work were included in SR release 23, and support food consumption surveys, food and nutrition policy, and consumer education.

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## 1. Introduction

Eggs are a relatively inexpensive source of high quality protein and other nutrients. Eggs are also a primary source of dietary cholesterol. Despite conflicting evidence about the role of cholesterol intake in cardiovascular disease (CVD) risk (Vos, 2010), calls for decreasing dietary cholesterol (e.g. Houston et al., 2011; Spence et al., 2010) have prompted developments in production to yield eggs with reduced cholesterol and enhanced levels of desirable nutrients including omega-3 fatty acids, vitamin E, and vitamin D (Cherian, 2009; Elkin, 2006, 2007; Kassis et al., 2010; Naber, 1993). Commonly used practices include targeted feed composition, poultry supplements such as Hy-D<sup>®</sup> 25-hydroxyvitamin D<sub>3</sub> (DSM Nutritional Products Europe Ltd., Basel, Switzerland), or free range vs. cage environments for hens. Variability in production practices means there is greater

potential variability in the composition of eggs in the current retail market.

In 2009 a study of the nutrient composition of eggs produced by controlled flocks of chickens, comparing cage vs. free range production (Anderson, 2011) was conducted. In that study the cholesterol content of whole large eggs was lower than reported in the United States Department of Agriculture (USDA) Nutrient Database for Standard Reference (SR) Release 22 (USDA, 2009). The SR22 data on eggs were based on a 2000–2001 nationwide sampling and analyses conducted by the USDA Nutrient Data Laboratory (NDL) as part of the National Food and Nutrient Analysis Program (NFNAP) (Haytowitz et al., 2007), in collaboration with the Egg Nutrition Center (ENC). Sampling and nutrient analyses for the ongoing NFNAP are conducted using statistical sampling plans, valid methods, and rigorous analytical quality control to assure the accuracy and precision of the results (Phillips et al., 2006). The data for cholesterol in eggs were reviewed in 2010 as part of the NFNAP, and a re-sampling and analysis of whole eggs was planned to update values based on potential changes in composition. Additionally, newly developed and validated methods for vitamin D would make accurate

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determination of this nutrient, including 25-OH-vitamin D<sub>3</sub> possible.

Because SR is the primary source of food composition data for many nutrient intake assessment programs, the accuracy of the resulting estimates depend on the accuracy and completeness of data in SR. It is also important to realize that changes in the average nutrient composition over time, or variability in the composition of a particular product within the food supply, may occur. Investigators must be aware of the impact of such changes on epidemiological assessments of the effect of diet on health. In fact, studies relating egg consumption or dietary cholesterol to CVD risk, such as the recent Health ABC study (Houston et al., 2011) use average nutrient concentrations from food composition databases merged with food intake records to estimate nutrient intake.

This report describes the estimation of cholesterol and vitamin D content of whole large eggs sampled in 2000–2001 and 2010 and the incorporation of the 2010 data into SR 23, with the new data for 25-OH-vitamin D<sub>3</sub> to be included in a future release of SR.

## 2. Materials and methods

### 2.1. Samples

Whole eggs were procured in November 2000/November 2001 and in March/April 2010 at 12 statistically determined supermarket locations identified for the NFNAP (Pehrsson et al., 2000; Perry et al., 2003), with the sampling plans for 2000/2001 and 2010 based on 1990 and 2000 census data, respectively. Fig. 1 shows the sampling locations in each year. Three to five cartons (one dozen eggs each) of white, large, grade A or AA eggs were obtained from each retail location. In 2010 shipment of eggs from three locations (CA1, CA2 and CO) was arranged by the ENC (Park Ridge, IL).

The eggs were shipped in their original cartons, by overnight express, on refrigerated cold packs to the Food Analysis Laboratory Control Center (FALCC) at Virginia Tech (Trainer et al., 2010) where they were composited, homogenized and subsampled for analysis. Eggs were inspected for integrity immediately upon receipt. Any damaged eggs were discarded. The eggs were stored refrigerated ( $4 \pm 3$  °C) and composited prior to their labeled sell-by date (within 1–21 days of receipt) as described below (except in three cases where the samples were composited no more than 9 days past the sell-by date).

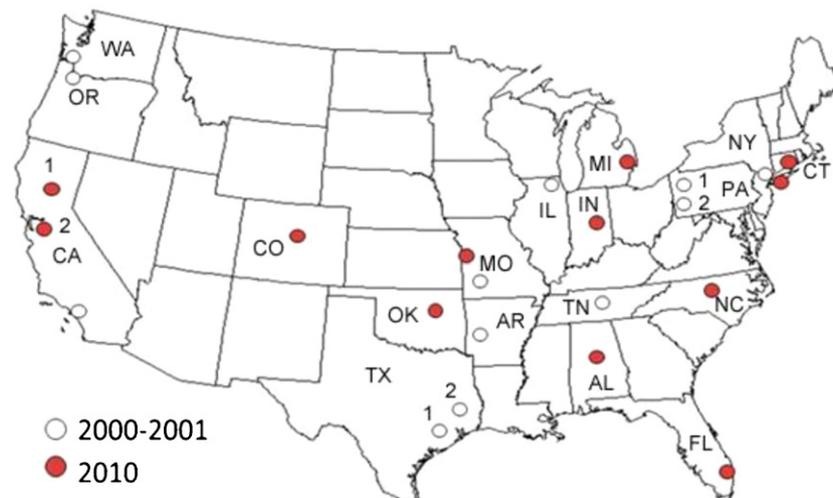
Eggs were composited as follows. In 2010, 12 single-city and 6 random city-pair composites were prepared. For the single-location composites, 9–12 eggs (450–700 g) from each location were used. For the 6 city-pair composites 9–12 eggs from each outlet were combined (total of 900–1300 g). In 2000–2001, 11 single-city composites, four regional composites of samples from three (triad) or four locations, and a national composite of samples from all locations were prepared using an equal number of eggs from each outlet (total of 800–1400 g per composite).

For each composite, eggs were homogenized in a stainless steel bowl using a hand blender (CSB-1C, Cuisinart<sup>®</sup>, Stamford, CT). Subsamples were dispensed, while maintaining homogeneity of the mixture, into 1-oz clear straight sided glass jars with Teflon<sup>®</sup>-lined lids (GLC-07098, Qorpak<sup>®</sup>, Bridgeville, PA). Each subsample was sealed under nitrogen and stored at  $-60$  °C prior to analysis.

### 2.2. Control composites

An egg control composite (Egg CC) was prepared in October 2008. Two cartons of eggs (1.5 dozen each) labeled as vitamin D enriched, were purchased locally (Kroger, Blacksburg, VA) and stored refrigerated ( $4 \pm 3$  °C) until composited two days after purchase. Samples were homogenized as described above and subsamples were dispensed into 2-oz clear straight sided glass jars (GLC-08640, Qorpak<sup>®</sup>, Bridgeville, PA), sealed under nitrogen, and stored at  $-60$  °C prior to analysis.

A pork and egg control composite (Pork/Egg CC) was prepared as follows. Two cartons of eggs (1.5 dozen each) labeled as vitamin D-enriched, and five packages (16 oz each) of pork bratwursts were purchased locally (Kroger, Blacksburg, VA). The samples were stored refrigerated ( $4 \pm 3$  °C) and composited one day after receipt. Eggs were separated and only the yolks were included in the composite. Bratwursts were cut into pieces of  $\sim 1.25$  cm. The cut bratwursts, egg yolks, and distilled deionized water (4.5:14.5:1, w/w/w) were homogenized in a 6 L industrial food processor (Robot Coupe<sup>®</sup> Blixer BX6V, Robot Coupe USA, Inc., Jackson, MS). The homogenized material was gradually added to a stainless steel bowl containing liquid nitrogen. After all of the material was sufficiently frozen, it was re-homogenized in a second 6 L industrial food processor, yielding a fine powder. Subsamples were dispensed into 1-oz clear straight sided glass jars with Teflon<sup>®</sup>-lined lids (GLC-07098, Qorpak<sup>®</sup>, Bridgeville, PA), sealed under residual nitrogen, and stored at  $-60$  °C prior to analysis or shipment.



**Fig. 1.** Sampling locations in 2000–2001 and 2010. AL = Alabama; AR = Arkansas; CA = California; CO = Colorado; CT = Connecticut; FL = Florida; IL = Illinois; IN = Indiana; MI = Michigan; MO = Missouri; NC = North Carolina; NY = New York; OK = Oklahoma; OR = Oregon; PA = Pennsylvania; TN = Tennessee; TX = Texas; WA = Washington.

The homogeneity of the Egg CC and Pork/Egg CC were validated as previously described (Phillips et al., 2006), using moisture and/or cholesterol and vitamin D as indicator nutrients.

### 2.3. Nutrient analyses

Egg samples along with control composites and reference materials were analyzed at qualified laboratories as described below, and were shipped from FALCC frozen ( $-60^{\circ}\text{C}$ ), on dry ice, via overnight express delivery. The 2010 samples from all 12 individual locations were assayed for cholesterol at each of three laboratories that used independent, validated methods (FALCC and two commercial laboratories). Vitamin  $\text{D}_3$  was analyzed in the six city-pair composites from both studies at Lab A, and vitamin  $\text{D}_3$  and 25-OH- $\text{D}_3$  were determined in the 12 single-city composites from 2010 at Lab B.

#### 2.3.1. Cholesterol

At FALCC total lipid extracts were prepared and total lipid was quantified gravimetrically as previously described (Phillips et al., 2008b). Cholesterol was analyzed after alkaline saponification of the total lipid extract, by gas chromatography of the trimethylsilyl ether derivative, with epicholesterol as the internal standard, using previously reported methodology for sterols (Phillips et al., 2005). The limits of detection (LOD) and quantitation (LOQ) were 1.5–5.0 and 7.5–25 mg/100 g fresh weight. At the commercial laboratories cholesterol was assayed using gas chromatography after saponification of the sample (AOAC, 2011a). Total fat was determined by acid hydrolysis methods for both the 2000–2001 study (AOAC, 2011b) and the 2010 study (AOAC, 2011c).

#### 2.3.2. Vitamin D

The city pair composites were analyzed for vitamin  $\text{D}_3$  at Lab A by high performance liquid chromatography with ultraviolet detection (HPLC-UV) as previously described in detail (Phillips et al., 2008a; Byrdwell, 2009). Lab B determined vitamin  $\text{D}_3$  and 25-OH- $\text{D}_3$ , in the single-city composites using ultra high performance LC-MS-MS (UHPLC-MS-MS) (Huang et al., 2009; Huang and Laluzerne, 2011).

#### 2.3.3. Method validation

Recovery studies of vitamin  $\text{D}_3$  were done at Lab A, where subsamples (4.5–5.0 g) of the Egg CC were assayed with ( $n = 5$ ) and without ( $n = 5$ ) addition of 54.4 ng vitamin  $\text{D}_3$ . Recovery studies of 25-OH- $\text{D}_3$  were conducted at the second laboratory, where subsamples of the Egg CC were assayed without ( $n = 4$ ) and with ( $n = 2$ ) addition of 100% of the estimated endogenous level of 25-OH- $\text{D}_3$  ( $\sim 0.5$  mg/100 g).

Repeat analyses of the Egg CC established matrix-specific precision for vitamin  $\text{D}_3$  and 25-OH- $\text{D}_3$ . The purity of the vitamin  $\text{D}_3$  peak in the egg matrix was confirmed by independent analysis of the Egg CC and two of the city-pair composites using HPLC-MS/MS at the USDA Food Composition and Methods Development Laboratory (FCMDL), Beltsville, MD (Byrdwell, 2009, 2010).

#### 2.3.4. Control and reference materials

Control composites (CC) were prepared and implemented as previously reported (Phillips et al., 2006) and included the Egg CC, and Pork/Egg CC described above. Certified reference materials (RM) for cholesterol were procured from the National Institute of Standards and Technology (Gaithersburg, MD) and included SRM 1546 Meat Homogenate, SRM 1845 Whole Egg Powder, and SRM 1563-2 Fortified Coconut Oil for cholesterol, and an in-house pork material supplied by Health Canada Food and Nutrition Laboratory (Québec) having target values for vitamin  $\text{D}_3$  and 25-OH- $\text{D}_3$  (Bilodeau et al., 2011). A vitamin D fortified processed cheese control material (Cheese CC) previously described (Phillips et al., 2008a) was also analyzed.

Results for RM were compared to the certified ranges, and Z-scores were calculated as described by Jorhem et al. (2001). Results for the Cheese CC were compared to the previously established validated tolerance limits (Phillips et al., 2008a).

For additional validation of the consistency in measurements between those conducted in 2000–2001 and 2010, selected composites from each year were assayed in the same analytical batch in 2010. Archived samples of the individual egg composites assayed in 2000–2001 were no longer available, but triad composites of the same samples were; therefore, the assayed value for each composite was compared to the mean of the concentrations assayed previously in the individual samples.

**Table 1**

Quality control results for (A) vitamin D ( $\mu\text{g}/100\text{g}$ ) and (B) cholesterol ( $\text{mg}/100\text{g}$ ). n/a = not applicable. Relative standard deviation (RSD) and number of analyses ( $n$ ) are shown in parentheses. Within-laboratory Z-scores<sup>a</sup> for assayed values are given in brackets. Values shown in bold font were from analyses conducted with 2010 egg samples. Values in bold italics were from analyses conducted with 2000–2001 and 2010 egg samples.

(A)					
Material	Certified or target concentration	Lab A	Lab B	Mean (RSD)	
<i>Vitamin D<sub>3</sub></i>					
Cheese control composite <sup>b</sup>	7.55 ± 1.35	<b>7.89</b> (n/a; 1) [0.4]	<b>7.10</b> (n/a; 1) [−0.5]	7.70 (3.1%)	
Egg control composite <sup>b</sup>		<b>0.69</b> (5.8%; 5) [0.2]	<b>0.64</b> (3.3%; 2) [−0.5]	0.66 (5.3%)	
Pork/egg control composite <sup>b</sup>	4.72 ± 0.51		<b>4.25</b> (n/a; 1)	n/a	
Health Canada pork reference material <sup>d</sup>	0.36	<b>0.37</b> (6.9%; 4) [0.2]	<b>0.37</b> (4.2%; 2) [0.3]	0.37 (0.0%)	
<i>25-OH-vitamin D<sub>3</sub></i>					
Health Canada pork reference material <sup>c</sup>	0.16		<b>0.14</b> (5.0%; 2) [−0.16]	n/a	
(B)					
Material	Certified or target concentration	Lab B	Lab C	Lab D	Mean (RSD)
<i>Cholesterol</i>					
NIST 1546 meat homogenate <sup>d</sup>	75.0 ± 7.2		<b>74.0</b> (1.3%; 2) [−0.6]	<b>64.1</b> (0.4%; 2) [−7.0]	71.0 (8.5%)
NIST 1845 whole egg powder <sup>d</sup>	1864 ± 39	<b>1800</b> (n/a; 1) [−1.9]		<b>1840</b> (n/a; 1) [−0.7]	1835 (1.8%)
NIST 1563-2 fortified coconut oil <sup>d</sup>	63.8 ± 0.8			<b>64.2</b> (n/a; 1) [0.2]	n/a

<sup>a</sup> Jorhem et al. (2001).

<sup>b</sup> In-house control materials (see text): egg control composite, cheese control composite, and pork/egg control composite (Phillips et al., 2006).

<sup>c</sup> Health Canada Nutrition Research Division (Québec, Canada) in-house reference material, with “target” concentration being the published value (Bilodeau et al., 2011).

<sup>d</sup> Commercially available Standard Reference Material<sup>®</sup> from National Institute of Standards and Technology (Gaithersburg, MD, US).

## 2.4. Data analysis

Means, standard deviations, and relative standard deviations (RSD) were calculated using Microsoft® Office Excel (Professional Plus edition, 2010; Microsoft Corporation, Redmond, WA), and analysis of variance ( $\alpha = 0.05$ ) and pair-wise comparison of means using the Student–Newman–Keuls test (Ott and Longnecker, 2008) with a 95% confidence interval were performed with XLSTAT (version 2011.2.06; Addinsoft, New York, NY). Precision was evaluated using ratio of obtained/expected RSD (HORRAT) by the approach of Horwitz et al. (1980).

## 3. Results and discussion

### 3.1. Quality control

#### 3.1.1. Vitamin D recovery

The mean recovery of vitamin D<sub>3</sub> added to the Egg CC was 98.9% with 4.8% relative standard deviation (RSD) and a range of 93.5–106% for the 5 replicates. The mean assayed concentration of 25-OH-D<sub>3</sub> ( $n = 4$ ) in the Egg CC was 0.516  $\mu\text{g}/100\text{ g}$  (3.6% RSD) and the recovery of 25-OH-D<sub>3</sub> was 91% and 105% for the duplicate analysis (mean, 97.5%).

#### 3.1.2. Results for control and reference materials

The results for control composites and standard reference materials for vitamin D and cholesterol are summarized in Table 1. The assayed concentrations of vitamin D<sub>3</sub> in the Cheese CC and Pork/Egg CC and in the Health Canada pork material determined at both laboratories were within the previously established tolerance limits of 6.2–8.9  $\mu\text{g}/100\text{ g}$  for the Cheese CC and 3.74–5.78  $\mu\text{g}/100\text{ g}$  for the Pork/Egg CC, and within the published range for the Health Canada pork material, with Z-scores  $\leq |0.5|$  in all cases. The result for 25-OH-D<sub>3</sub> in the Health Canada pork material (0.14  $\mu\text{g}/100\text{ g}$ ) was also in good agreement with the published mean (0.16  $\mu\text{g}/100\text{ g}$ ; Z-score of 0.14) (Bilodeau et al., 2011). Vitamin D<sub>3</sub> was analyzed by Lab A with the 2000–2001 egg samples and by Lab B with the 2010 egg samples. The mean assayed concentration of vitamin D<sub>3</sub> in the egg control composite assayed at these laboratories (Table 2) was 0.69 ( $n = 5$ ) and 0.64 ( $n = 2$ ), respectively, with no statistically significant difference based on 95% confidence intervals.

Results for cholesterol in NIST SRM 1845 Whole Egg Powder were consistent with the certified concentration at both laboratories that analyzed this RM, with Z-scores of  $-0.7$  and  $-1.9$ . The HORRAT for the inter-laboratory ( $n = 3$ ) analysis of cholesterol in the individual 2010 egg samples ranged from 0.4 to 2.7 (mean, 1.7), indicating acceptable inter-laboratory precision (Horwitz et al., 1980).

**Table 2**

Results for vitamin D<sub>3</sub> ( $\mu\text{g}/100\text{ g}$ ) an egg control composite assayed with the 2000–2001 and 2010 egg samples at two laboratories.

Replicate	Assayed with	Assayed
	2000–2001 samples	with 2010 samples
	Lab A	Lab B
1	0.64	0.65
2	0.67	0.62
3	0.73	
4	0.70	
5	0.72	
Mean	0.69 <sup>a</sup>	0.64 <sup>a</sup>
Standard deviation	0.04	0.02
Relative standard deviation	5.8%	3.3%

<sup>a</sup> Means do not differ significantly ( $\alpha = 0.05$ ).

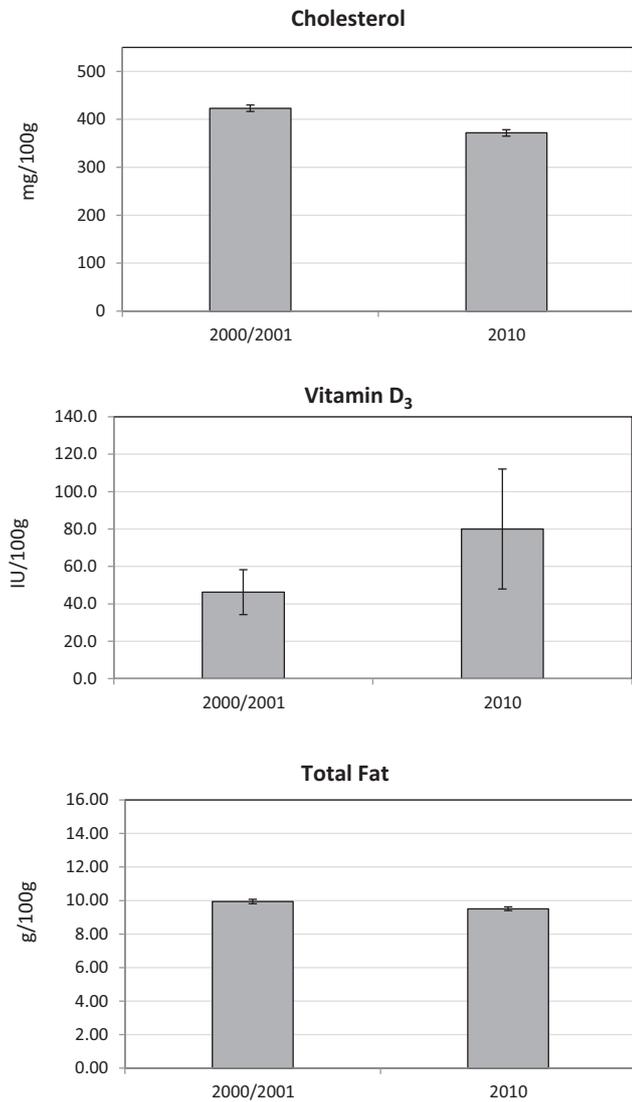
For the two selected egg composites from each of 2000–2001 and 2010 that were assayed in the same analytical batch in 2011 there was no statistically significant difference in the means for the original and re-assayed cholesterol concentrations. An exception was a lower value from the re-analysis of the AR, MO, TN composite, compared to the average of the cholesterol concentrations originally assayed in the individual samples from the those three locations (513 mg/100 g). However, the overall mean cholesterol content of eggs in 2000–2001 calculated including the original concentration of 513 mg/100 g was 446 mg/100 g, compared to 412 mg/100 g without that value, which was much closer to the result of 396 mg/100 g concentration from the later analysis of the triad composite containing that sample. Therefore, although the result of 513 mg/100 g may have been a deviation in the earlier analysis, the mean cholesterol content across all locations was not substantially affected, being 434 mg/100 g when the value of 513 was included and 426 mg/100 g when the result for the AR,MO,TN composite was substituted for the individual values. Therefore, it can be reasonably concluded that there was not an analytical bias between the analysis of eggs in 2000–01 and in 2010.

### 3.2. Comparison of egg composition: 2000–2001 vs. 2010

The cholesterol, vitamin D<sub>3</sub>, and total fat content of eggs sampled in 2000/2001 and 2010 are illustrated in Fig. 2. The average cholesterol concentrations in 2000/2001 and 2010 were 423 and 370 mg/100 g, respectively, representing a 53 mg/100 g (12.4%) decrease ( $p < 0.0001$ ). Although there are no definitive reasons for the reduced cholesterol content of eggs in the U.S. food supply, research shows alteration in the diet fed to poultry can change the composition of the egg yolk (Elkin, 2006, 2007; Rahimi, 2005) and practices to optimize the nutrient profile may be increasing in the poultry industry.

The mean vitamin D<sub>3</sub> concentration of eggs in 2000/2001 and 2010 were 1.40 and 2.05  $\mu\text{g}/100\text{ g}$ , respectively, but this difference was not statistically significant due to the high variability in vitamin D<sub>3</sub> among individual samples within each period, as discussed below. The higher mean vitamin D content in 2010 was a result of eggs from specific suppliers having markedly higher vitamin D content than other eggs. This higher value may reflect the introduction into the retail food supply of eggs produced by feeding or supplementation practices that increase vitamin D in the yolk. Although direct supplementation of the chicken feed with vitamin D has been used for this purpose, vitamin D content could also be elevated as an indirect effect in cases where fishmeal or fish oil is incorporated in poultry feed to enhance the omega-3 fatty acid content of the yolk (Küçükersan et al., 2010), if those particular fish products are high in endogenous vitamin D.

The 25-OH-D<sub>3</sub> content was determined only in the 2010 egg samples. The mean content of 0.65  $\mu\text{g}/100\text{ g}$  (range 0.43–1.32) was nearly double the average of 0.36  $\mu\text{g}/100\text{ g}$  (range, 0.16–0.55) measured in 6 samples of eggs in Canada by Bilodeau et al. (2011) using similar methodology and the same Health Canada pork control material. In the Canadian study the vitamin D<sub>3</sub> (mean, 0.92  $\mu\text{g}/100\text{ g}$ ; range 0.70–1.20) was also lower than the average of 2.0  $\mu\text{g}/100\text{ g}$  (range 0.97–9.22) in the U.S. egg samples in this study. The highest vitamin D<sub>3</sub> value in this study, 12.1  $\mu\text{g}/100\text{ g}$ , was for a sample collected from a New York supermarket. That product was not labeled as having enhanced vitamin D content. Without this sample, the range would be 0.97–1.8. While the number of samples was not as large in the Canadian study, these data nonetheless illustrate how vitamin D in particular egg samples might differ significantly from the mean value in food composition tables.



**Fig. 2.** Cholesterol, vitamin D<sub>3</sub> and total fat content of eggs sampled in the U.S. in 2000/2001 and 2010 (1 IU vitamin D<sub>3</sub> = 0.025 μg). Error bars indicate ±standard error. Within each figure the 2000/2001 and 2010 means differ significantly only for cholesterol ( $p < 0.0001$ ).

3.3. Between-sample variability in cholesterol and vitamin D in eggs

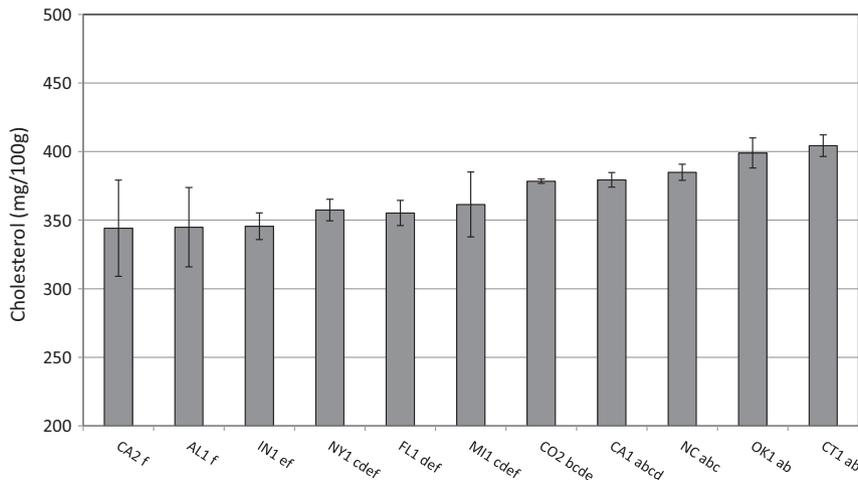
Figs. 3 and 4 illustrate the variability in cholesterol and vitamin D among eggs sampled at the 12 individual retail locations in 2010. The eggs from the NY location had notably higher vitamin D content. Additional sample units from the same NY store were re-sampled on 2 additional occasions within four months and analyzed. These samples had lower vitamin D content than the initial sample of eggs from this location, but the NY location mean (9.22 μg/100 g) was still significantly greater than in eggs from any of the other locations. The variation in cholesterol (339–405 mg/100 g) and vitamin D<sub>3</sub> (0.73–9.22 μg/100 g) in eggs from different locations probably reflects industry efforts to introduce eggs with modified nutrient composition that have been studied for positive benefits on serum cholesterol (Elkin, 2006, 2007; Garwin et al., 1992).

3.4. Incorporation of results in SR

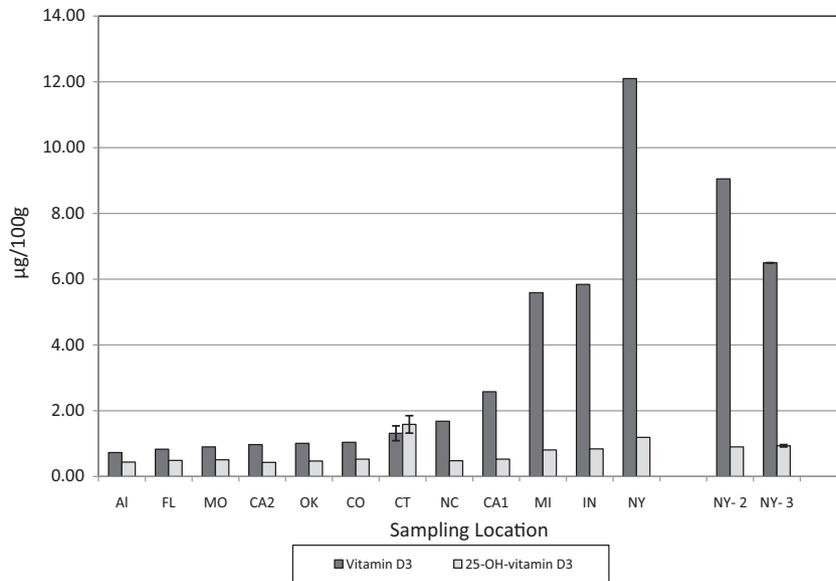
Table 3 summarizes the cholesterol, vitamin D<sub>3</sub>, and total fat contents of eggs sampled in 2010, which are the basis for values in SR23 (USDA, 2011) for whole large eggs (NDB#01123). It is anticipated that 25-OH D<sub>3</sub> values will be disseminated in a future release of SR. Reliable data on the vitamin D content of eggs were lacking prior to the 2010 NFNAP sampling and analysis. Values for vitamin D<sub>3</sub> in SR prior to release 23 in 2011 were based on the 2000–2001 samples. In 2001 variable data and lack of assurance of method validation in the initial analyses of the eggs was part of the rationale for the method development work conducted (Phillips et al., 2008a,b; Byrdwell, 2009, 2010) prior to the current study.

The value for vitamin D<sub>3</sub> in large, whole eggs reported in SR23 (NDB#01123) (USDA, 2011) is for regular eggs (i.e. having no label information regarding the vitamin D content). Therefore, the values for vitamin D<sub>3</sub> in the two samples that were labeled as having enhanced vitamin D content (IN and MI) were not included in the mean. The rationale for exclusion was that the presence of a claim could influence the selection of that brand by the consumer and bias the representativeness of the mean for eggs in general. The new vitamin D<sub>3</sub> value of 2.0 μg/100 g in SR23 (USDA, 2011) is 60% higher than the previous value of 1.25 μg/100 g in SR22 (USDA, 2009).

In SR23 data for products that contain egg yolk (where the fat, cholesterol, and vitamin D<sub>3</sub> are found) were updated to reflect the



**Fig. 3.** Variability in cholesterol content among eggs sampled from retail outlets in locations across the U.S. (Fig. 1) in 2010. Each bar shows the mean ± standard error (SE) from analysis at 3 independent laboratories. Interval for mean ± 2SE: 358–383 mg/100 g. Samples from locations with different lower case letters differ statistically ( $\alpha < 0.05$ ).



**Fig. 4.** Variability in vitamin D content among eggs sampled from retail outlets in locations across the U.S. (Fig. 1) in 2010. NY location was re-sampled twice within four months to confirm the value (NY-2 and -3). Error bars show the range for the mean in cases where a sample was analyzed in duplicate. Estimated relative standard deviation for single values, based on control sample data: vitamin D<sub>3</sub>, 3.3%; 25-OH vitamin D<sub>3</sub>, 5.0% (1 µg vitamin D<sub>3</sub> = 40 IU).

**Table 3**

Total fat, total lipid, cholesterol, and vitamin D content of retail eggs in the U.S. in 2010.<sup>a</sup>

Location <sup>b</sup>	Total fat g/100 g	Total lipid g/100 g	Cholesterol mg/100g <sup>c</sup>	Vitamin D <sub>3</sub> µg/100 g	25-OH vitamin D <sub>3</sub> µg/100 g
AI	9.25	10.2	345 <sup>F</sup>	0.73	0.44
FL	9.45	10.3	355 <sup>DEF</sup>	0.83	0.49
MO	10.3	11.3	405 <sup>A</sup>	0.90	0.51
CA2	9.48	10.4	344 <sup>F</sup>	0.97	0.43
OK	9.61	10.7	399 <sup>AB</sup>	1.01	0.47
CO	9.58	10.5	378 <sup>BCDE</sup>	1.04	0.53
CT	9.95	10.9	404 <sup>AB</sup>	1.09	1.32
NC	9.30	10.2	385 <sup>ABC</sup>	1.68	0.48
CA1	9.72	10.7	379 <sup>ABCD</sup>	2.58	0.53
MI	9.06	10.1	36 <sup>CDEF</sup>	5.59 <sup>d</sup>	0.81
IN	8.78	9.62	346 <sup>EF</sup>	5.84 <sup>d</sup>	0.84
NY	9.60	10.5	35 <sup>CDE</sup>	9.22 <sup>e</sup>	0.99
Mean	9.51	10.5	372	2.00	0.65
Range	8.78–10.3	9.62–11.3	344–405	0.73–12.1 <sup>e</sup>	0.43–1.32

<sup>a</sup> Estimated relative standard deviation for single value, based on control sample data: total fat, 1.6%; total lipid, 0.6%; vitamin D<sub>3</sub>, 3.3%; 25-OH vitamin D<sub>3</sub>, 5.0%.

<sup>b</sup> See Fig. 1.

<sup>c</sup> Values within a column with a different capital letter superscript differ statistically ( $\alpha=0.05$ ) based on  $n=3$  independent laboratory analyses for each sample.

<sup>d</sup> Value not included in average reported in the United States Department of Agriculture database (USDA, 2011) (see Section 3 in text).

<sup>e</sup> 9.22 is the mean of values for three samples obtained over 4 months for this location, with vitamin D<sub>3</sub> concentrations of 6.50, 9.05, and 12.1 µg/100g.

values determined in the 2010 sampling and analyses. The new cholesterol and vitamin D<sub>3</sub> values for whole eggs were used to calculate the composition of egg-containing food items where the values are derived from formulations and recipes.

#### 4. Conclusions

The estimated cholesterol value for eggs as a result of this study was lower than previously reported and the vitamin D<sub>3</sub> value was higher.

The inclusion of consistent and complete quality control in analyses of samples from both sampling periods was essential to ensure that the changes observed were not due to analytical variability, and to validate the accuracy of the measured concentrations.

In the 2010 nationwide sampling of eggs 2010 there was a difference of 61 mg/100 g in the cholesterol content of eggs with

the highest and lowest concentration. In eggs from one location there was no statement about enhanced vitamin D content or any vitamin D value given in the nutrition facts panel of the carton label, although eggs from this location had a consistently higher vitamin D content than the national average and that of brands that were labeled as vitamin D enhanced.

It is critical that researchers recognize the potential for significantly greater cholesterol and vitamin D intake in individuals consuming eggs from a particular source or brand, and take this into account when considering intake in specific cases. Another sampling of whole eggs is tentatively planned as part of the NFAP in 2013–2014 to monitor levels of vitamin D.

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