

CONTINUING EDUCATION

Bias and error in the determination of common macronutrients in foods: Interlaboratory trial

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Abstract Chemical analyses of nutrient values in foods form the basis of much of the science and practice of nutrition and dietetics, but little is known about the accuracy and precision of common macronutrient analyses. Therefore, an interlaboratory study was set up. One batch each of egg powder, full-fat milk powder, whole rye flour, whole wheat flour, biscuits, and French beans (snap beans, "haricots verts") was thoroughly homogenized. Samples were sent to 19 leading food analysis laboratories in Europe and the U.S., and each performed analyses of macronutrients by its own routine methods. Most were government or semi-government laboratories and major contributors to national nutrient data banks. The results for dry matter content and ash agreed well between laboratories. For protein, the coefficient of variation between laboratories ($CV_{between}$) ranged from 2.8% to 6.4%. The reproducibility within laboratories was sometimes quite poor. The $CV_{between}$ for total fat ranged from 5.4% to 54%. For "available" carbohydrates, the $CV_{between}$ ranged from 9% to 27%. The $CV_{between}$ for total dietary fiber ranged from 23% to 84%. Only part of the variability could be explained by the use of methods of different principle. It is concluded that leading laboratories produce widely different values for macronutrients in common foods. Quality control programs and reference materials of certified nutrient concentration are urgently needed. *J Am Diet Assoc* 88:556, 1988.

Nutrition research and counseling rely heavily on analytical data for the nutrient content of foods. However, surprisingly little is known about the quality of routine nutrient analyses. In contrast, in medicine the quality of chemical determinations in, e.g., blood plasma is monitored by extensive quality control programs. What information there is about laboratory accuracy and precision

for food analysis in the open literature often deals with contaminants or other regulated substances rather than with nutrients. (For a review, see reference [1].)

Substantial information is available on the precision attainable with specified analytical procedures, e.g., methods described by the Association of Official Analytical Chemists and the International Organization for Standardization. Those data are collected by means of collaborative studies, in which selected laboratories all use the same accurately described method for analysis of identical samples. Sometimes calibration materials or reagents are also distributed.

However, it is a well-known fact that for routine analyses, different laboratories actually use different methods to determine a certain nutrient in a certain food. Even if the same methodological principles are followed, subtle differences in procedure and in calibration materials could still cause large differences in outcome.

We now report on the reproducibility of the determination of protein, fat, carbohydrate, and fiber within and between laboratories under real-life conditions. Leading laboratories, one American and 18 European, that regularly contribute nutrient values to nutrient data banks participated in this study. Participants were encouraged to apply the methods of analysis and calculation used routinely in their respective laboratories. The trial arose from the Eurofoods (2) endeavor toward compatibility of nutrient data banks in Europe.¹

Method

Materials

Six foods were used:

1. Egg powder: commercially available spray-dried whole egg powder
2. Full-fat milk powder: commercially available spray-dried full cream milk powder
3. Whole rye flour: whole rye grains (RIVRO Institute, Wageningen), ground, 100% extraction
4. Whole wheat flour: whole wheat grains (TNO/IGMB Institute, Wageningen), ground, 100% extraction

¹A report with detailed data may be obtained from Peter C. H. Hollman, State Institute for Quality Control of Agricultural Products, Bornsesteeg 45, NL-6708 PD Wageningen, The Netherlands.

5. Biscuits: commercially available dry cookies, ground
 6. French beans: commercially available freeze-dried French beans (snap beans, "haricots verts"), ground

Three kilograms of each food was ground to pass a sieve of 0.5-mm openings, carefully homogenized, and divided into samples of about 100 gm, using a sample divider. The samples were packed into airtight black bottles with screwcaps. Homogeneity was tested by analyzing 10 random sample bottles of each product for protein. This yielded a coefficient of variation between bottles ranging from 0.11% to 0.25%, which could be ascribed to analytical error. All sample bottles of one food thus had the same protein content. It seems legitimate to assume that other nutrients were also homogeneously distributed. Bottles were labeled with the name of the product, vacuum-sealed in airtight plastic foil, and sent to the participants, together with instructions.

Participants and method

Only laboratories that were regular contributors of data to national food tables were asked to participate (Table 1). Many were government or semi-official institutes and were considered highly authoritative within each respective country. Participants were instructed to treat the samples in the same way as any other sample received for routine analysis and to use their own routine methods for analysis, calculation, and reporting. However, all laboratories were asked to perform all analyses in duplicate, with one technician on one day providing one value and another technician on a second day providing the second value. This made it possible to calculate the variation within each laboratory.

In addition, each laboratory was requested to determine dry weights, using a vacuum stove method provided with the samples, and to report all results as grams per 100 gm dry matter as determined by that method. This prevented confounding of the results by changes in moisture content of the samples during transport or storage.

Statistical analysis

Statistical analysis followed the principles of the International Organization for Standardization norm ISO 5725 (3) for the calculation of the standard deviations(s) and coefficients of variation (CV) of overall reproducibility, variation within laboratories (s_{within} , CV_{within}), and variation between laboratories (s_{between} , CV_{between}). Contrary to ISO 5725, outliers were not rejected, because ISO 5725 applies only to interlaboratory tests using one method described in detail. In the present trial, rejecting an outlying laboratory implied an unwarranted judgment on the correctness of methods and values; the outlying value could theoretically still be the "true" value. Moreover, the aim of this interlaboratory trial was to investigate the influence of different laboratory procedures.

Horwitz (4) examined the results of more than 150 collaborative studies, organized by the Association of Official Analytical Chemists, and found an empirical equation that relates CV_{between} to the concentration (C) of the analyte, expressed in negative powers of 10:

$$CV_{\text{between}} = 2^{(1 - 0.5 \log C)}$$

This value represents the variation between laboratories that can be obtained when all laboratories use the same rigidly defined standardized methods.

Table 1. Participating laboratories*

participant	laboratory	city
Eckelmanns, V.	Ministry for Economic Affairs, Central Laboratory	Brussels, Belgium
Gheorghiev, G. K.	Medical Academy, Institute of Gastroenterology and Nutrition	Sofia, Bulgaria
Bergstrøm-Nielsen, M.	National Food Institute	Søborg, Denmark
Hartmuth-Hoene, A. E.	Institute of Biochemistry, Federal Research Center for Nutrition	Karlsruhe, Federal Republic of Germany
Hyvönen, L.	EKT Department of Chemistry and Technology, University of Helsinki	Helsinki, Finland
Dworschák, E.	National Institute of Food Hygiene and Nutrition	Budapest, Hungary
Fidanza, F.	Institute of Nutrition and Food Science, University of Perugia	Perugia, Italy
Van de Bovenkamp, P.	Department of Human Nutrition, Wageningen Agricultural University	Wageningen, The Netherlands
Dukel, F.	TNO-CIVO Food Analysis Institute	Zeist, The Netherlands
Van der Veen, N. G.	State Institute for Quality Control of Agricultural Products	Wageningen, The Netherlands
Roomans, H.	Food Inspection Service	Maastricht, The Netherlands
Kunachowicz, H.	National Food and Nutrition Institute	Warsaw, Poland
Amaral, E.	National Institute of Health	Lisbon, Portugal
Valdehita, T.	Institute of Nutrition, University of Madrid	Madrid, Spain
Torelm, I.	National Food Administration	Uppsala, Sweden
Florence, E.	Food Research Institute	Reading, United Kingdom
Faulks, R.	AFRC Food Research Institute	Norwich, United Kingdom
Cooke, J. R.	Laboratory of the Government Chemist, Department of Industry	London, United Kingdom
Wolf, W.	Nutrient Composition Laboratory, U.S. Department of Agriculture	Beltsville, USA

*The order of the laboratories does not correspond to the laboratory numbers used in the text and figures.

Table 2. Variability of the determination of protein, total fat, carbohydrates, and total dietary fiber within and between laboratories when identical samples of six* different foodstuffs were analyzed

<i>nutrient</i>	<i>egg</i>	<i>milk</i>	<i>rye</i>	<i>wheat</i>	<i>biscuits†</i>	<i>French beans‡</i>
protein						
no. of laboratories	17	18	19	19	19	18
mean (gm/100 gm dry weight)	53.0	28.1	10.2	12.7	7.8	15.1
range (gm/100 gm dry weight)	49.7-56.9	25.7-32.8	9.2-11.8	11.1-14.3	7.2-9.5	11.7-15.8
CV (%)	3.1	6.0	7.0	6.7	7.9	6.3
CV _{within} (%)	1.4	3.1	2.9	2.0	4.8	1.3
CV _{between} (%)	2.8	5.2	6.4	6.4	6.2	6.2
CV _{between} (%)#	2.7	5.4	4.7	5.2	5.0	6.2
total fat						
no. of laboratories	18	18	19	19	19	18
mean (gm/100 gm dry weight)	37.8	27.3	2.6	3.0	11.6	2.7
range (gm/100 gm dry weight)	29.4-44.2	24.5-30.0	1.6-4.5	1.8-5.8	9.9-15.4	1.2-5.8
CV (%)	8.9	5.7	43.9	42.4	10.6	59.8
CV _{within} (%)	2.0	2.0	24.5	30.6	2.7	25.7
CV _{between} (%)	8.7	5.4	36.4	29.3	10.3	54.0
carbohydrates¶						
no. of laboratories		16	16	16	16	15
mean (gm/100 gm dry weight)		34.7	69.6	69.3	75.2	42.4
range (gm/100 gm dry weight)		14.9-44.4	38.4-94.0	35.7-82.1	63.4-89.3	28.3-67.5
CV (%)		20.1	20.8	18.0	9.9	27.4
CV _{within} (%)		4.8	4.2	4.5	3.3	3.1
CV _{between} (%)		19.4	20.4	17.5	9.3	27.3
CV _{between} (%)		19.5	18.5	15.6	6.9	22.6
total dietary fiber						
no. of laboratories	4	7	14	14	14	14
mean (gm/100 gm dry weight)	0.4	0.3	15.4	13.1	3.1	27.6
range (gm/100 gm dry weight)	0-0.8	0-0.8	10.0-22.0	8.7-19.8	0.7-10.9	15.6-35.8
CV (%)	117	130	26.3	26.9	84.3	23.8
CV _{within} (%)	22.6	15.6	9.5	5.1	7.1	6.8
CV _{between} (%)	115	129	24.6	26.4	84.0	22.8

*Five different foodstuffs for carbohydrate determination.

†Commercially available dry cookies, ground.

‡Snap beans, haricots verts.

#Recalculated using uniform Kjeldahl factors.

¶Carbohydrates were determined by direct analysis of free sugars plus starch in 13 laboratories and by difference (dry weight - ash - protein - fat - "fiber") in three other laboratories. Two of the latter used the AOAC method (11) for dietary fiber, and one used a crude fiber method.

||No. = 13; in this case, all data were expressed as monosaccharides, and "by difference" values were excluded.

Results

Dry weight

The results of the dry weight determination by the prescribed vacuum stove method agreed very well: the coefficient of variation between laboratories (CV_{between}) ranged from 0.3% to 0.6%. Thus, packing and storage conditions of the samples proved to be adequate to protect against changes in moisture content.

Optional dry weight methods, performed by a number of laboratories in addition to the prescribed method, yielded results quite similar to those of the prescribed method.

Protein

The results for protein showed an overall coefficient of variation (CV) of 3% to 8% (Table 2). The CV_{between} was somewhat higher than the achievable CV_{between} predicted by the Horwitz equation (4). That finding was to be expected, as the equation was derived from collaborative studies using a uniform method.

Part of the variation between laboratories was caused by variation in Kjeldahl nitrogen-to-protein conversion factors. When all results were recalculated with the Kjeldahl factors recommended by FAO/WHO (5), the variation between laboratories indeed showed some decrease, especially for rye and wheat (Table 2). The chemical methods used differed in choice of catalyst and in procedures for digestion, distillation, and determination of the ammonia formed. No significant effects of those materials and procedures on the results was evident.

Finally, differences between duplicate values were surprisingly high in some laboratories.

Total fat

The reproducibility of the fat determination was rather poor, especially for products low in fat (Table 2). Thus, fat contents reported for whole wheat flour ranged from 1.8 to 5.8 gm/100 gm dry weight. For egg powder, the range was 29 to 44 gm/100 gm. The CV_{between} ranged from 5.4% for milk powder to 54% for French beans (snap beans, "haricots verts").

Methods were mostly based on acid hydrolysis followed by extraction with petroleum ether or diethylether. Laboratories using the Schmid-Bondzynski-Ratzlaf method (6) or variations of it found on average higher results than laboratories using the Weibull method (7) in its various modifications. These differences were significant ($p < .05$) for wheat, rye, biscuits, and French beans (snap beans, "haricots verts"). Milk powder was analyzed by most participants with Röse-Gottlieb methods (8). For milk powder, Schmid methods gave higher results than Röse-Gottlieb methods, which in turn gave higher results than Weibull methods. However, those differences did not prove significant ($p > .05$).

A number of laboratories used a Folch-type (9) direct extraction technique with solvents such as chloroform/methanol or dichloromethane/methanol. The results of those methods were not quite consistent. Thus laboratories Nos. 1, 3, and 6, using Folch-like methods, obtained a high value for fat in egg powder, but laboratory No. 15, also using Folch, reported a lower value than average (Figure 1). In other products, laboratory No. 6 instead of

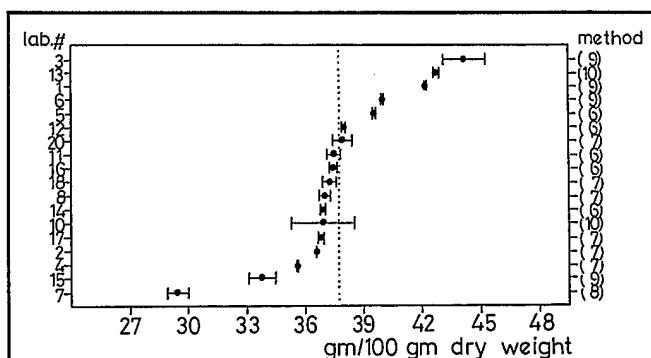


FIG. 1. Total fat content in identical samples of whole egg powder analyzed by different laboratories. References to the methods used by the laboratories are indicated at the right. However, each laboratory used its own modification of those methods. Vertical dashes refer to the two different values obtained by two technicians on different days within one laboratory. Black dots represent the means of the duplicates per laboratory. Figures in parentheses at the righthand side of the figure refer to the references.

laboratory No. 15 consistently found lower-than-average fat contents. Thus, only part of the differences between laboratories in the results for fat could be explained by the use of analytical methods of different principle.

Within-laboratory variations were again relatively large, especially for products low in fat.

"Available" carbohydrates

For the purpose of this trial, "available" carbohydrates were defined as free sugars (mono- and di-saccharides and other oligosaccharides up to approximately 10 monosaccharide units) plus starch. The reproducibility of the "available" carbohydrate determinations between laboratories was very poor, with CV_{between} ranging from 9% to 27% (Table 2). A small part of the variability was due to the different modes of expression. Seven laboratories expressed their results as monosaccharides, four as polymeric starch, and two as "carbohydrates." When all data were expressed as monosaccharides, the variability between laboratories was slightly reduced (Table 2).

The 13 laboratories that performed direct analysis of carbohydrates used a wide variety of methods. Three additional laboratories did not use an analytical method to determine carbohydrates but calculated the value by difference, as dry weight - (ash + protein + fat + fiber). That produced values close to the mean of the other participants, except for one laboratory which determined crude fiber instead of total dietary fiber. As a result, certain fiber components were counted as carbohydrate, and the resulting carbohydrate value was higher than average.

Total dietary fiber

Fourteen laboratories reported values for total dietary fiber (Table 2). Two more had determined crude fiber; those values were not used in the statistical analysis, because crude fiber is a small and variable part of total dietary fiber. There was a large variability in dietary fiber values between the different laboratories. This was probably largely a result of the well-known differences between methods.

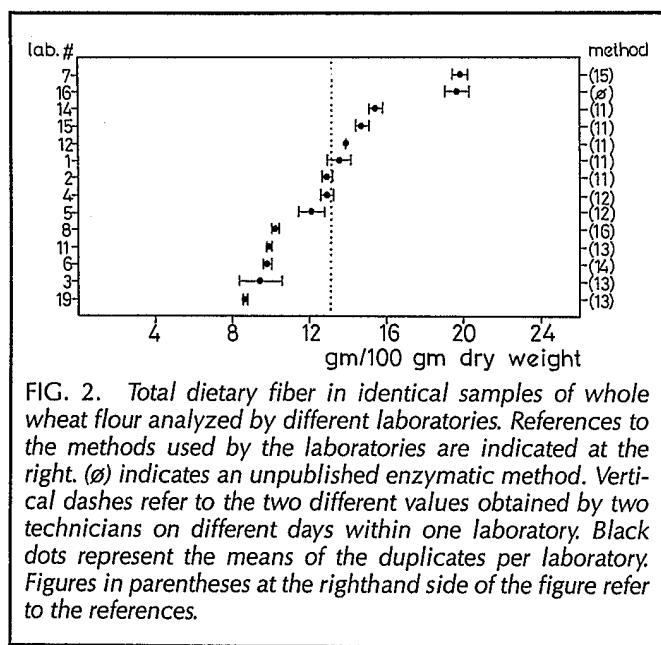


FIG. 2. Total dietary fiber in identical samples of whole wheat flour analyzed by different laboratories. References to the methods used by the laboratories are indicated at the right. (ø) indicates an unpublished enzymatic method. Vertical dashes refer to the two different values obtained by two technicians on different days within one laboratory. Black dots represent the means of the duplicates per laboratory. Figures in parentheses at the righthand side of the figure refer to the references.

The method described by Prosky et al. (11), used by five laboratories, and the related method of Asp (12), used by two participants, resulted in values that agreed well. Laboratory Nos. 3, 11, and 19 used the Englyst (13) method or a modification of it. They tended to report lower dietary fiber values than the trial mean (Figure 2). Prosky et al. (11) also found that the Englyst method gave lower values than their method and pointed out that dietary fiber as determined by the Englyst method does not include lignin. The low values of laboratory No. 6 (Figure 2) was to be expected, because that laboratory used the neutral detergent method (14), which determines only the water-insoluble fiber components.

Ash

Methods used show various pre-ashing procedures and ashing times and temperatures. The coefficient of variation between laboratories ranged from 3.3% to 6.7%. With standardized methods, a CV_{between} of 3.0% to 3.7% as calculated by the Horwitz equation (4) can be achieved. Thus, the results for ash agreed rather well between laboratories, although outliers did occur.

Discussion

Results of this trial

This trial has shown that prominent laboratories in various countries produced widely different values for the concentration of fat, carbohydrates, and fiber and, to a lesser extent, of protein in everyday foods.

It should be noted that several sources of error that occur commonly in routine analyses of foods had already been reduced or eliminated beforehand in the present trial. Thus, the foods were supplied as stable, well-ground powders of uniform particle size, easy to store, handle, and sample. Also, the samples had been carefully packaged and clearly marked and identified. Thus, the analyst could find out whether his/her values were more or less

correct by simply consulting a food table. Last, but not least, the trial samples may have been analyzed with more than usual care and attention.

Therefore, values produced in daily routine analyses of unknown samples will probably show an even larger variation between and within laboratories than the values reported here.

Causes of variability

As for the causes of the discrepancies, differences in methods probably play an important role. Elkins (17) reported a much lower interlaboratory variability for protein and fat in the cooperative study of the Committee of Canning Industry Chemists. A main difference from the present study was that the participants in the Canning Industry study all used the same methods, as defined and described in detail by the Association of Official Analytical Chemists. The differences in fiber values (Table 2) are to a large extent due to differences in definition of and methodology for dietary fiber. However, differences in methodological principles are not the full explanation of the variability in other nutrients, as laboratories using similar methods sometimes still reported widely diverging results. The cause for that is unknown.

Variability in protein values caused by differences in Kjeldahl nitrogen-to-protein conversion factors was present but small. Widespread use of standard Kjeldahl factors is to be recommended. There was also a clear effect of differences in conventions for expressing carbohydrate content, i.e., as polymeric starch vs. as weight of the monosaccharides produced from starch by hydrolysis. Although the variability caused by the different modes of expression was small compared with total variability, better standardization is again desirable.

Consequences for food table users

The consequences of the analytical variability for users of nutrient data banks depend on the particular application. Individual dietary recalls are subject to large errors in the recollection of amount and identity of foods consumed and to large day-to-day variability within one subject or patient. Therefore, errors in food analyses, even of the size reported here, are less important for use with dietary recalls.

The errors do become influential in other applications, e.g., in deciding which individual foodstuffs are allowed for a patient on a certain prescribed diet and in estimation of group mean intakes, when individual errors tend to cancel out. Such group means are typically used in epidemiological studies in which diet and disease prevalence are compared between countries. For such studies, better standardization of food analysis procedures is required.

Possible remedies

The trial has brought to light two types of variability.

First, within-laboratory variation was rather large for certain laboratories when they analyzed certain nutrients and products. Such variability could be monitored and controlled by the use of internal control pools, using standard quality control techniques.

Second, differences in level between laboratories were

(Continued on page 563)

SELF-ASSESSMENT QUESTIONNAIRE

After reading the continuing education article, "Bias and error in the determination of common macronutrients in foods: Interlaboratory trial," please answer the following questions by indicating your responses on the self-assessment questionnaire form located on the next page.

This activity has been approved for 1 hour of continuing education credit for Registered Dietitians by the Commission on Dietetic Registration. Answers to the self-assessment questionnaire can be found on page 652.

ADA members should cut out the completed form and return it, with a check for \$8 each (non-members \$12) to cover processing, to: The American Dietetic Association, P.O. Box 10960, Chicago, IL 60610-0960.

Questionnaires must be returned within 1 year of their appearance in the *Journal* in order to be eligible for credit. Notification will not be sent if hour is approved.

Items 1 to 9

For items 1 to 9, select the *best* answer or completion to each question or statement.

1. In this study, participating laboratories performed food analyses under which conditions?
 - A. All used identical standardized protocols provided by the authors
 - B. All used the protocols that were routine for their own laboratories
 - C. One-half used standardized protocols and one-half used routine ones
 - D. None of the above
2. How did the authors estimate variation *within* the participating laboratories for food determinations?
 - A. Analyses were done on two separate days by the same technician
 - B. Analyses were done on two separate days by different technicians
 - C. Ten random samples were analyzed for protein only
 - D. Ten random samples were analyzed for all nutrients of interest
 - E. None of the above
3. The authors found food products that were low in fat tended to produce larger differences for which type of comparison?
 - A. Between-laboratory
 - B. Within-laboratory
 - C. Both of the above
 - D. Neither of the above
4. From the data reported, the best overall reproducibility was for which macronutrient determination?
 - A. Available carbohydrate
 - B. Total dietary fiber
 - C. Total fat
 - D. Protein
 - E. None of the above

5. When the authors standardized the mode of expression for carbohydrate determinations, how did the coefficient of variation between laboratories change?
 - A. Increased
 - B. Decreased slightly
 - C. Stayed the same
 - D. Cannot be determined from the information presented in the article
6. The smallest range of values for coefficients of variation between laboratories was reported by the authors for which nutrient determination?
 - A. Fat
 - B. Carbohydrate
 - C. Dietary fiber
 - D. Protein
 - E. Crude fiber
7. The authors concluded that the procedures they used to prepare, package, and distribute the food products to the participating laboratories could be expected to have which effect on variability?
 - A. Decreased coefficients of variation-between
 - B. Decreased coefficients of variation-within
 - C. Both of the above
 - D. Neither of the above
8. The authors concluded that the coefficients of variation between laboratories found in this study could have been reduced, at most, to what values if uniform analytical protocols were used?
 - A. <5%
 - B. 5% to 10%
 - C. 15% to 20%
 - D. 25% to 50%
 - E. >50%
9. Which of the following were suggested by the authors to increase reproducibility among/within laboratories for food determinations?
 - A. Use of within laboratory quality control programs
 - B. Use of external reference materials of known nutrient concentrations
 - C. Both of the above
 - D. Neither of the above

Items 10 to 12

For items 10 to 12, select:

- A. If 1, 2, and 3 only are correct
- B. If 1 and 3 only are correct
- C. If 2 and 4 only are correct
- D. If 4 only is correct
- E. If *all* are correct

10. Characteristic features of the laboratories that participated in this study included which of the following?
 1. Most were major contributors to national nutrient databanks
 2. Most were located in the United States
 3. All performed the food determinations using their own routine methods
 4. Most were private laboratories

11. The current status, according to the authors, of what is known relevant to laboratory accuracy and precision for food analysis is characterized accurately by which of the following?

1. Little is actually known about the quality of routine food analyses
2. Substantial knowledge is available about the precision of food analyses achievable with specified analytical procedures
3. Most available information about laboratory quality for food analyses concerns contaminants or other regulated substances
4. The quality of chemical determinations in food analyses is monitored extensively through quality control programs

12. The Horwitz equation for food determinations:

1. Is derived from more than 150 collaborative studies
2. Relates the coefficient of variation-between to the concentration of the analyte
3. Represents the variation between laboratories that could be obtained when all use the same standardized methods
4. Produces estimates of achievable coefficient of variation-between that are higher than those obtained in the type of study done by the authors

Items 13 to 17

Items 13 to 17 below consist of a set of lettered headings followed by a list of numbered statements. Select the lettered heading that is most clearly related to each numbered statement. Each lettered heading may be used once, more than once, or not at all within a set.

Approximate ranges of observed coefficients of variation-between

- A. 3% to 6%
- B. 5% to 54%
- C. 9% to 27%
- D. 23% to 129%
- E. None of the above

Macronutrients studied

13. Total fat.
14. Protein.
15. Total dietary fiber.
16. Available carbohydrate.
17. Crude fiber.

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Continuing Education Article

"Bias and error in the determination of common macronutrients in foods: Interlaboratory trial"

*Journal, May 1988***SELF-ASSESSMENT QUESTIONNAIRE ANSWER FORM**

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After reading each statement, please select the one best answer or completion:

1.	A	B	C	D	E
2.	A	B	C	D	E
3.	A	B	C	D	E
4.	A	B	C	D	E
5.	A	B	C	D	E
6.	A	B	C	D	E
7.	A	B	C	D	E
8.	A	B	C	D	E
9.	A	B	C	D	E
10.	A	B	C	D	E
11.	A	B	C	D	E
12.	A	B	C	D	E
13.	A	B	C	D	E
14.	A	B	C	D	E
15.	A	B	C	D	E
16.	A	B	C	D	E
17.	A	B	C	D	E

(Continued from page 560)

responsible for most of the variability observed in this trial. Such differences can be detected by regular inter-laboratory trials (18) or by using external reference materials with a certified concentration of the nutrient of interest. This trial has shown that the production and use of such reference materials should have a high priority.

Finally, standardization of methods is urgently required. Collaborative trials have shown that for analytes present in high concentrations, such as the macronutrients studied here, the use of uniform analytical protocols can reduce the variability between laboratories to some 2% to 4% at the most (4). Collaborative studies are always encouraged. Organizations such as the Association of Official Analytical Chemists (AOAC) and the International Organization for Standardization (ISO) are continuously engaged in testing and improving methods for the analysis of just about every nutrient in every kind of food. The methods that are finally accepted and published (8) have proved their worth in extensive collaborative trials. Laboratories engaged in food analysis ought to be familiar with those methods and should use the available methods unless they have strong reasons not to.

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