SCIENCE INQUEST
Excellent in sharing scientific knowledge

Open Access
Research Article

Volume 3 Issue 1 *Journal of Nutrition and Diet Supplements*

Associations Between %AA (20:4 n6) and Percentages of EPA (20:5 n3), DPA (22:5 n3), and DHA (22:6 n3) Are Distribution Dependent in Breast Muscle Lipids of Chickens

Høstmark AT[1*], and Haug A[2]

[1]*Faculty of Medicine, Institute of Health and Society, University of Oslo, Norway*
[2]*Department of Animal and Aquacultural Sciences, the Norwegian University of Life Sciences, Norway*

[*]**Corresponding Author:** Høstmark AT, Faculty of Medicine, Institute of Health and Society, University of Oslo, Box 1130 Blindern, 0318 Oslo, Norway, Fax: +47 22850590, Tel: +47 22844629, E-mail: a.t.hostmark@medisin.uio.no

Citation: Høstmark AT, Haug A (2019) Associations Between %AA (20:4 n6) and Percentages of EPA (20:5 n3), DPA (22:5 n3), and DHA (22:6 n3) Are Distribution Dependent in Breast Muscle Lipids of Chickens. J Nutr Diet Suppl 3(1): 103

Abstract

Since eicosapentaenoic acid (EPA, 20:5 n 3) and arachidonic acid (AA, 20:4 n6) have antagonistic actions, it is of interest to assess their relative abundances. We recently reported a positive association between %AA and %EPA, and observed that the correlation was related to the *distribution* of the fatty acids. Body concentrations of AA and EPA are influenced by diet. The aim of the present work was to investigate whether fat tissue %AA might be positively associated also with %DPA (22:5 n3) and %DHA (22:6 n3), and whether their distributions may explain the outcome. We reanalysed data from a previous diet trial, involving 163 chickens. Breast muscle was collected, and the concentration of fatty acids in muscle lipids was determined using gas chromatography. We made scatterplots of %EPA (DPA, DHA) vs. %AA, and did linear regressions for the %EPA (DPA, DHA) vs. %AA association. We next computed R=S–EPA (DPA, DHA)–AA, where S is the sum of all fatty acids (g/kg) and R is concentration of all fatty acids, except EPA (DPA, DHA) and AA. From histograms, we found the physiological distributions (g/kg/wet weight): 0.125-0.225 for EPA; 0.25-0.39 for AA; 0.21-0.43 for DPA; 0.11-0.32 for DHA; and 5-15 for R. Then we generated 163 RANDOM numbers for each of these variables, keeping their true distributions, and studied the above-mentioned associations using the random numbers. Finally, we explored whether a narrowing or broadening of the random number distributions might change the relationship between their relative abundances. With scatterplots and correlation analyses, we found these positive associations: %AA vs. % EPA: $r=0.719$, $p<0.001$; AA vs. DPA: $r=0.861$, $p<0.001$; and AA vs. DHA: $r=0.741$, $p<0.001$. Also with random numbers, we found positive associations between %"AA" and percentages of "EPA" ("DPA", "DHA"), all with $p<0.001$, and with scatterplots (and regression lines) similar to the corresponding ones obtained with the true values. The scatterplots showing the association between %"AA" and percentages of "EPA" ("DPA", "DHA") were improved when narrowing the distribution, and became much poorer when broadening the distribution.

Conclusions: %AA is positively associated with percentages of EPA (DPA, DHA), but also percentages of random numbers, generated within the biological distributions of the fatty acids, have positive relationships, similar to those found with corresponding real values. The associations between percentages of "AA" and "EPA" ("DPA", "DHA") are sensitive to changes in the distribution of the fatty acid surrogate, random numbers. Thus, distribution *per se* governs a positive association between the relative abundance of AA and DPA (DHA, EPA), i.e. there seems to be a ***Distribution Dependent Regulation*** of the association between relative abundances of some fatty acids, raising the question of whether this correlation outcome might be a novel regulatory mechanism in physiology.

Keywords: *Eicosapentaenoic acid; Arachidonic acid; Docosapentaenoic acid; Docosahexaenoic acid; Random numbers; Muscle fat; Chickens*

List of Abbreviations: *AA: Arachidonic acid (20:4 n6); "AA": a random number variable with distribution like that of AA; EPA: Eicosapentaenoic acid (20:5 n3); "EPA": a random number variable with distribution like that of EPA; DPA: Docosapentaenoic acid (22:5 n3); "DPA": a random number variable with distribution like that of DPA; DHA: Docosahexaenoic acid (22:6 n3); "DHA": a random number variable with distribution like that of DHA*

Definitions

Distribution: In this article, we use "distribution" when referring to a particular range, a - b.

Uniform distribution: Every value within the range is equally likely. In this article, for random numbers with uniform distribution we write "Distribution was from a to b", or "Distributions of A, B, and C were a - b, c - d, and e - f, respectively". "Small–number variables" have small numbers compared with "high-number variables".

Introduction

Arachidonic acid (20:4 n6) is formed in the body from linoleic acid (LA, 18:2 n6), a major constituent in many plant oils, and is converted by cyclooxygenase and lipoxygenase into various eicosanoids, i.e. prostacyclines, thromboxanes and leukotrienes [1-3]. AA derived thromboxane A_2 (TXA_2) and leukotriene B_4 (LTB_4) have strong proinflammatory and prothrombotic properties. Furthermore, endocannabinoids, which are derived from arachidonic acid, may have a role in adiposity and inflammation [4]. It is well known that eicosapentaenoic acid (EPA, 20:5 n3) and AA are metabolic antagonists [1-3]. Eicosanoids derived from EPA may decrease inflammatory diseases [5,6], development of cardiovascular diseases [7], and cancer [8]. When considering the beneficial health effects of foods rich in EPA, many of the positive effects would be anticipated if the fatty acid works to counteract effects of AA. It has been reported that a decreased level of the serum EPA/AA ratio may be a risk factor for cancer death [8]. It would appear, accordingly, that a coordinated regulation of the relative abundances of EPA and AA could be of physiological interest, so that an increase (decrease) in the percentage of one of these fatty acids would be accompanied by a concomitant increase (decrease) in percentage of the other. In accordance with these considerations, we recently reported that percentages of AA and EPA were indeed positively associated in breast muscle lipids of chickens [9]. We additionally found that the relationship is related to the particular *distributions* of EPA and AA. Since docosapentaenoic acid (DPA, 22:5 n3) and docosahexaenoic acid (DHA, 22:6 n3) may be retro-converted to EPA [1,10], thereby serving as a store of EPA, it occurred to us that also percentages of DPA and DHA might possibly be positively associated with %AA. If so, we reasoned that distribution might also explain an association between %AA and %DPA (DHA), and that a change in distributions should alter the relationships. There is, however, a methodological concern when correlating percentages of for example DHA (DPA, EPA) and AA, since the same sum appears in the denominator when calculating the percentages. Thus, the relative abundances of DHA (DPA, EPA) and AA could be correlated without having any biological explanation. However, if so it is not *a priori* apparent whether the correlation will be positive or negative, and to what extent the association may be attributed to the concentration *distribution* and/or to specific biological regulatory mechanisms. Therefore, we have extended our previous study [9] to investigate whether %AA is positively associated with %DPA (DHA), and whether changes in distributions might influence these associations, using random numbers instead of the true (measured) concentrations of the fatty acids. By this approach, we anticipated to circumvent possible biological feedback mechanisms.

Body concentrations of AA and EPA are influenced by diet. The aim of the present work was to investigate whether fat tissue %AA is positively associated with percentages of DPA and DHA, and whether a narrowing or broadening of the distribution of AA, DPA, and DHA might alter the relationship between relative abundances. We also included a repeat of our previous analysis of the %AA vs. %EPA association.

Methods

The present study is a spin-off of a previously published diet trial [11], in which groups of chickens were fed different types of diet. The work was carried out at The Norwegian University of Life Sciences (diet trial, AH), and at The University of Oslo, Norway (random number analyses, ATH).

Chickens and diet

We refer to our previous article [11] for details concerning the diet trial. In brief, from day 1 to 29 one-day-old Ross 308 broiler chickens from Samvirkekylling (Norway) were fed wheat-based diet containing 10 g fat per 100 g diet. ALA (18:3 n3; a precursor of EPA) provided 15% of the fatty acids, and LA (18:2 n6; a precursor of AA) provided 21%. The n6/n3 ratio was 1.4. Energy content of the feed was about 19 MJ/ kg. ALA provided 2.5% of the energy, and LA 4%. Other components in the feed were: Histidine 0.1%, choline chloride 0.13%, mono-calcium phosphate 1.4%, ground limestone 1.3%, sodium chloride 0.25%, sodium bicarbonate 0.2%, vitamin A, E, D, K, B 0.18%, L-lysine 0.4%, DL-methionine 0.2%, and L-threonine 0.2%.

Calculations and statistical analysis

We first re-investigated our previously reported association between %EPA and %AA [9], but in the present analyses we only studied the outcomes in the diet group with 163 chickens, i.e. birds fed high ALA/low LA diet. We extended

the analyses to include investigation of the association between %AA and percentages of DPA and DHA. From histograms, we found physiological distributions (g/kg wet weight) for the fatty acids: 0.125 - 0.225 for EPA; 0.25-0.39 for AA; 0.21-0.43 for DPA; 0.11-0.32 for DHA; and 5-15 for R. Next we computed S, the sum (g/kg wet weight) of all fatty acids, and R, the remaining sum when omitting AA and EPA (DPA, DHA). In our computer analyses we had variables, as shown by the equation R=S–AA–EPA (DPA, DHA) where S and R relate to which of the n3-fatty acids we studied. Then we generated uniformly distributed RANDOM numbers with the main physiological distributions for AA, EPA, DPA, DHA, and R. Since the diet trial had 163 birds, for each of the variables AA, EPA, DPA, DHA, and R we generated 163 random numbers with the particular distributions shown above. We use quotation marks when working with random numbers. Thus, percentages of the fatty acids were: %EPA=(EPA/S)*100; %AA=(AA/S)*100; %DPA=(DPA/S)*100; %DHA=(DHA/S)*100; %;R=(R/S)*100. Note that we use "AA", "EPA" ,"DPA"; "DHA", "R", and "S" with random numbers to keep in mind that the aim of our analyses was to mimic results with real values of fatty acids. Additionally, upper case letters are used (RANDOM) in the figure texts to clarify. Next, we made histograms to show the distributions of percentage values of the fatty acids, and of R (not presented, to reduce number of figures). Our aim was to investigate associations between percentages of AA and each of the long-chain n3-fatty acids. Therefore, we always calculated R ("R") as the sum (or random number surrogate) of all fatty acids, except AA and the n3 fatty acid under investigation. Dependency between percentages is shown by the equations %EPA+%AA+%R=100; %DPA+%AA+%R=100; %DHA+%AA+%R=100. Therefore, R should be different for calculations with EPA, DPA, and DHA. However, the differences in R-values were small, due to great similarity between values for the fatty acids. Using the random numbers, we made scatterplots for (RANDOM number) %"EPA" vs. %"AA". Finally, we studied how alterations in the distributions for random number "AA", "EPA", "DPA", and "DHA" might change the relationship between %"AA" and percentages of random numbers representing each of the n3-fatty acids. With random numbers, there are many ways to define distribution ranges. In this work, we limit our analyses to narrowing or broadening of the physiological distributions. For each analysis, we made several repeats with new sets of random numbers; the general outcome of the repeats was always the same, but the correlation coefficients (Pearson), and scatterplots, varied slightly. We present the results as correlation coefficients, scatterplots, and regression analyses. SPSS 25.0 was used for the analyses, and for making figures. We also did the above-mentioned analyses using random numbers having a *normal* distribution, where the sampling was based upon mean values and standard deviations. The outcomes were qualitatively the same with both types of random numbers; in this work, we only present results with uniformly distributed random numbers. The significance level was set at $p<0.05$.

Authors' contributions

The present study is a spin-off study of a previously published diet trial, conceived and conducted by AH. ATH conceived and designed the present study, analyzed and interpreted the data, conceived the hypothesis of Distribution Dependent Regulation, and wrote the article. ATH emphasizes that the excellent diet trial of AH - and the nice correlations observed-were crucial for the hypothesis. AH contributed substantially to the interpretation of data and revising the article critically for important intellectual content. Both authors read and approved the final manuscript.

Ethics approval

The diet trial in chickens was performed in accordance with National and international guidelines concerning the use of animals in research (Norwegian Animal and Welfare Act, European Convention for the protection of Vertebrate Animals used for Experimental and other Scientific Purposes, CETS No.: 123 1986). The Regional Norwegian Ethics Committee approved the trial, and the experimental research followed internationally recognized guidelines. There are no competing interests.

Results and Discussion

Descriptive data

Descriptive data for the fatty acids under investigation are shown in Table 1.

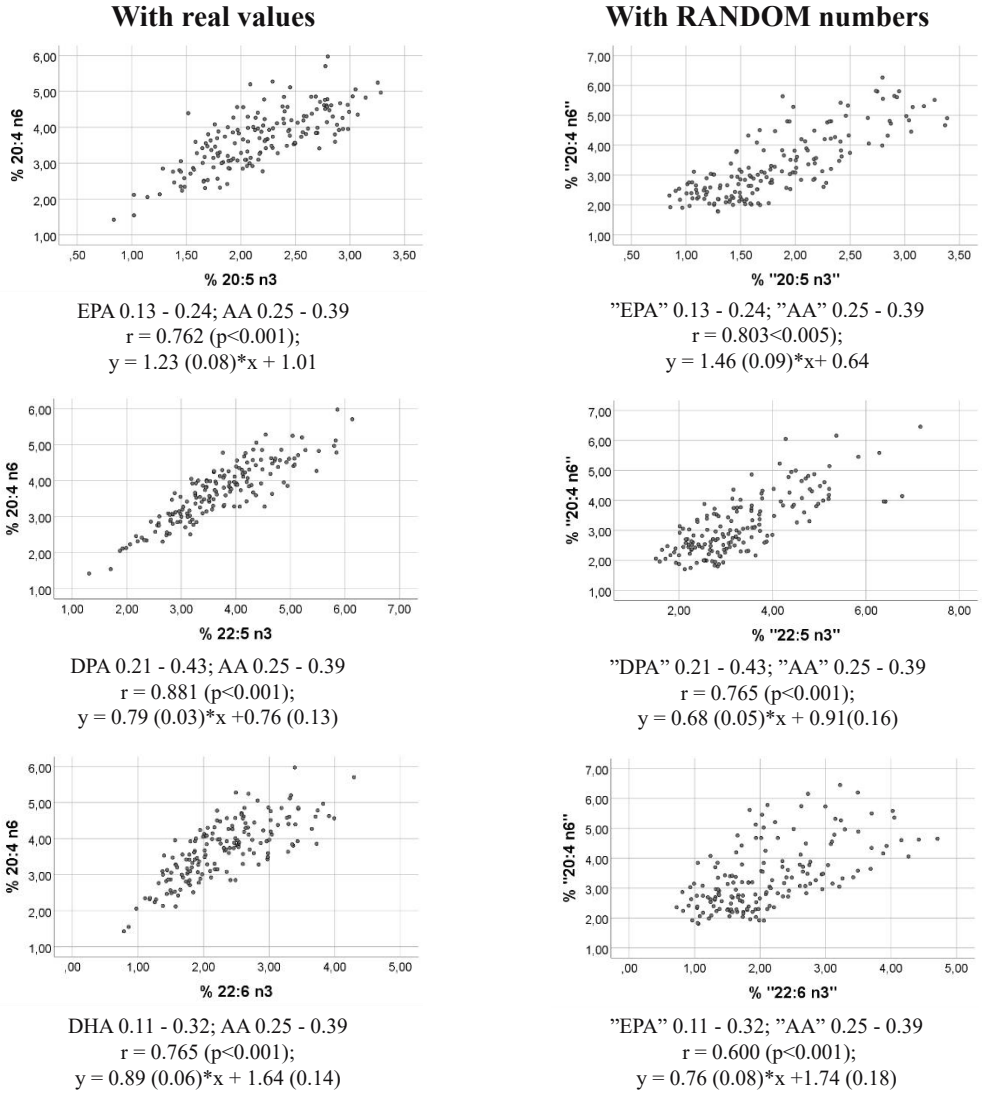
Fatty acid	Min	Max	Mean	SD	%	CV (%)
20:4 n6	0.25	0.39	0.31	0.03	3.4	9.7
20:5 n3	0.13	0.24	0.18	0.02	2.0	11.1
22:5 n3	0.21	0.43	0.31	0.04	3.5	12.9
22:6 n3	0.11	0.32	0.19	0.04	2.3	21.1

Table 1: Descriptive statistics for the fatty acids under investigation (n=163); minimum and maximum values, mean values (g/kg), with SD, % of total weight, and CV=(SD/Mean)*100

All of the investigated fatty acids from breast muscle lipids in chickens had low-number mean values, varying from 2.0% (of total weight) for EPA, to 3.5% for DPA. The coefficient of variation (CV) varied from 9.7 for AA to 21.1% for DHA. Total weight of all fatty acids was 8.86±2.62 g/kg wet weight (mean±SD).

Scatterplots of the positive associations between %AA and percentages of EPA (DPA, DHA) as compared with scatterplots of corresponding random numbers

Percentage of AA (20:4 n6) correlated positively ($p<0.001$ for all correlations) with percentages of 20:5 n3; 22:5 n3; and 22:6 n3 (Figure 1; below each panel: distribution, correlation coefficient, and equation of the regression line). In Figure 1, *right* panels we show the association between percentages of *random* numbers of the fatty acids; these numbers had a *uniform* distribution and were collected within the true distribution of the fatty acids in question. We additionally investigated these associations using random numbers with a *normal* distribution. The outcomes were qualitatively the same (not shown) with uniform and normal distributions. Additionally, the outcomes were similar with Pearson's correlation coefficient, and with Spearman's non-parametric correlations (not shown). Results with %AA vs. %EPA (Figure 1, upper panels) also appear in our previous work [9], but with a different sample of random numbers; inclusion here is to make a more complete picture of how %AA is positively associated with percentages of all of the long-chain n-3 fatty acids. Also the scatterplots between random number %"AA" and percentages of random numbers representing each of the other long-chain n3-fatty acids (DPA and DHA) were similar to the true scatterplots (Figure 1, right panels). Note that, in each of the panels, we use uniformly distributed random numbers, not only for the couple of fatty acids, but also for the sum of the remaining fatty acids (R), when calculating percentages (see Methods).



EPA 0.13 - 0.24; AA 0.25 - 0.39
r = 0.762 ($p<0.001$);
y = 1.23 (0.08)*x + 1.01

"EPA" 0.13 - 0.24; "AA" 0.25 - 0.39
r = 0.803<0.005);
y = 1.46 (0.09)*x+ 0.64

DPA 0.21 - 0.43; AA 0.25 - 0.39
r = 0.881 ($p<0.001$);
y = 0.79 (0.03)*x +0.76 (0.13)

"DPA" 0.21 - 0.43; "AA" 0.25 - 0.39
r = 0.765 ($p<0.001$);
y = 0.68 (0.05)*x + 0.91(0.16)

DHA 0.11 - 0.32; AA 0.25 - 0.39
r = 0.765 ($p<0.001$);
y = 0.89 (0.06)*x + 1.64 (0.14)

"EPA" 0.11 - 0.32; "AA" 0.25 - 0.39
r = 0.600 ($p<0.001$);
y = 0.76 (0.08)*x +1.74 (0.18)

Figure 1: Associations between true values of %20:4 n6 (AA) and percentages of 20:5 n3 (EPA), 22:5 n3 (DPA), and 22:6 n3 (DHA), left panels; and between percentages of corresponding RANDOM numbers, right panels (see Methods). Distributions of the fatty acids, and corresponding RANDOM numbers, Pearson correlation coefficient (r), and equation for the regression line are shown below each of the panels; the general formula y=a (SE) *x+b (SE) is used, where y is the ordinate, and x the abscissa. Note that the outcomes were similar when using Spearman's non-parametric correlations, and random numbers with normal instead of uniform distribution

How will a narrowing and broadening of the distribution influence the association between percentages of random numbers representing the fatty acids?

We reasoned that, if distribution of the fatty acids is crucial for obtaining the positive associations between the presented relative amounts, then an alteration in the physiological distributions should change the appearance of scatterplots, correlation coefficients, and the equations of the regression lines. Since there are infinite many ways to alter the distributions, we decided to limit our studies to consider the effect of narrowing and broadening of the distributions. As shown in Figure 2, left panels, a **narrowing** of the distribution improved the association between relative abundances of AA and the long-chain n3-fatty acids. Note that we use quotation marks with random number surrogate variables for the fatty acids. Thus, for the %"AA" vs. %"EPA" association, when narrowing "EPA" distribution from 0.16 to 0.18, and "AA" distribution from 0.30 to 0.35, we found an improved scatterplot, with r=0.984 (p<0.001), and regression line y=1.89 (0.03)*x+0.03(0.05). Corresponding values for "DPA" with distribution 0.15 to 0.20, and "AA" from 0.30 to 0.35 were: r=0.961 (p<0.001), and y=1.84 (0.04) *x+0.11(0.09); and for "DHA" with distribution 0.16 to 0.20, and "AA" 0.30 to 0.35: r=0.979, p<0.001; y=1.78 (0.03)*x+0.04(0.06). Conversely, when **broadening** the distributions (Figure 2, right panels), we obtained poorer scatterplots, and correlation coefficients: With "EPA" distribution 0.05-0.40, and "AA" 0.10-0.60, we found r=0.316 (p<0.001), and y=0.41 (0.10)*x+2.47 (0.27). With "DPA" 0.10–0.80, and "AA" 0.10–0.60: r=0.084 (p=0.287); y=0.07 (0.06) *x+3.35 (0.31), and with "DHA" 0.02–0.40, and "AA" 0.10–0.60: r=0.069 (p=0.383), and y=0.09 (0.11)*x+3.45 (0.28).

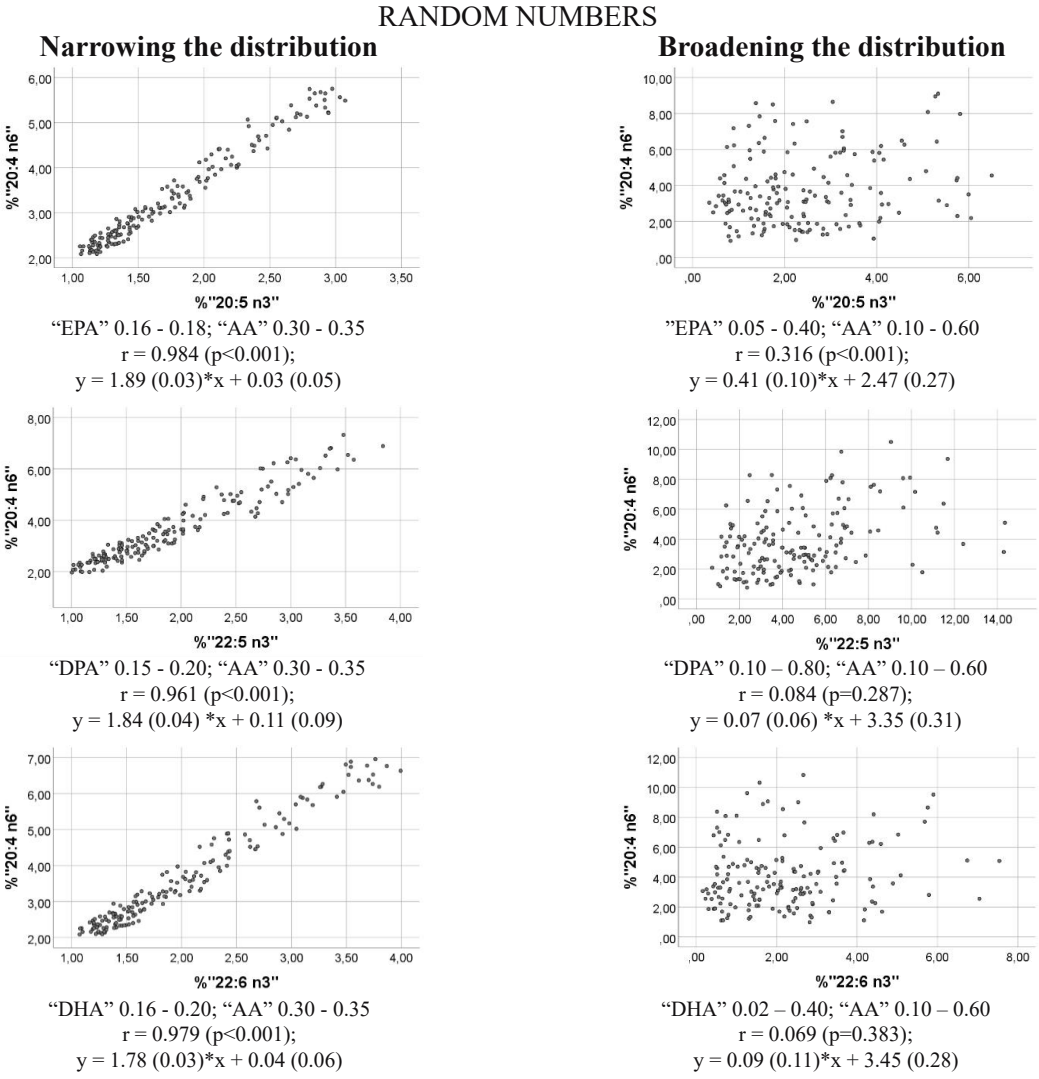


Figure 2: Effect of narrowing (left panels) and broadening (right panels) the distribution of EPA, DPA, DHA, and AA. Scatterplots of % of RANDOM numbers of "20:4 n6" and % of RANDOM numbers of "20:5 n3", "22:5 n3", and "22:6 n3"), see Methods. Distribution of the fatty acids are shown below each panel, as well as the correlation coefficient (r), and equation for the regression line, using the general formula y=a (SE) *x+b (SE), where y is the ordinate, and x the abscissa

Percentages of each of the n3 fatty acids (EPA, DPA, and DHA) correlated positively with percentages of the remaining two of them (all with p < 0.001, results not shown).

Suggested Explanation of the Results

Algebraic reasoning

We define 3 scale variables, A, B and C, giving %A+%B+%C=100, i.e. %A=- % B+(100-% C). If %C consists of high values (close to 100) and corresponding values of %B and %C are such that (100%-%C)>%B, then the equation will approach %A=%B, showing a linear positive association between %A and %B, with a slope close to1. That the requirement (100-%C)>%B is indeed satisfied follows from this example: Suppose that %C could reach 99% (or any higher value, e.g. 99.999%), then the remaining percentages (1 or 0.001%) must be divided between %A and %B. Hence, %B will be positive. One example to approximate this situation is to let A and B both have a distribution involving small numbers, for example from 0.10 to 0.15, and C a distribution involving high numbers as compared with A and B, e.g. from 1.0 to 10.0. A computer-check verified that - with these distributions - the requirements above were valid; i.e. the major %C-distribution was high (95 to 97%), and (100 -%C)>%B (output not shown). We show the association between percentages of A and B in Figure 3, r=0.941, p<0.001: The equation of the regression line is %B = 0.97 (0.02)*%A + 0.09 (0.07).

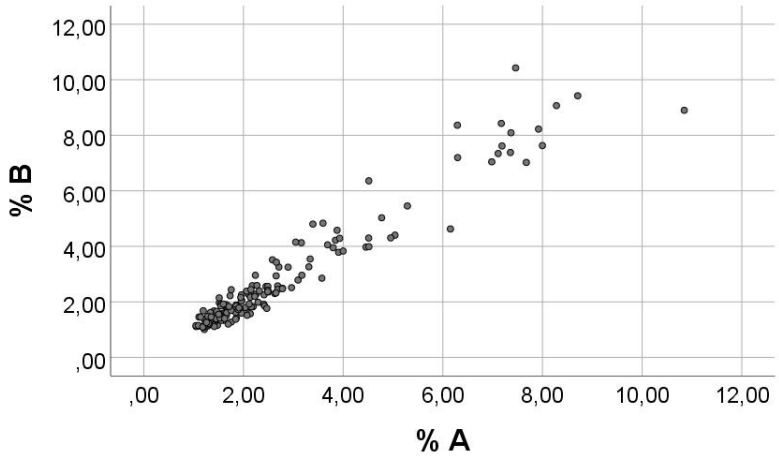


Figure 3: Association between percentages of two small-number variables, A and B, both with distribution 0.10-0.15, and one high-number variable C with distribution 1.0 - 10.0; r=0.941 (p<0.001); regression line: %B=0.97 (0.02)*%A+0.09 (0.07)

This example seems to reflect the positive correlations that we observed between %AA and percentages of the long-chain n3-fatty acids. For all of these associations, one of the 3 variables had a distribution involving high values (R, with distribution 5 to 15), whereas the other fatty acids, with percentage amounts correlating positively with %AA, had their distribution (g/kg) involving much lower values. Distributions for the fatty acids were for 20:4 n6 (0.25 to 0.39); for 20:5 n3 (0.13 to 0.23); for 22:5 n3 (0.21 to 0.43); and for 22:6 n3 (0.11 to 0.32).

We rewrite the equation above to be %A=- %C+(100-%B). Since we have given B (and accordingly %B) low values, we could roughly approximate the equation to %A=-%C+100%, which would give a perfect inverse linear association between %A and %C, and a regression line crossing both axes at 100%. The finding that extrapolation of the regression line did cross %C axis at 100 (figure not shown) is confirmed by the equation of the regression line: %A=-0.50 (0.01)*%C+49.9 (0.5). However, the assumption that %B approached zero was incorrect, since extrapolation of the regression line crosses the %A axis at 50%; % C vs. %A (B): r=-0.905 (0.965); p<0.001 for both.

Probability reasoning

We consider two variables (A, B) with distribution involving small numbers (e.g. 0.10 to 0.15), and one variable (C) with distribution among high numbers (e.g. 1 to 10). Computer analysis with random numbers shows that, with these values we obtain a distribution of percentages of A and B going from 1 to 10%, and for C: from 82 to 98 %. A positive association between %A and %B would happen if %B increases (decreases) when %A increases (decreases). With the current example, this will be the case since a continuing increase (decrease) in %C from the lowest (highest) value must be accompanied by a concomitant decrease (increase) in both %A and %B, to compensate for the complete %C - increase (decrease). Thus, %A should be positively correlated with %B; and %C should be negatively associated with %A and %B. Computer analysis showed (n=163): %A vs. %B, r=0.942 (p<0.001); %A vs. %C, r=- 0.986 (p<0.001); %B vs. %C, r=- 0.985 (p<0.001).

Geometric reasoning

We may think of a cake that is divided into three pieces (Figure 4), two of which (A and B) being small, and one (C)

large. Thus: Piece A+Piece B+Piece C=The whole cake. The size of each of the 3 pieces should vary, but only within defined boundaries.

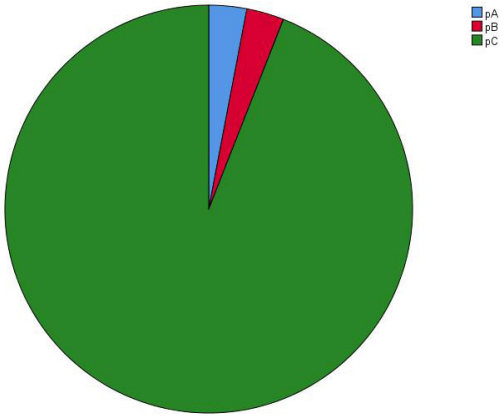


Figure 4: Pie chart to show percentages of two small-number variables (A in blue; B in red, both with size distribution 0.10 to 0.15) and percentage of one large-number variable (C, in green, with distribution 1 to 10)

If piece C progressively increases from its lowest value, then A and B will progressively decrease, in order to compensate for the C-expansion. Since the sum of the pieces is always 100%, C will be negatively correlated with each of A and B. Furthermore, since piece A and B both decrease (increase) as piece C increases (decreases); there will be a positive correlation between A and B. Furthermore, in response to progressively extending the C-distribution, A and B will be increasingly compressed, thereby improving the positive association between them. Conversely, a narrowing of the C-distribution will decrease piece C, increase A and B, and cause a poorer scatter for the size A vs. size B association. Additionally, a narrowing of the distributions for A, B, or both will increase piece C, decrease piece A and piece B, and improve the association between them. The opposite will happen when increasing the A- or B- distributions. These considerations may serve to explain the presented positive correlations between %AA and percentages of each of the long-chain n3-fatty acids.

Suggested Interpretation of the Results

Comment on the correlation between percentages

It is not surprising that percentages of fatty acids may be correlated, since they are all computed from the same sum. Indeed, as early as in 1897 Karl Pearson [12] reported that there would be a spurious correlation between two indexes with the same denominator, even if the variables used to produce the indexes were selected at random with no correlation between them. This general rule raises the question of whether also the present findings represent a correlation bias. Our analyses certainly show that it is easy to be biased if relying on correlation coefficients only, and their levels of significance. Thus, with random numbers we many times observed p-values less than 0.001 for a relationship between percentages of fatty acids, in spite of poor scatterplots. This observation is a reminder that scatterplots should always be made when relating percentages of the same sum. Below we will argue that our results could be interpreted as physiologically interesting, and not as a correlation bias.

Conceivably, percentages of two fatty acids can be inversely related since an increase in the percentage of one particular fatty acid must be accompanied by a reduced percentage of one or more of the remaining ones. However, percentages of two fatty acids may both be reduced-when the percentage of the remaining fatty acids increases. In this case, the percentages of the two former ones can be positively related. We did indeed encounter this situation with EPA (DPA, DHA) and AA. While it is easy to conceive these general considerations, it is not so simple to predict whether percentages of two particular fatty acids (among several others) will be negatively or positively associated. As explained above, we may get some idea about the outcome by considering the distribution of percentages of the fatty acids, the equation %"AA"=-%"EPA"+(100–%"R"), and by a probability and geometric reasoning.

Do the results have any implications for physiology?

The finding that %EPA is positively associated with %AA is in accordance with our previous report [9]. Since this couple of fatty acids has antagonistic metabolic actions [1-3], we might expect biological regulatory mechanisms to ensure that an increase (decrease) in %AA is accompanied by a concomitant increase (decrease) in %EPA, as was indeed found in our previous and present work. The finding that percentage of AA is positively associated with percentages of DPA and DHA as well could be related to the fact that these fatty acids can be retro-converted to EPA

[1,10], thereby serving as a store of EPA. The present study still raises the question of how these positive associations are brought about, i.e. which metabolic regulatory mechanisms are involved. Our results strongly suggest that the ***distribution per se*** of the fatty acids is crucial for the outcome. The finding that percentages of random number surrogate variables for the fatty acids produced similar associations as those found with true variables is one argument in favor of this suggestion. Additionally, if a particular concentration distribution were crucial for the correlation outcome, then we would anticipate that alterations in distributions would change the scatterplots and correlation coefficients for the association between relative amounts. Indeed, this result was just what happened in response to narrowing and broadening the distribution of the fatty acids. We hypothesize that evolution might have developed mechanisms to ensure that some fatty acids exist in low concentrations whereas others are more abundant. Regulatory mechanism could–in general–be related to synthesis of enzymes of fatty acid metabolism, allosteric regulation of the activities of particular enzymes, and/or interconversion between phosphorylated and dephosphorylated forms of key enzymes. We have, however, no data to suggest any of these possibilities. In any instance, the present results suggest that a regulation of the distribution of fatty acids can ensure positive correlations between percentages of some fatty acids, such as between %AA and %EPA (DPA, DHA). We have presented three ways of reasoning to understand the phenomenon of Distribution dependent correlation/- regulation. Studies are currently in progress with the aim of improving the explanation of this suggested novel regulatory principle in physiology.

Keeping in mind that the biological part of the present analyses originated from a diet trial in chickens, we still briefly present some general considerations related to health. Eicosanoids derived from EPA and AA may have antagonistic actions. It is well known that AA can promote inflammation and thrombosis, and thereby increase the risk of cardiovascular diseases [1-3]. The thromboembolic risk should be decreased by increasing the EPA concentration, thereby lowering the percentage of AA in platelet phospholipids and subsequently the production of TXA2 and platelet aggregation. In keeping with this, it has been reported that EPA modifies platelet-signaling responses [13]. From the present results it may be hypothesized that a disturbance in the ***Distribution Dependent Regulation*** so that the positive association between percentages of EPA and AA is disturbed, and perhaps even lost, could increase the risk of AA related conditions and diseases, but we do not have data to corroborate this hypothesis.

Suggestion of how to increase the EPA/AA ratio

Since a reduced EPA/AA ratio could be unfavourable, it is of interest to increase this ratio. To increase EPA, the consumer could increase the intake of foods rich in fatty fish, such as salmon, sardines, mackerel, and fish oils/fish oil supplements, rapeseed oil, walnuts, and flaxseeds/-oil. Similarly, to reduce the amount of AA we would advise reducing the intake of oils with high amounts of linoleic acid, such as safflower oil, sunflower oil, soybean oil, corn oil, and foodstuffs rich in these oils, for example, many processed foods, grain-based products, such as breads, pizza, and - additionally - meat, eggs and milk from animals fed a diet rich in LA, such as feeds based on corn, sunflower, wheat, barley, oat and soybean.

Suggested general rule

The present observations with fatty acids seem to imply the following, general rule: If encountering three scale variables, one of which with distribution among high numbers, and the remaining two with a narrow distribution among much lower numbers, then percentages of the small-number variables will be positively associated. Additionally, percentage of the high-number variable will be negatively associated with percentages of each of the low-number variables. We are currently working with the aim of generalizing this rule, to include other distributions. It is tempting to speculate whether the mathematical rules governing the phenomenon of Distribution Dependent Correlation (-Regulation) might have a general relevance when studying associations between relative abundances, in biology, physics, chemistry, and in social sciences. Thus, if we know distributions, then we may possibly predict whether relative abundances are positively or negatively associated, or non-existing.

Limitations of the study

Since this work was confined to studying the association between percentages AA and EPA (DPA, DHA), we do not know to what extent the phenomenon of distribution dependent regulation is valid for other fatty acids as well. Furthermore, the analyses was based upon the fatty acid pattern in breast muscle lipids of chickens and we do not know the generalizability of our results, as related to different organs, tissues or compartments, and to various species, including man. Future work in this field should include studies to explore whether the fatty acid distribution might also govern the association between percentages of other fatty acids. To investigate whether our findings have a more general validity, comparable studies should be done in other animals and in humans as well.

Conclusion

The present analyses show that percentages of AA and EPA (DPA, DHA) are positively associated. This association seems to be explained by the distribution *per se* of the fatty acid concentrations, i.e. there is a ***Distribution Dependent Regulation*** governing the association between relative abundances of AA and each of the long-chain n3-fatty acids. The present results confirm our previous observation with surrogate random numbers for fatty acids [9]. We speculate whether a disturbance in the Distribution Dependent Regulation could increase the risk of AA- associated conditions and diseases. Further studies are required to explore the generalizability of this work.

Acknowledgements

The study of which the present one is a spin-off, was funded by grant no 190399 from the Norwegian Research Council, and Animalia; the Norwegian Meat and Poultry Research Centre. We thank the collaborators at the Norwegian University of Life Sciences, and at the Animal Production Experimental Center, Ås, Norway, especially Nicole F. Nyquist, Therese Mosti and Malin Andersen.

References

1. Mayes PA (2000) Metabolism of unsaturated fatty acids and eicosanoids. In: Harper's Biochemistry, McGraw-Hill, New York, USA.

2. Smith WL, Murphy RC (2008) The eicosanoids: cyclooxygenase, lipoxygenase and epoxygenase pathways. In: Biochemistry of Lipids (4th Edn.), Elsevier, UK.

3. Gogus U, Smith C (2010) n-3 Omega fatty acids: a review of current knowledge. Int J Food Sci Tech 45: 417-36.

4. Alvheim AR, Malde MK, Osei-Hyiaman D, Lin YH, Pawlosky RJ, et al. (2012) Dietary linoleic acid elevates endogenous 2-AG and anandamide and induces obesity. Obesity 20:1984-94.

5. Kremer JM, Bigauoette J, Michalek AV (1985) Effects of manipulation of dietary fatty acids on manifestations of rheumatoid arthritis. Lancet: 184-7.

6. Lorenz R, Weber PC, Szimnau P (1989) Supplementation with n-3 fatty acids from fish oil in chronic inflammatory bowel disease - a randomized, placebo-controlled, doubleblind cross-over trial. J Intern Med Suppl 731: 225-32.

7. Kromhout D, Bosschieter E, Coulander CL (1985) The inverse relation between fish consumption and 20-year mortality from coronary heart disease. N Engl J Med 312: 1205-9.

8. Nagata M, Hata J, Hirakawa Y, Mukai N, Yoshida D, et al. (2017) The ratio of serum eicosapentaenoic acid to arachidonic acid and risk of cancer death in a Japanese community: The Hisayama Study. J Epidemiol 27: 578-83.

9. Høstmark AT, Haug A (2018) The Fatty Acid Distribution per se Explains Why Percentages of Eicosapentaenoic Acid (20:5 n3) and Arachidonic Acid (20:4 n6) are Positively Associated; a Novel Regulatory Mechanism? J Nutr Diet Suppl 2: 103.

10. Allaire J, Harris WS, Vors C, Charest A, Marin J, et al. (2017) Supplementation with high-dose docosahexaenoic acid increases the Omega-3 Index more than high-dose eicosapentaenoic acid. Prostagland Leukotrienes Essential Fatty Acids 120: 8-14.

11. Nyquist NF, Rødbotten R, Thomassen M, Haug A (2013) Chicken meat nutritional value when feeding red palm oil, palm oil or rendered animal fat in combinations with linseed oil, rapeseed oil and two levels of selenium. Lipids Health Dis 12:69.

12. Pearson K (1897) Mathematical contributions to the theory of evolution. On a form of spurious correlation which may arise when indices are used in the measurement of organs. Proc R Soc Lond 60: 489-96.

13. McCue B, Shaull L, Kollasch A, Ye S, Whiteheart S, et al. (2014) The effect of EPA and DHA on platelets treated with multiple agonists found in the thrombus core. FASEB J 28: Supplement 1.