

Relative Amounts of Eicosanoid and Docosanoid Precursor Fatty Acids Are Positively Associated: A Distribution Dependent Regulation

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Abstract:

We recently reported a positive association between %EPA (20:5 n3) and %AA (20:4 n6) in muscle lipids of chickens. In the present work we investigate whether relative amounts of other fatty acids, which are precursors of eicosanoids and docosanoids, might also be positively correlated. The present results seem to verify this suggestion. Furthermore, the correlation outcome might be explained by the particular concentration distributions of the fatty acids under investigation. Thus, similar results were obtained using true values and substitute, random numbers for the fatty acids, sampled with the true ranges. Additionally, when we hypothetically altered distributions in computer experiments, we observed appreciable changes in the strength of the associations. Thus, there seems to be Distribution dependent positive associations between eicosanoid and docosanoid fatty acid precursor percentages, possibly serving to ensure that effects of important regulatory molecules in physiology will be balanced. We suggest that Distribution Dependent Correlations could be a novel evolutionary regulatory principle.

Keywords: arachidonic acid; eicosapentaenoic acid; docosahexaenoic acid; dihomo-gammalinolenic acid; chickens; random numbers; muscle fat; chickens

Definitions and abbreviations:

Variability: the width or spread of a distribution, measured e.g. by the range and standard deviation.

Range: showing the highest and lowest values.

Distribution: graph showing the frequency distribution of a scale variable within a particular range. In this article, we also use *distribution* when referring to a particular range, a – b, on the scale.

Uniform distribution: every value within the range is equally likely. In this article, we may write “Distribution was from a to b”, or “Distributions of A, B, and C were a – b, c – d, and e – f, respectively”.

“Low-number variables” have low numbers relative to “high-number variables”.

OA = Oleic Acid (18:1 n7); LA = Linoleic Acid (18:2 n6); ALA = Alpha Linolenic Acid (18:3 n3); AA = Arachidonic Acid (20:4 n6); EPA = Eicosapentaenoic Acid (20:5 n3); DPA = Docosapentaenoic Acid (22:5 n3); DHA = Docosahexaenoic Acid (22:6 n3); DGLA = dihomo-gammalinolenic acid (20:3 n6)

Introduction

Fatty acids in blood and tissues are important in health and disease, and body amounts are influenced by diet [1-3]. Poly-unsaturated fatty acids with 20 or 22 carbon atoms serve as precursor molecules for physiologically important regulatory molecules, i.e. the eicosanoids and docosanoids, which are produced in most organs and cell types, in

reactions catalyzed by cyclooxygenases, lipoxygenases, and epoxygenases [4]. It is well known that EPA (20:5 n3) and AA (20:4 n6) are metabolic antagonists [1 -3]. Eicosanoids derived from EPA may decrease inflammatory diseases [5, 6], improve coronary heart diseases [7, 8], and cancer [9], although a systematic Cochrane Review of selected studies questioned the beneficial effects of long-chain n3 fatty acids on all-cause and cardiovascular mortality [10].

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. This latter fatty acid 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. prostaglandins, prostacyclin, thromboxane, and leukotrienes [1,2]. AA derived thromboxane A₂ (TXA₂) and leukotriene B₄ (LTB₄) have strong proinflammatory and prothrombotic properties, and are involved in allergic reactions and bronchoconstriction [1, 2, 4]. Furthermore, endocannabinoids, which are derived from AA, may have a role in adiposity and inflammation [11]. Additionally, it has been reported that a decreased level of the serum EPA/AA ratio was a risk factor for cancer death in the general Japanese population [9]. However, not only the eicosanoids, but also docosanoids, originating from C22 fatty acids (DPA, DHA), have strong metabolic effects. Among these latter compounds are protectins, resolvins, and maresins, which may strongly counteract immune- and inflammatory reactions [4]. Also eicosatrienoic acid, i.e. 20:3 n6 (dihomo-gammalinolenic acid, DGLA) may serve as precursor for eicosanoids [4]. However, to our knowledge,

there are less data on eicosanoids derived from three other C20 fatty acids: the two eicosatrienoic acids 20:3 n3 and 20:3 n9 (Mead acid, not detected in the present work), and eicosadienoic acid (20:2 n6).

In order to achieve a balance between the metabolic influences of the many eicosanoids and docosanoids, we would expect a coordinated regulation of precursor fatty acid percentages, e.g. of % EPA, %AA, %DGLA, and of other relevant fatty acid percentages. Indeed, we might expect in general that these particular percentages of the total sum of fatty acids were positively associated, so that an increase (decrease) in e.g. %AA would be accompanied by a concomitant increase (decrease) in other fatty acid precursor percentages as well, in order to obtain the required balance. We previously reported that that %AA, %EPA, and %DHA were positively associated in breast muscle lipids of chickens [12, 14]. We also showed that this correlation outcome was related to the particular *concentration distributions* of the fatty acids, as suggested by similar outcomes with true values and surrogate random numbers, however sampled with the true ranges [13 -17]. Furthermore, experimentally altering the ranges in computer experiments strongly influenced the correlation outcomes. The aim of the present work was to further investigate whether relative amounts of eicosanoid and docosanoid precursor fatty acids are positively associated, and whether the correlations are related to the concentration distributions.

Materials and Methods

Chickens and Diet

We refer to a previous article [18] 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

Correlations. The following 22 fatty acids were determined in chicken breast muscle lipids: 14:0; 14:1; 15:0; 16:0; 16:1; 17:0; 18:0; 18:1 t6,11; 18:1 c9; 18:2 c11; 18:2 n6; 20:0; 18:3 n6; 18:3 n3; 20:1 n9; 20:2 n6; 20:3 n6; 20:3 n3; 20:4 n6; 20:5 n3; 22:6 n3; and 22:5 n3. The sum (S) of all these fatty acids (SD), i.e. 8.85 (2.62) g/kg wet weight (n = 163) was used in the denominator when calculating relative amounts of the fatty acids. For example, percentage arachidonic acid was computed as:

$$\%AA = (AA/S)*100, \text{ and } \%EPA = (EPA/S)*100.$$

To obtain percentage amounts of other fatty acids, the calculation procedure was as shown for AA and EPA. With all fatty acids serving as potential precursors for eicosanoids and docosanoids, we computed correlation coefficients (Pearson's r and/or Spearman's rho) to assess associations between the fatty acid percentages. The following 7 precursor fatty acids were investigated: 20:4 n6, 20:3 n6, 20:3 n3, 20:2 n6, 22:5 n3, 20:5 n3, and 22:6 n3. We additionally made scatterplots to illustrate associations between relative amounts of these fatty acids, but only some examples are included in this work.

Calculations Performed To Possibly Explain the Correlation Outcome

Range and variability.

We computed ranges, mean values, and variabilities (coefficient of variation, SD) of the fatty acids under investigation. For example, the range was 0.13 - 0.24 g/kg for EPA, and 0.25 - 0.42 g/kg for AA. To further examine concentration (g/kg) distributions of the various fatty acids, we made histograms; only some examples are shown.

Simplification.

To understand how associations between fatty acid percentages are brought about, we previously simplified the analyses by considering 3 variables only, i.e. the two fatty acids under investigation, and sum (R) of the remaining fatty acids. For example, R = S - DPA - DGLA, if DPA (22:5 n3) and DGLA (20:3 n6) are the fatty acids under investigation. Thus, %DPA + %DGLA + %R = 100, or %DPA = -%DGLA + (100 -%R). With high %R-values, this equation will approach %DPA = %DGLA, showing a positive association between relative amounts of the variables; with a positive slope determined by the ranges of DPA and DGLA, as explained in more detail previously [13 -17]. A similar reasoning is valid when considering the association between relative amounts of all other fatty acids. We present further details under Results and Discussion.

Are the correlation outcomes related to distributions of the fatty acids? A random number approach.

As reported previously [13, 14], with AA and EPA the distribution per se seems to be crucial for the correlation outcome between the fatty acid percentages. If this conclusion is valid for the current analyses as well, we should anticipate similar correlation results with true and surrogate, random numbers for the fatty acids, however sampled with the true concentration ranges. Furthermore, the strength of the associations should be changed if we altered the distributions. We accordingly generated uniformly distributed RANDOM numbers with the physiological distributions of the couple of fatty acids under investigation, and of R. Since the diet trial had 163 birds, for each of the analyses below we generated 163 random numbers with the particular fatty acid distributions shown in Table 1. To clarify, we use upper case letters (RANDOM) or quotation marks in Figures or figure texts when working with random numbers.

Using random numbers in a previous computer experiment, we suggested [15] that, with 3 positive scale variables, two of which having low-number distribution, and low variability, as compared with the third variable, we might expect a positive association between relative amounts of the two low-number variable, and a negative association between percentage high-number variable and each of the low-number variable percentages. Furthermore, a decrease (increase) in the variability of either or both of the two low-number variables seemed to improve (make poorer) the association between their relative amounts. In contrast, a narrowing (broadening) of the distribution of the high-number variable seemed to make poorer (improve) the association between the two low-number variable percentages.

In the present work, it seems that we have two low-number variables (the pair of fatty acids under investigation) relative to a high-number variable (R). Therefore, the previous rules should apply for the current

analyses. Thus, R is sum of the remaining fatty acids when omitting the two fatty acids that were under correlation analysis, i.e. $R = S - A - B$, if S is the total sum of fatty acids, whereas A and B are the fatty acids under investigation. This means that

$$\%A + \%B + \%R = 100, \text{ or } \%B = -\%A + (100 - \%R).$$

Conceivably, R should be different for each of the calculations, since different pairs of fatty acids were used. It turned out, however, that variation in R was small, due to great similarity between the ranges of the low-number fatty acids under investigation; the R variability was generally 5 -15 g/kg. We used the range 5 -15 for R in some computer experiments to investigate whether we might obtain the same correlation outcome with true values and random numbers, sampled with the true ranges. However, we used the true R-ranges when computing correlations shown in Table 3.

Additionally, by experimentally changing ranges with hypothetical values in computer experiments, we aimed at further clarifying whether the concentration ranges do govern the association between particular fatty acid percentages of the same sum. Since there are infinite many ways to change the distributions, 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's r and/or Spearman's rho), and scatterplots, varied slightly. We present the results as correlation coefficients, scatterplots, and regression analyses. SPSS 26.0 was used for the analyses, and for making figures. The significance level was set at $p < 0.05$. The

experimental conditions are presented in more detail under "Results and Discussion".

Authors' Contributions

This work is a spin-off study of a previously published diet trial, conceived and carried out by AH. ATH conceived the present study, did the calculations, statistical analyses, and wrote the article. Both authors participated sufficiently - intellectually or practically - in the work, to take public responsibility for the content of the article. 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 were no competing interests.

Results and Discussion

Descriptive Data

The 7 fatty acids under investigation had in general low numbers, and low variability (Table 1).

Fatty acid	Min	Max	Mean	SD	CV
20:2 n6	0.04	0.06	0.05	0.01	20.0
20:3 n6	0.06	0.11	0.08	0.01	12.5
20:3 n3	0.04	0.09	0.05	0.01	20.0
20:4 n6	0.25	0.42	0.31	0.03	9.7
20:5 n3	0.13	0.24	0.18	0.02	11.1
22:5 n3	0.21	0.43	0.31	0.04	12.9
22:6 n3	0.11	0.32	0.19	0.04	21.1

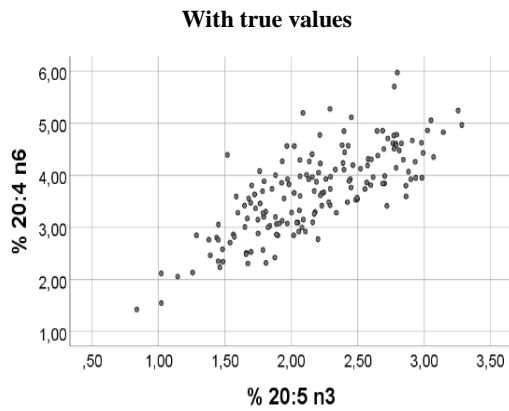
Note: Some of the values appear as zero due to the number of decimals.

Table 1. Descriptive data for the 7 fatty acids under investigation: minimum and maximum values, means (g/kg), with SD, and variability, $CV = (SD/mean) \times 100$.

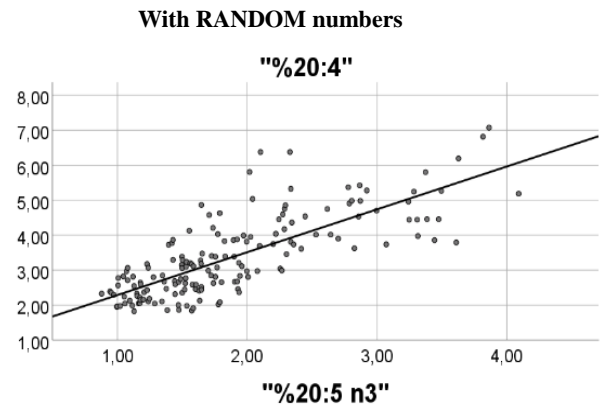
Will we obtain similar associations with true and random numbers for the fatty acids?

We previously reported a positive association between %EPA and %AA; the outcomes were similar with true values and with surrogate, random numbers, provided that the numbers were sampled with the true ranges for the fatty acids [13]. A repeat of one of these analyses (with a

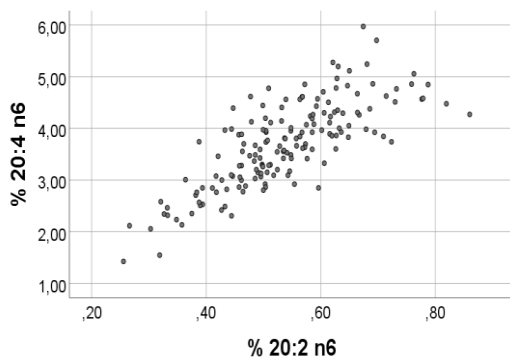
new set of random numbers) is shown in Figure 1, upper panel. In the repeat analysis (Figure 1, top panels) we found that the regression lines were very similar when using true values and random numbers; the equations being $y = 1.23 (0.08) \cdot x + 1.01(0.18)$ with true values (top, left panel), and $y = 1.23 (0.08) \cdot x + 1.06(0.16)$ with random numbers (top, right panel). In Figure 1, middle and lower panels, we present two other examples among the altogether 42 possible scatterplots.



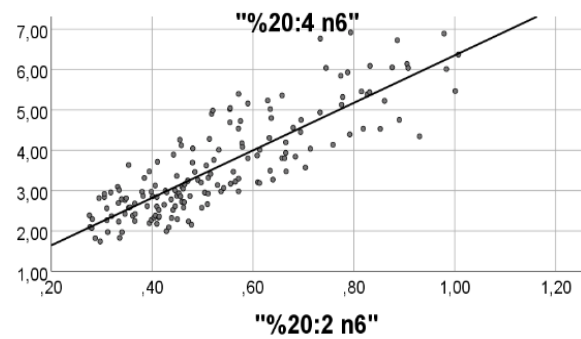
rho = 0.750 (p<0.001);
Y = 1.23 (0.08)*X + 1.01(0.18)



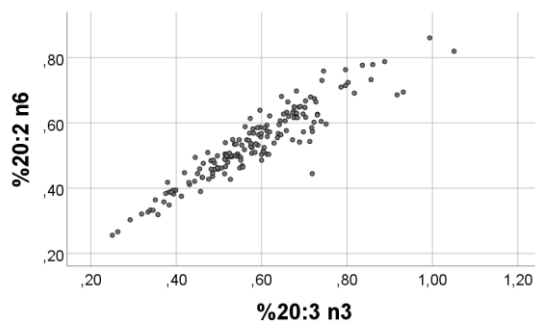
rho = 0.771, p<0.001);
Y = 1.23 (0.08)*X + 1.06(0.16)



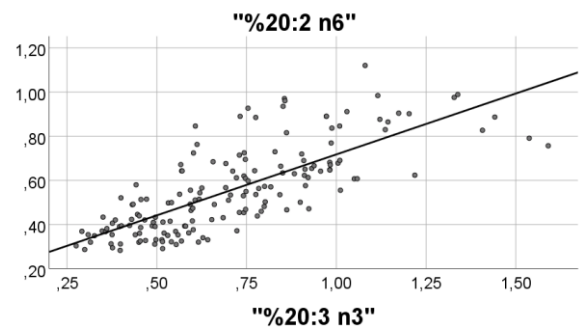
rho = 0.768 (p<0.001);
Y = 5.61(0.36)*X + 0.68 (0.20)



rho = 0.839 (p<0.001);
Y = 5.89 (0.29)*X + 0.46(0.16)



rho = 0.916 (p<0.001);
Y = 0.75 (0.02)*X + 0.10 (0.01)



rho = 0.785 (p<0.001);
Y = 0.55 (0.04)*X + 0.17(0.03)

Figure 1. Scatterplots of %AA (20:4 n6) vs. % EPA (upper panel, left), %AA vs. % eicosadienoic acid (20:2 n6, middle panel left), and %eicosadienoic acid (20:2 n6) vs. %eicosatrienoic acid (20:3 n3), lower panel, left. Right panels show scatterplots of the association between percentage surrogate RANDOM numbers, computed from RANDOM numbers sampled with the true concentration ranges of the same fatty acids, and of R. The RANDOM number variables are shown in question marks. Spearman's rho values appear below each panel, and also the equation of the regression line, using the general formula $Y = a (SE) *X + b (SE)$, where Y is the ordinate, and X is the abscissa, n = 163.

A correlation analysis showed that relative amounts of all of the selected 7 precursor fatty acids for eicosanoids and docosanoids correlated positively with high significance (Table 2). However, as shown in Table 3 we also obtained positive correlations when replacing the true values with RANDOM numbers, sampled within the true ranges for the fatty acids (and R, i.e. sum of the remaining fatty acids when omitting the

couple under investigation). Indeed, strengths of correlations were comparable to those obtained with true values, as also confirmed by scatterplots (only some examples are shown in Figure 1). These results strongly suggest that distributions per se of the fatty acid concentrations are crucial for the correlation outcomes.

Fatty acid	%20:3 n6	%20:3 n3	%20:2 n6	%22:5 n3	%20:4 n6	%22:6 n3	%20:5 n3
%20:3 n6	1						
%20:3 n3	0.823	1					
%20:2 n6	0.884	0.931	1				
%22:5 n3	0.659	0.642	0.714	1			
%20:4 n6	0.761	0.635	0.774	0.881	1		
%22:6 n3	0.550	0.542	0.609	0.842	0.765	1	
%20:5 n3	0.807	0.652	0.684	0.726	0.762	0.550	1

Table 2. Correlations (Pearson’s correlation coefficients) between relative amounts of fatty acids (see text).
All correlations are with $p < 0.001$, $n = 163$.

Fatty acid	%20:3 n6	%20:3 n3	%20:2 n6	%22:5 n3	%20:4 n6	%22:6 n3	%20:5 n3
%20:3 n6	1						
%20:3 n3	0.834	1					
%20:2 n6	0.865	0.823	1				
%22:5 n3	0.788	0.782	0.869	1			
%20:4 n6	0.888	0.819	0.899	0.813	1		
%22:6 n3	0.772	0.738	0.721	0.754	0.760	1	
%20:5 n3	0.854	0.774	0.894	0.827	0.867	0.777	1

Table 3. Correlations (Pearson’s correlation coefficients) between relative amounts of surrogate, random numbers representing the fatty acids (see text).

All correlations are with $p < 0.001$, $n = 163$.

An Algebraic Approach to Explain the Results

The above results raise the question of how to explain why the concentration ranges seem to govern that relative amounts of the selected fatty acids are positively associated.

Some general considerations

We first consider - in general- three positive scale variables, A, B and R, giving $\%A + \%B + \%R = 100$, i.e. $\%B = - \%A + (100 - \%R)$. This equation has three unknown variables, each of which with a particular distribution (range). It is therefore hard to predict whether or not there is a significant association between relative amounts of e.g. A and B. We may, however, simplify the equation by approximations, so as to involve two variables only. This may be achieved in two particular situations: 1)

if the expression $(100 - \%R)$ approaches zero, or 2) if $\%R$ approaches zero. Thus, if $\%R$ consists of high values (close to 100) and the low-number, corresponding values of $\%A$ and $\%R$ are such that $(100 - \%R) > \%A$, then the equation would approach $\%B = \%A$, showing a linear positive association between $\%A$ and $\%B$. The requirement $(100 - \%R) > \%A$ is indeed satisfied, since the remaining value when calculating $(100 - \%R)$ would have to be divided between $\%A$ and $\%B$. For example, suppose that $\%R$ could reach 99%, then the remaining percentage is to be divided between $\%A$ and $\%B$. Hence, the slope must be positive.

On the other hand, if $\%R$ consists of very small values, we should expect a negative $\%A$ vs. $\%B$ association, since the equation in this case would approach $\%B = - \%A + 100$. Additionally, we might anticipate positive or negative correlations between A and B percentages also within a certain boundary around the above-mentioned conditions, but

with poorer outcomes as the above-mentioned conditions are decreasingly complied with. This reasoning raises the question of how far from the “mathematically ideal”, but “physiologically extreme” (if relating the A, B, and C variables to physiologically ones) conditions we may go and still obtain a positive (negative) %B vs. %A association. Furthermore, this reasoning implies that there must be a **Turning Point** where a positive (negative) correlation between percentages of A and B turns to become negative (positive), as we previously demonstrated in computer experiments [15, 17]. We previously showed that the association between %A and %B was strongly influenced by altering R [16]. However, also changes in the A and/or B ranges should influence correlations between percent A (B, R). Thus, when narrowing the A or B ranges, this means lower values of %A (B). Since $\%A + \%B + \%R = 100$, a decrease in %A (B) must be accompanied by an increase in %R, thereby approaching the above Condition 1), i.e. improving the positive association between %A and %B. Conversely, an increase in A (B) caused increased values of %A (B), and accordingly lower values of %R, would make the %A vs. %B association poorer. A decrease/increase in %A (B) values is obtained by altering the A (B)

ranges, see below. Below we will experimentally show these effects, but first we will give a brief comment on the range of R, and the *slope* of the regression line.

Range of R: the remaining sum when omitting the pairs under investigation.

To explain the correlation outcome, we need the distribution of R (sum of the remaining fatty acids when omitting the two fatty acids being under investigation). Therefore, we calculated R for a large number of pairs. The general outcome is illustrated by the examples shown in Figure 2. Conceivably, the R distribution should not be much different with different pairs of the current fatty acids, since all of them had low-number ranges as compared with the high-number fatty acids included in R (mainly oleic acid with range 1.0 – 8.6 g/kg). This means that values of R (and accordingly also of %R) were not much altered by varying the couple of fatty acids under investigation; the range of was generally close to 5 – 15 g/kg wet weight (Figure 2).

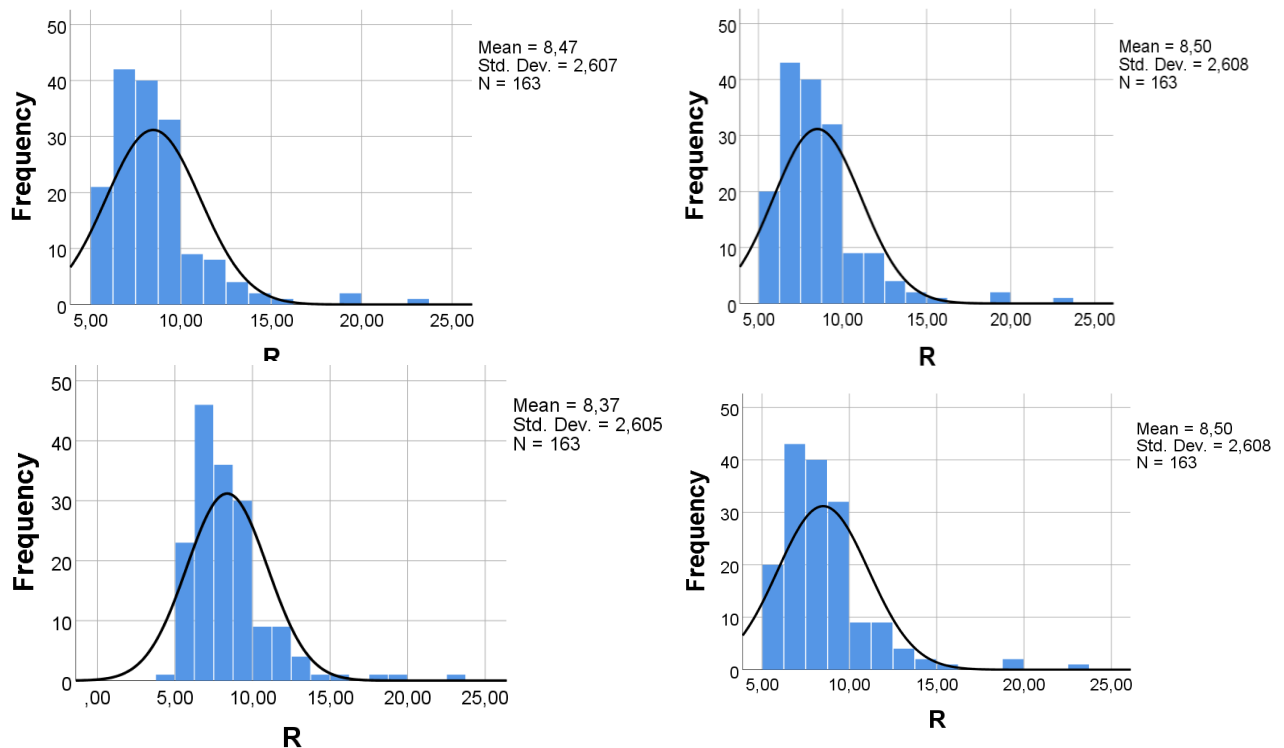


Figure 2. Example of histograms of the remaining fatty acids (R) when omitting the two fatty acids being under investigation (see text). *Upper panel, left* shows distribution of R = S - AA - 20:3 n6; *upper panel, right:* R = S - AA - 20:3 n3. *Lower panel, left:* R = S - AA - 20:2 n6; *lower panel, right:* R = S - AA - 20:5 n3; *lower panel, right* R = S - AA - 22:6 n3.

Slope of the regression line

Above we argued that there should be a positive association between %B and %A, if %R values were very high so that the expression $(100 - \%R)$ approached zero. However, in this case it is inappropriate to write $\%B = \%A$, like $Y = X$. In the latter case, both the abscissa and the ordinate may have any value on the scale, and the Y vs. X graph would have slope = 1. In contrast to this, %B and %A – values are limited by the B and A distributions (ranges), respectively. A more general equation would be: $\%B_{(p-q)} = -\%A_{(r-s)} + (100 - \%R_{(t-u)})$ where the

subscript parentheses indicate ranges of A, B, and R. The slope of the %B vs. %A regression line will accordingly be determined by the ranges of A (%A) and B (%B). Thus, if A- and also B - have the same distribution (range), then the slope should be close to 1. Indeed, in an experiment with range 0.10 – 0.15 for both A and B, and 1 – 10 for R, we did find slope =1, [15]. With *differing* ranges for A and B, e.g. for A 0.20 - 0.40, and for B 0.10 – 0.15, and for R 1 - 10, we found that the equation of the regression line was: $\%B = 0.38 (0.01)* \%A + 0.22 (0.10)$.

Applying the Above Algebraic Approach to Explain the Association between Eicosanoid (Docosanoid) Precursor Fatty Acid Percentages

We apply the above general consideration to understand the current correlation outcome between percentage eicosanoid (docosanoid) precursor fatty acids from breast muscle lipids of chickens. With the 7 fatty acids under investigation, we arbitrarily choose one of the pairs shown in Figure 3, i.e. for B in the equation $%B = -\%A + (100 - \%R)$ we use eicosadienoic acid (20:2 n6) and for A eicosatrienoic acid (20:3 n3); thus, the equation of the regression line would be: $%B_{(0.04-0.06)} = -\%A_{(0.04-0.09)} + (100 - \%R_{(5-15)})$ which is approaching $%B_{(0.04-0.06)} = \%A_{(0.04-0.09)}$, due to high $\%R_{(5-15)}$ values, i.e. there should be a positive association between %B and %A, as was also observed (rho about 0.9). Similarly, the observed negative association between $%B_{(0.04-0.06)}$ (or $%A_{(0.04-0.09)}$) and $\%R_{(5-15)}$, i.e. $\rho = -0.951 (-0.887)$, $p < 0.001$ for both ($n = 163$) may be explained by approximations of the equations 1) $%B = -\%R + (100 - \%A)$ and 2) $%A = -\%R + (100 - \%B)$. Eq. 1) may be approximated to $%B = -\%R + 100$, since %A is small compared with %R. Similarly, eq. 2) may be approximated to $%A = -\%R + 100$. Thus, %R should be negatively associated with both %B and %A, as we did observe. A similar way of reasoning should be valid for all of the current 42 correlations. Therefore, we suggest that the concentration distributions alone can explain all of the positive associations observed.

Experiments to further clarify the influence of range upon RANDOM number % 20:3 n3 vs. RANDOM number % 20:2 n6

If the concentration ranges of the fatty acids really determine the strength of the association between their relative amounts, then we should expect that a change in ranges would cause a disturbance in the associations, as suggested by scatterplots and verified by correlation coefficients. We accordingly did some computer experiments where we changed the ranges of two arbitrarily chosen pair of fatty acids, for example eicosadienoic acid (20:2 n6) and eicosatrienoic acid (20:3 n3). As expected, in response to a moderate narrowing of ranges, we obtained that the association between their relative amounts improved (compare the top panels of Figure 3); the improved association was verified by the change in Spearman's rho, i.e. $\rho = 0.737 (p < 0.001)$ before narrowing, against $\rho = 0.918$ after narrowing ($p < 0.001$). This outcome was further corroborated by the greatly improved scatterplot obtained after a strong narrowing of the ranges (Figure 3, middle panel, left; ρ after narrowing: 0.992 ($p < 0.001$)). In contrast to these appreciable improved associations caused by narrowing the ranges of the fatty acids, we observed a much poorer scatterplot between the relative amounts in response to broadening the ranges, as illustrated by the scatterplot (Figure 3, lower panel, right), and by the poorer correlation coefficient ($\rho = 0.337, p < 0.001$).

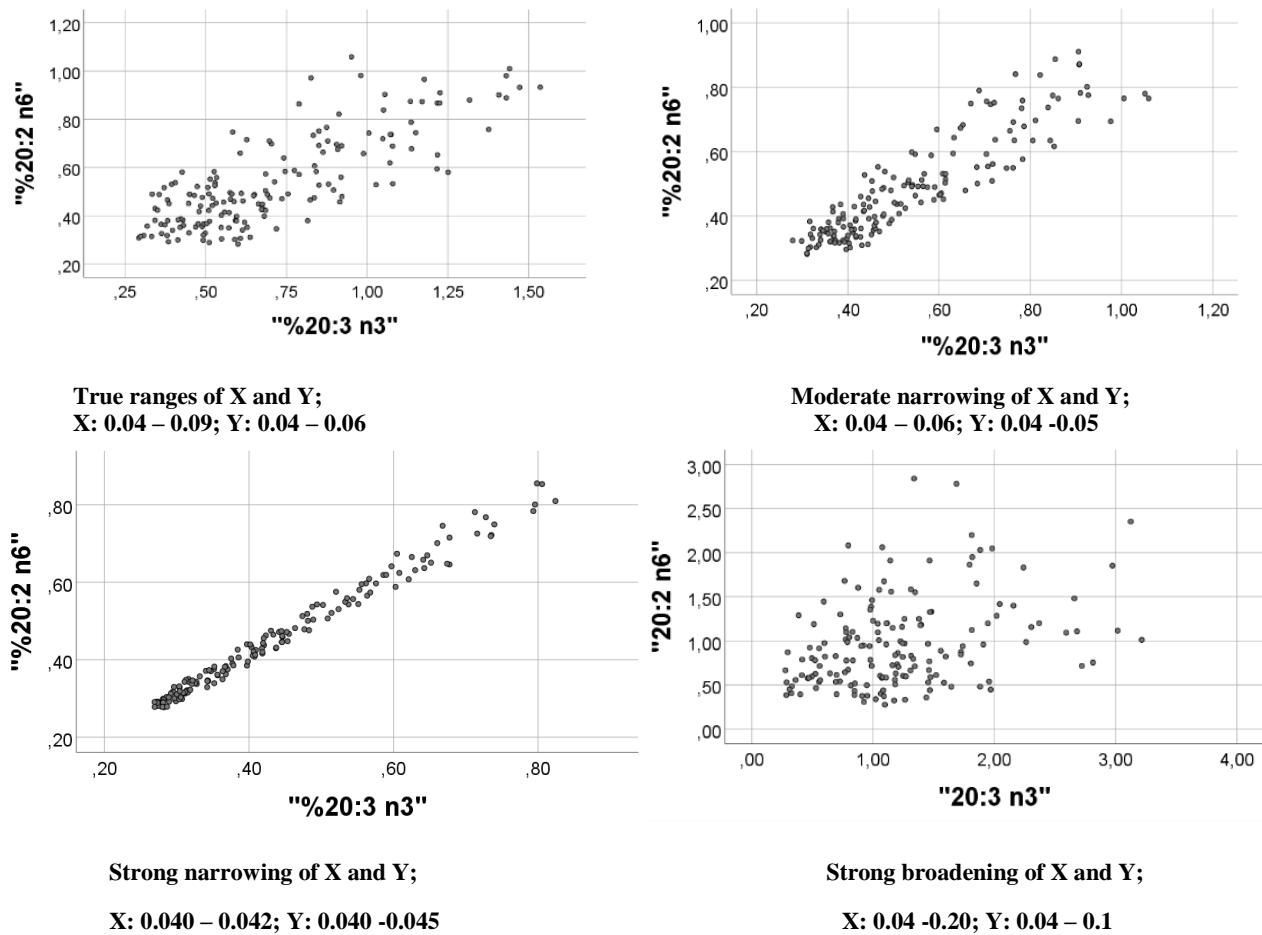


Figure 3. Scatterplots showing effects of hypothetically narrowing and broadening ranges of fatty acids. The figure relates to the general eq. $\%X + \%Y + \%R = 100$, or $\%Y = -\%X + (100 - \%R)$, where X and Y represent various fatty acids, and R is sum of the remaining fatty acids when omitting X and Y, see text. Note that we have used only RANDOM numbers to produce all panels of this figure; the random numbers had uniform distribution and were generated on the basis of the hypothetical ranges shown. We use X for RANDOM number surrogate values of 20:3 n3 and Y for RANDOM numbers of

20:2 n6. The fatty acids appear with quotation marks on the axes, since we apply random number. Upper panel, left: The *physiological ranges* of X (0.04 - 0.09) and Y (0.04 - 0.06) were used. Spearman's rho for %Y vs. %X, rho = 0.737, %X (Y) vs. %R, rho = -0.955(-0.894); p<0.001 for all, n =163. Quartiles of the %R distribution were: 98.4, 98.9, and 99.1, respectively. Eq. of regression line: $y = 0.51 (0.03)x + 0.18 (0.02)$. Upper panel, right: Moderate narrowing of ranges: X: 0.04 - 0.06; Y: 0.04 - 0.05. Spearman's rho for %Y vs. %X, rho = 0.918, %X (Y) vs. %R, rho = -0.981(-0.974); p<0.001 for all, n =163. %R quartiles were: 98.6, 99.0, and 99.2, respectively. Eq. of regression line: $Y = 0.80 (0.03)X + 0.05(0.02)$. Lower panel, left: Effect of a *strong narrowing of the ranges*; X: 0.040 - 0.042; Y: 0.040 - 0.045. Spearman's rho for %Y vs. %X: 0.992, %X (Y) vs. %R, rho = -0.998(-0.998); p<0.001 for all, n =163. %R quartiles were: 98.9, 99.1, and 99.3, respectively. Effect of an appreciable *broadening* of the ranges is shown in lower panel, right; X: 0.04 - 0.20; Y: 0.04 - 0.16. Spearman's rho for %Y vs. %X: 0.337, %X (Y) vs. %R, rho = -0.845(-0.763); p<0.001 for all, n =163. %R quartiles were: 97.4, 98.0, and 98.5, respectively. Eq. of the regression line. $Y = 0.29 (0.06)X + 0.61 (0.08)$. Note: the same range (5 - 15) was used for R in all panels, see text.

Further Details to Explain Figure 3

The scatterplots shown in Figure 3 relate to the general eq. $\%Y = -\%X + (100 - \%R)$, where X and Y represent fatty acids, and R is sum of the remaining fatty acids when omitting X and Y, see text. To produce this figure, we have used RANDOM numbers only; the numbers had uniform distribution and were generated on the basis of the hypothetical ranges shown. We use X for RANDOM number surrogate values of 20:3 n3 and Y for RANDOM numbers of 20:2 n6. The fatty acids appear with quotation marks in the panels, since we apply random number. We first show the outcome with the *physiological ranges* (Figure 3, upper panel, left) of X (0.04 - 0.09) and Y (0.04 - 0.06); Spearman's rho for %Y vs. %X: rho = 0.737; %X(Y) vs. %R, rho = -0.955(-0.894); p<0.001 for all, n =163. %R quartiles were: 98.4, 98.9, and 99.1, respectively. Equation of the regression line was: $y = 0.51 (0.03)x + 0.18 (0.02)$. In response to a *moderate narrowing* of ranges (Figure 3, upper panel, right), i.e. X: 0.04 - 0.06; Y: 0.04 - 0.05, the Spearman's rho for %Y vs. %X was improved; rho = 0.918; %X (Y) vs. %R, rho = -0.981(-0.974), p<0.001 for all, n =163. %R quartiles were: 98.6, 99.0, and 99.2, respectively. Thus, the %R distribution had moved slightly towards higher values, an effect that should improve the association between relative amounts of the surrogate fatty acids, see above. Equation of regression line was: $Y = 0.80 (0.03)X + 0.05(0.02)$. Effect of a *strong narrowing of the ranges*; X: 0.040 - 0.042; Y: 0.040 - 0.045 is shown in Figure 3, lower panel, left. Spearman's rho for %Y vs. %X was further improved: rho = 0.992; %X (Y) vs. %R: rho = -0.998(-0.998); p<0.001 for all, n =163. %R quartiles were: 98.9, 99.1, and 99.3, respectively, showing a further minor movement towards higher values of the %R distribution, thereby possibly explaining the improved scatterplot and correlation coefficient (as explained above). Effect of a *broadening the ranges* is shown in Figure 3, lower panel, right; X: 0.04 - 0.20; Y: 0.04 - 0.16. Spearman's rho for %Y vs. %X was: 0.337; %X (Y) vs. %R, rho = -0.845(-0.763), p<0.001 for all, n =163. %R quartiles were: 97.4, 98.0, and 98.5, respectively. Thus, in line with the above reasoning, in response to a broadening of the X(Y) distribution, we observe a movement of the %R histogram towards lower values. This effect should make the %X vs. %Y association poorer, as illustrated in the scatterplot, and verified by the poorer correlation coefficient. Equation of the regression line after broadening the ranges of X and Y was: $Y = 0.29 (0.06)X + 0.61 (0.08)$. Note that we use the same range (5 - 15) for R in all panels, as explained above. The strong inverse association between %A(B) and %R is explained by the equation $\%B = -\%A + (100 - \%R)$, which may be approximated to $\%B = -\%R + 100$ (showing an inverse %B vs. %R relationship), and by $\%A = -\%R + 100$ (showing an inverse %A vs. %R association).

An additional comment on how to explain positive and negative correlations between percentages.

Above we simplified the equation $\%A + \%B + \%C = 100$, i.e. $\%B = -\%A + (100 - \%C)$, in two ways: 1) by increasing %C to very high values (giving positive correlations), or 2) by decreasing %C to very low values (giving negative correlations). However, the denominator, i.e. (A + B + C) is always there when dealing with percentages, but the significance

of C (%C) is quite different in 1) and 2). With *positive* correlations between %A and %B, C is defined to be very high, thereby governing the denominator, and accordingly also the A and B percentages. Thus, we may approximate to $\%A = (A/C)*100$, and $\%B = (B/C)*100$. Since A and B percentages would both decrease with increasing C-values, it follows that %A and %B will be positively associated. In contrast to this, with *negative* correlations between %A and %B, C is defined to be very low, making the denominator approach A + B, thereby giving $\%A = 100*A/(A+B)$, and $\%B = 100*B/(A+B)$. With two variables only, their relative amounts must vary inversely.

Will body weight and body fat influence the present results?

Body weight

When diving the 163 chickens into subgroups according to body weight, we obtained qualitatively the same correlation outcome in each of the weight subgroups (results not shown). Thus, body weight does not seem to modify the correlations.

Total fat

Body fat (g/kg wet weight) is essentially the sum of all fatty acids. We did not measure fat in other tissues/organs than breast muscle. The possibility exists that the correlation outcome in other tissues/organs might differ from that observed in muscle. The cornerstone of the idea of *Distribution Dependent Correlations* is that the particular concentration ranges (possibly caused by evolution) of the many fatty acids will ensure that some of their percentages must be positively associated, whereas others are negatively correlated, by mathematical rules. Since even minor changes in ranges may strongly influence the correlations, we might raise the question of how stratifying according to total fat might influence correlations. For example, if we make 3 groups according to amount fat, then the high-fat group would have more than the other groups of e.g. the *highest values* of oleic acid (OA, 18:1 c9) and ALA (18:3 n3) being major "high-number" fatty acid (OA with range 1.04 - 8.56, and ALA 0.12 - 2.40 g/kg). A computer analysis confirmed this reasoning. For example, in the "high-fat group", OA range was 2.50 - 8.56 g/kg, against 1.04 - 2.07 g/kg in the "low-fat group". Corresponding ranges for ALA were 0.55 - 2.40, and 0.12 - 0.40 g/kg, respectively.

Thus, in a high-fat subgroup we should have more of e.g. OA and ALA than in the other groups. We apply our previous general equation $\%A + \%B + \%R = 100$, or $\%B = -\%A + (100 - \%R)$, where A and B represent eicosanoid precursor fatty acids, and R is sum of the remaining fatty acids when omitting A and B. With increasing %R, the expression (100 - %R) will move towards zero, thereby favoring a positive %A vs. %B association. Accordingly, in the high-fat group we should obtain increased %R values (since OA and ALA are main R- components); this should improve %A vs. %B association. In line with this reasoning, when dividing the current population into subgroup by amount body fat, we did find improved positive associations, as corroborated by

scatterplots and correlation coefficient (not shown). We previously reported that increased OA improved the positive association between %EPA and %AA [16].

Possible physiological interpretations of the results

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 [19] reported that there will be a spurious correlation between two indexes with the same denominator, even if the variables used to produce the indexes are 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 results show that significant correlations (positive and negative) between percentages of the same sum can indeed be obtained, but not always, and add that *range* of the variables is essential for the outcome. In our opinion, such correlations may serve as a novel regulatory mechanism in biology, rather than being “spurious correlations”.

Relation to fat intake?

We do not know whether the above correlation outcome, related to amount body fat, might have any physiological significance. One possible interpretation of the results could be that type of dietary fat might influence the associations between eicosanoid and docosanoid precursor percentages, but we do not have direct experimental data to verify this hypothesis.

Distribution Is the Crucial Point

Due to both stimulatory and inhibitory effects of various eicosanoids and docosanoids [2-4], we should expect a balance between their relative amounts. For example, from current knowledge of physiology we would anticipate a positive association between relative amounts of EPA and AA, due to the antagonistic actions of eicosanoids synthesized from these fatty acids [1-4]. It is well known that AA can promote inflammation and thrombosis, and thereby increase the risk of cardiovascular diseases [1-4]. The thromboembolic risk should be decreased by increasing the EPA concentration, thereby lowering percentage AA in platelet phospholipids and subsequently the production of TXA₂ and platelet aggregation. In keeping with this, it has been reported that platelet signaling responses are modified by EPA [20].

EPA, AA, DGLA, and DHA may serve as precursor fatty acids for eicosanoids and docosanoids [4]. Hypothetically, our finding that relative amounts of 20:3 n₃ and 20:2 n₆ as well were positively associated, an effect well explained by their particular concentration distributions, could possibly imply that also these fatty acids give eicosanoids that need to be balanced, but we have no data to substantiate this suggestion. In any instance, the present results suggest that the body might have developed regulatory mechanisms to ensure a proper balance between eicosanoids (docosanoids) having antagonistic metabolic effects. One way to achieve this task could be to regulate the relative amounts of precursor fatty acids for the synthesis of these regulatory molecules, as observed with 7 candidate fatty acids in the present work. Our results indicate that the crucial point in this biological regulation is regulation of the *concentration ranges* of the precursor molecules: i.e. a **Distribution Dependent Regulation**. This suggestion is further supported by our finding that *alterations* in distributions may strongly change associations between relative amounts of the precursor fatty acids, as illustrated in scatterplots and verified by changes in the correlation coefficients. It is tempting to speculate whether a disturbance of this regulation, making the positive association between e.g. %EPA

and % AA poor, could possibly increase the risk of AA related conditions and diseases, but we do not have data to corroborate this hypothesis.

Thus, our results lead to the intriguing question of whether evolution might have “chosen” particular concentration ranges for some types of fatty acids, to ensure that percent amounts of some of them must be negatively associated whereas the relative amounts of others are positively correlated. Furthermore, from the results of the present work it is tempting to speculate whether the mathematical rules governing the phenomenon that we have named *Distribution Dependent Correlations/-Regulation* might also have relevance in other contexts where associations between relative amounts of the same sum are studied, in biology, physics, chemistry, and in social sciences. Thus, if we know distributions (range, variability), then we may possibly predict whether or not relative amounts are positively or negatively associated, or non-existing.

Limitations of the Study

This work was confined to studying the association between relative amounts of fatty acids being precursors for eicosanoids and docosanoids. We do not know to what extent the suggested phenomenon of Distribution dependent correlations/-regulation is valid for other fatty acids as well. Furthermore, the analyses were based upon fatty acids found 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 relative amounts of other fatty acids. Comparable studies should be done in other animals and in humans as well.

Conclusion

The present analyses show that relative amounts of fatty acids being precursors of eicosanoids and docosanoids, are positively associated. The positive associations seem to be fully accounted for by the distribution per se of the fatty acid concentrations, suggesting a Distribution Dependent Regulation, possibly serving to balance metabolic effects of various eicosanoids and docosanoids. We speculate whether a disturbance in this type of regulation could increase the risk of e.g. AA associated conditions and diseases.

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