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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?

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Abstract

Introduction: 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 examined whether % EPA and % AA were correlated. However, percentages of the same sum could be correlated without involving biology. We therefore investigated whether random numbers, generated within the true concentration distributions for EPA and AA, may be associated.

Methods: We reanalysed data from a previous diet trial, involving 195 chickens. The concentration of fatty acids in breast muscle lipids was determined using gas chromatography. We made scatterplots of % EPA vs. % AA, and found two diet-related subgroups (A: n=32, and B: n =163). In each subgroup, we studied scatterplots, and linear regressions for the % EPA vs. % AA association. We next computed R = S – EPA – AA, where S is the sum of all fatty acids (g/kg) and R is concentration of all fatty acids, except EPA and AA. From histograms, we found the physiological distributions of EPA, AA and R in each subgroup. Then we generated random numbers for each of 3 variables with distributions like those found for the fatty acids; i.e. in subgroup A: for EPA (0.025-0.047), AA (0.54-0.75), and R (5-15), and in subgroup B: for EPA (0.125-0.225), AA (0.25-0.39), and R (5-15). Finally, we explored how a narrowing or broadening of the distributions might change the relationship between percentages of "EPA" and "AA".

Results: In each subgroup there was a positive correlation between percentages of EPA and AA (A: r=0.782, p<0.001; B: r=0.762, p<0.001). Regression lines (SE in parentheses) were for A: % AA=13.30 (1.94) *% EPA+2.07(0.79); for B: % AA=1.23 (0.08) *% EPA+1.01(0.18). Also with random numbers we found a positive association between their percentages (r= 0.769, p<0.001 and r=0.793, p<0.001 in-group A and B, respectively). Regression lines were; for A: %"AA"=11.71 (1.74)* %"EPA"+2.31 (0.69); for B: %"AA"=1.33 (0.08)*% "EPA"+0.88 (0.17). The lines involving random numbers did not differ significantly from corresponding ones with the real values for EPA and AA. The % EPA vs. % AA relationship changed appreciably in response to slightly altering distributions of the fatty acids.

Conclusion: Percentages of EPA and AA correlated positively, but also the random numbers had a positive relationship, which did not differ significantly from that found with the real values. The association between percentages of "EPA" and "AA" was sensitive to changes in their distributions. Thus, distribution per se governs the association between percentages of EPA and AA, i.e. a ***Distribution Dependent Regulation***, raising the question of whether this phenomenon is a novel regulatory mechanism.

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

Abbreviations: *EPA: Eicosapentaenoic acid (20:5 n3); "EPA": A random number variable with distribution like that of EPA; AA: Arachidonic acid (20:4 n6); "AA": A random number variable with distribution like that of AA; LA: linoleic acid (18:2 n6); ALA: Alpha linolenic acid (18:3 n3)*

Introduction

It is well known that eicosapentaenoic acid (EPA, 20:5 n3) and arachidonic acid (AA, 20:4 n6) are precursors of eicosanoids that have antagonistic actions [1-3]. Eicosanoids derived from EPA may decrease inflammatory diseases

[4,5], development of cardiovascular diseases [6], cancer [7], and possibly mental illnesses [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 arachidonic acid (AA, 20:4 n6). 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. prostacyclines, thromboxanes and leukotrienes [1,2]. AA derived thromboxane A_2 (TXA_2) and leukotriene B4 (LTB_4) have strong proinflammatory and prothrombotic properties [1,2,9]. Furthermore, endocannabinoids, which are derived from arachidonic acid, may have a role in adiposity and inflammation [10]. 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 [7]. It would appear, accordingly, that a coordinated regulation of the relative abundances of EPA and AA would be of physiological interest, so that an increase (decrease) in the percentage of one of these fatty acids would be accompanied by a concomitant balanced increase (decrease) in percentage of the other. These considerations raise the question of whether the relative abundances of EPA and AA are positively associated.

There is, however, a methodological concern when correlating percentages of for example EPA and AA, since the same sum appears in the denominator when calculating the percentages. Thus, the relative abundances of 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. In an attempt to shed some light on these questions, we have investigated the association between percentages of EPA and AA, and extended the study by replacing the observed concentrations for EPA and AA with random numbers, generated with the real distributions. By this approach, we anticipated to circumvent biological feedback mechanisms. Similarities and differences between results obtained with random and real numbers were visualized by scatterplots. As expected from the fact that percentages involve the same sum, preliminary analyses showed significant correlations also with random numbers. It then occurred to us that the relationship between relative abundances of EPA and AA might possibly be - at least partly - caused by the particular *distribution* of EPA, AA, and sum of the remaining fatty acids (R). If so, then a change in distributions should disturb the % EPA vs. %AA relationship. The main purpose of this work was accordingly to study the association between percentages of EPA and AA, and to explore whether *variations* in the distributions of EPA, AA, and R might influence the association between these percentages.

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 with; for a minor group A (low ALA/high LA group, n=32): about 4 % alpha linolenic acid (ALA, 18:3 n3) and 36% linoleic acid (LA, 18:2 n6) of the total fatty acid methyl esters (n6/n3 ratio=8.7).

Corresponding values of ALA and LA in the major group B (high ALA/low LA group, n=163) were 14 % ALA and 20 % LA (n6/n3 ratio=1.4). The percentage of 16:0, 18:0 and 18:1 were similar in both diet groups, being about 18%, 9% and 27%, respectively.

Calculations and Statistical Analysis

We first investigated the association between %EPA and %AA. Preliminary scatterplots showed that the 195 chickens were divided into two well-separated subgroups: a minor group (A, n=32) consisting of birds fed low ALA/high LA diet [11], and a major group (B, n=163) consisting of chickens fed high ALA/low LA diet. The main physiological distributions (g/kg/wet weight, found from histograms, Figure 3) were in group A: 0.025-0.047 for EPA, 0.54-0.75 for AA, and 5-15 for R. Corresponding distributions in group B were: 0.125-0.225 for EPA; 0.25-0.39 for AA; and 5-15 for R. Next we computed S, the sum (g/kg we weight) of all fatty acids, and R, the remaining sum when omitting EPA and AA. Thus, R=S - EPA-AA. For analyses with random numbers, we generated uniformly distributed random numbers with the main physiological distributions for EPA, AA, and R. Since the diet trial had 32 chickens in subgroup A, and 163 birds in group B, for each of the variables EPA, AA, and R we generated 32 (163) random numbers with the particular, group-specific distributions shown above. We computed percentages of the variables:

%"EPA"=("EPA"/"S")*100; %"AA"=("AA"/"S")*100; %"R"=("R"/"S")*100. Note that we use "EPA" , "AA", "R" and "S" with random numbers to keep in mind that the aim of our analyses was to mimic results with real values of this couple of fatty acids, but upper case letters was used (RANDOM) in the figure texts to clarify. Next, we made histograms to illustrate the distributions of percentage values of "EPA", "AA", and "R". Minimum and maximum values of the percentages were also controlled with manual calculation. Dependency between percentages is shown by the equation %"EPA"+%"AA"+%"R"=100. Using the random numbers, we made scatterplots for (RANDOM number) %"EPA" vs. %"AA". Finally, we studied how alterations in the distributions for EPA, AA, and R might change the relationship between %"EPA" and %"AA"; however, in these analyses we mainly generated 163 numbers, with distributions found in diet group B. Since there are infinite numbers of ways to change the distributions, we limit our analyses to narrowing or broadening of the physiological distributions, and generally to change distribution of only one of the variables each time. 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. 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. 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

Results obtained with observed (measured) values of EPA and AA (g/kg wet weight)

Considering the whole group we found a negative association between percentages of EPA and AA ($r=-0.443$, $p<0.001$, $n=195$). However, as shown in Figure 1 there were two distinct subgroups: one minor subgroup (A) consisting of the 32 chickens fed Low ALA/High LA (group to the left, see Methods), and a main subgroup B (n=163) fed High ALA/ Low LA (group to the right). Interestingly, percentages of EPA separated the subgroups completely. Thus, the apparent negative correlation between %EPA and %AA in the whole group was a spurious one. As illustrated in Figure 2,3, in each subgroup there was a positive relationship between percentages of EPA and AA; in the minor group A: $r=0.782$, $p<0.001$, $n=32$; in the major group B: $r=0.762$, $p<0.001$, $n=163$).

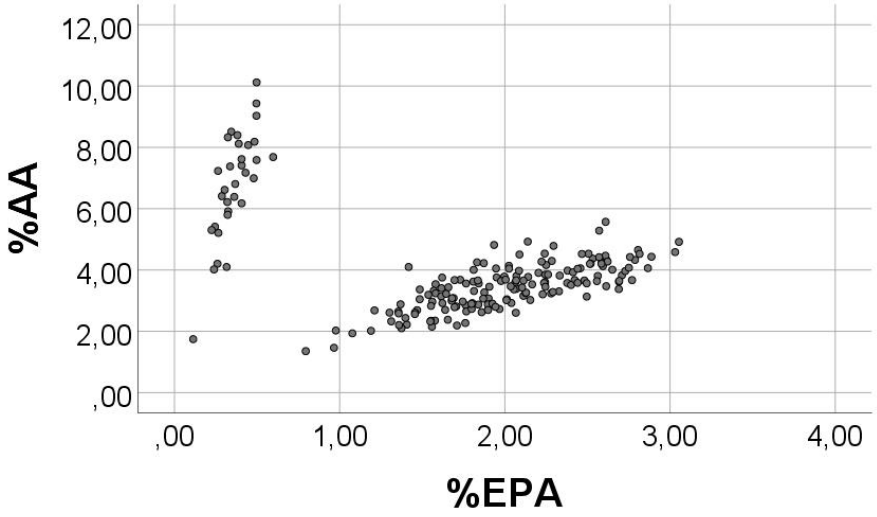

Figure 1: Scatterplot of the association between percentage values of EPA and AA in breast muscle lipids of 195 chickens divided into two diet groups

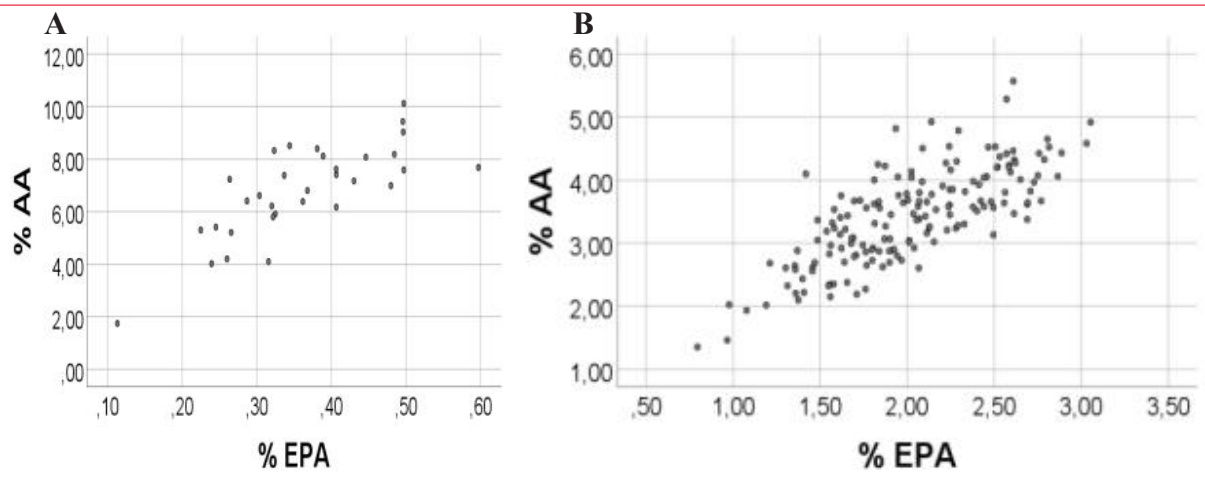

Figure 2: Association between %EPA and %AA in group A (left) and group B (right). A: r=0.782, p<0.001, n=32; B: r=0.762, p<0.001, n=163. Regression lines (SE in parentheses); A: %AA=13.3 (1.94)* %EPA+2.07(0.79); B: %AA=1.23 (0.08) *%EPA+1.01(0.18)

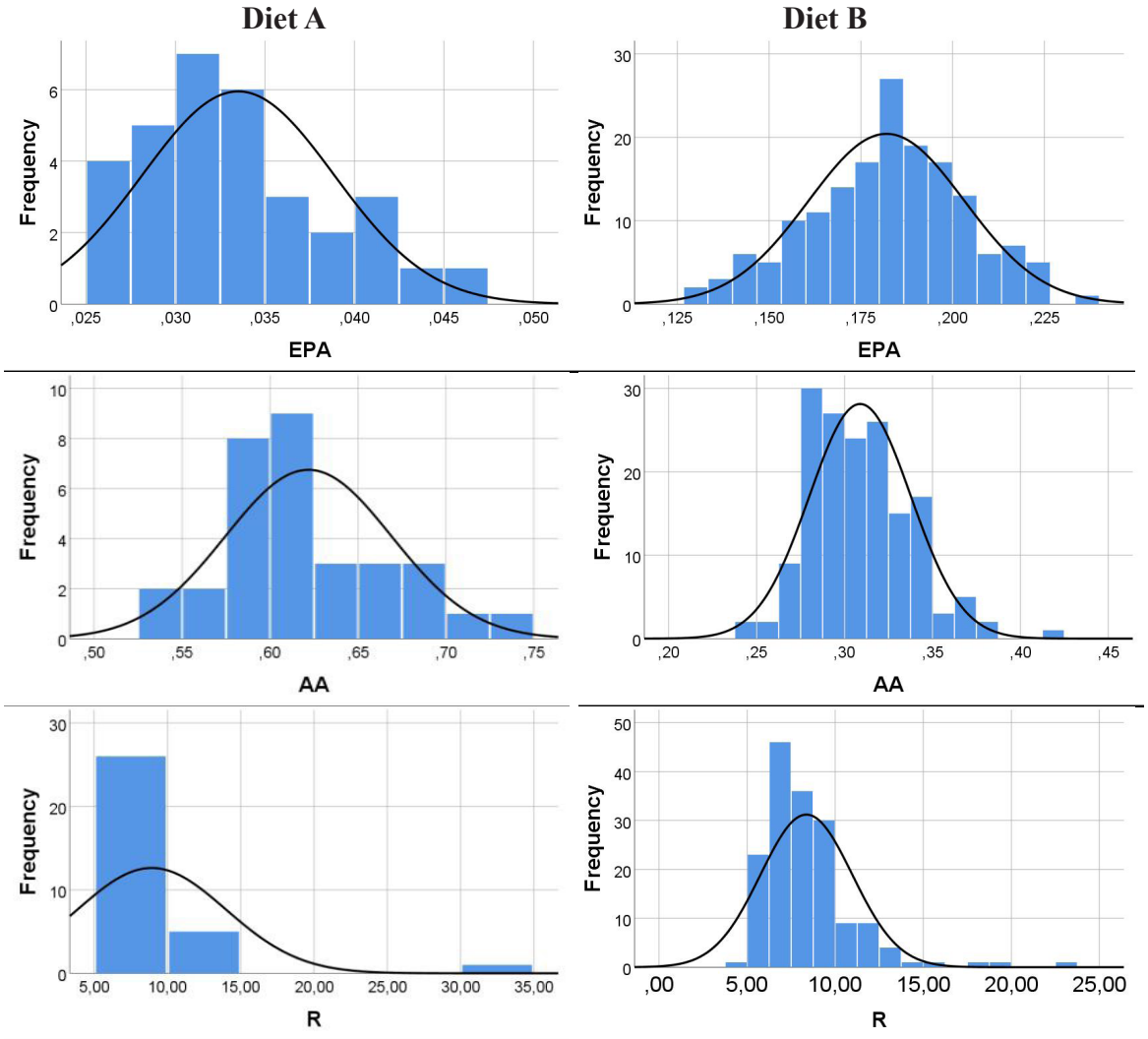

Figure 3: Concentration (g/kg wet weight) distribution of EPA (top panels) , AA (middle panels) , and sum of the remaining fatty acids (R, lower panels) in breast muscle lipids of chickens fed a low ALA/high LA diet (n=32, panels to the left) and a high ALA/low LA diet (n=163, panels to the right)

Relationship between percentages of RANDOM numbers, generated with the physiological (measured) concentration distributions of EPA, AA, and R (the remaining fatty acids)

As shown in Figure 4, the %"EPA" vs. %"AA" scatterplots (and regression lines) found with RANDOM numbers were similar to those obtained with the real values (Figure 2). Note that we use "EPA" and "AA" when presenting results with random numbers generated within the main distribution for EPA and AA, and use upper case RANDOM in the figure texts to clarify. Indeed, the lines obtained with real values and with random numbers did not differ

significantly. We did several repeats of these analyses with random numbers; the outcomes were qualitatively always the same, with minor variations in corresponding correlation coefficients, and regression lines.

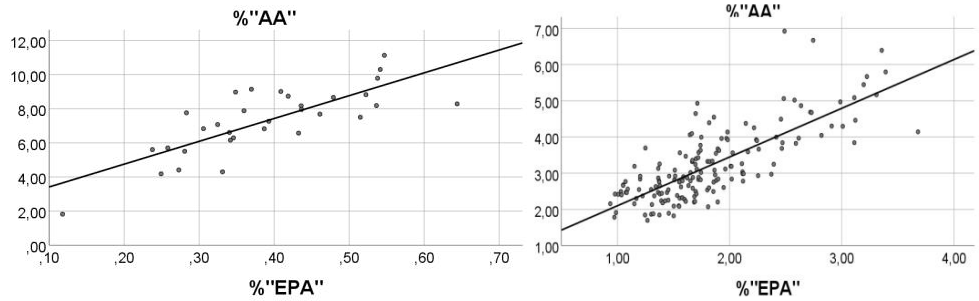

Figure 4: Scatterplot of the association between percentages of RANDOM numbers generated with the main distributions of EPA, AA, and R: Minor group (left): r=0.769, p<0.001, n=32; Major group (right), r=0.793, p<0.001, n=163. Linear regression lines (SE in parentheses); minor group: t= 6.6, p<0.001; equation: %"AA"=11.71 (1.74)* %"EPA"+2.31 (0.69); major group: t=15.8, p<0.001; equation: %"AA"=1.33 (0.08)*% "EPA"+0.88 (0.17). The slopes obtained with real values (Figure 2) and with random numbers did not differ significantly

How does the %"EPA" vs. %"AA" association respond to variations in the distribution of "EPA", "AA", and "R"?

Narrowing the Distribution of "EPA" or "AA": The %"EPA" vs. %"AA" relationship was sensitive to changes in the distribution of "EPA". A progressive narrowing of the "EPA" or "AA" distribution while keeping the physiological distributions of "AA" ("EPA") and "R" seemed to improve the scatterplots for the association, and the correlation coefficient. In Figure 5 we show the effect of narrowing "EPA" distribution to 0.16-0.18 instead of the physiologic al distribution of 0.125 to 0.225 (left panel), or narrowing the AA distribution to 0.30 -0.35 instead of 0.25-0.39 (right panel).

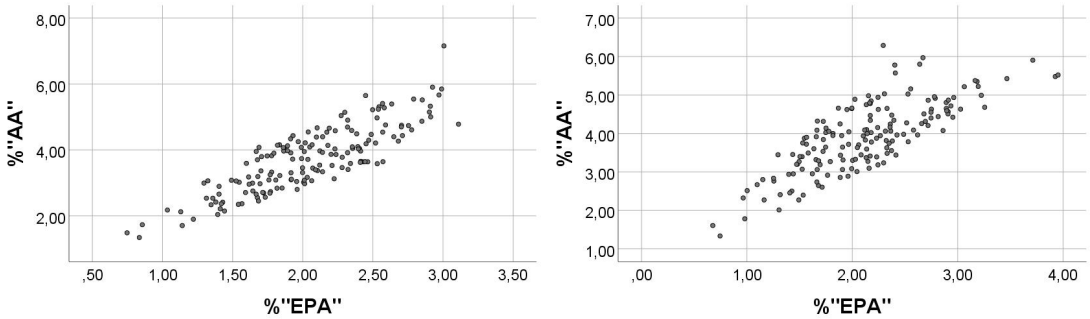

Figure 5: Effect of narrowing the "EPA" distribution (left panel) to 0.16 -0.18 (%"EPA" vs. %"AA": r=0.778, p<0.001; %"AA"=1.76(0.09) *%"EPA"+0.14(0.18); or narrowing the "AA" distribution (right panel) to 0.30-0.35 instead of 0.25-0.39; r=0.780, p<0.001; %"AA"=1.19 (0.08) *%"EPA"+1.38 (0.17)

In response to narrowing the distribution of "EPA" and "AA" simultaneously we obtained that the scatterplot improved further (not shown); r=0.984; %"AA"=1.90 (0.03)* %"EPA"+0.03(0.05), t=69.5, p<0.001.

Broadening the Distribution of "EPA" or "AA": When broadening the "EPA" distribution to 0.05-0.8 (instead of 0.13-0.22, and keeping "physiological" distributions of "AA" and "R", there was a much poorer scatter for the positive association between %"EPA" and %"AA (Figure 6); r=0.328 p=0.007. Regression line: %"AA"=0.13 (2.61)*%"EPA"+0.03 (0.13), t=4.4, p<0.001.

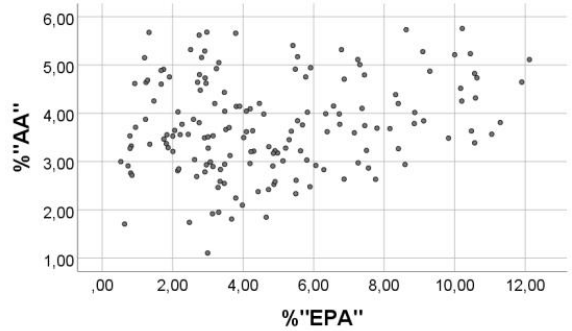

Figure 6: Effect of broadening the "EPA" distribution to 0.05 -0.8 (instead of 0.13 -0.22), and keeping "physiological" distributions of "AA" and "R"; r=0.328, p=0.007. Regression line: %"AA"=0.13 (2.61)*%"EPA"+0.03 (0.13), t=4.4, p<0.001

Also broadening of the "AA" distribution, to 0.05-1.5 (instead of 0.25-0.39), made the scatterplot poorer (not shown); r=0.412, p<0.001. The association was even poorer when broadening both the "EPA" and "AA" distributions simultaneously (not shown); r=0.160, p=0.041. %"AA"=0.16 (0.08) * %"EPA"+4.26 (0.47); t=2.1, p=0.041.

Narrowing or Broadening the Distribution of "R"

We finally studied how a change in the "R" distribution might alter the relationship between percentages of "EPA" and "AA". Narrowing the "R" distribution to 8 to 10 (from its "normal" distribution 5 to 15), while keeping physiological distributions of "EPA" and "AA", made the scatterplot become much poorer, giving a non-significant association, scatterplot not shown); r=-0.015, p=0.845. In contrast, broadening the "R"-distribution to 0.5-30.0 (instead of 5-15) greatly improved the scatterplot (not shown); r=0.937, p<0.001; %"AA"=1.94 (0.06) *%"EPA"-0.20 (0.17).

These analyses with 163 random numbers for each of three variables "EPA", "AA", and "R", show that the distribution *per se* determines whether relative abundances of "EPA" and "AA" will be significantly correlated , and to what extent the points will scatter close to the regression line. Thus, our results strongly suggest that there is a ***Distribution Dependent Regulation*** of the association between percentages of scale variables with a distribution like EPA, AA, and the remaining (R) amount of fatty acids. We did some additional analyses to mimic distributions of the 3 variables in the minor diet group A (n=32): also in this case similar patterns emerged with real and random numbers. Furthermore, changing distributions around the physiological ones caused appreciable alterations in the %"EPA" vs %"AA" relationship (results not shown).

A Practical Approach to Explain the Distribution Dependent Regulation: Our observations may be explained by probability mathematics, but it is beyond the scope of this work to provide a comprehensive mathematical explanation. Below is a practical approach to explain our findings. The present results strongly suggest that the positive relationship between percentages of scale variables with distributions like EPA and AA can change appreciably by slightly changing distributions. First we consider the equation "EPA"+"AA"+"R"="S", where "EPA" and "AA" represent random numbers, generated with physiological distributions for EPA and AA, and "R" is random numbers made in the distribution of the remaining fatty acids. Thus, the "S" distribution depends on distributions of "EPA", "AA", and "R". In our analyses, a new set of random numbers were generated for each of our 163 cases, and each of the variables. Thus, there are an infinite number of combinations of values for "EPA", "AA", and "R". There is, however, the important limitation that we used the physiological distributions, i.e. 0.13-0.24 for "EPA", 0.25-0.39 for "AA", and 5-15 for "R". Dependency between the percentages is shown by the equation %"EPA"+%"AA"+%"R"=100%.

To better understand how the extreme values for percentages are brought about, we did manual calculations to find the lowest and highest percentages for the observed distributions of EPA, AA, and R (Table 1).

Variable	Absolute distribution (g/kg)	Distribution of percentage values
EPA	0.13-0.23	Lowest: 100* 0.13/(0.13+0.39+15)=0.8 Highest: 100* 0.23/(0.23+0.25 +5)=4.2
AA	0.25-0.39	Lowest: 100* 0.25/(0.25+0.23+15)=1.6 Highest: 100* 0.39/(0.39+0.13+5)=7.1
R	5-15	Lowest: 100* 5/(5+0.23 +0.39)=88.9 Highest: 100*15/(15+0.13+0.25)=97.5

Table 1: Lowest and highest absolute values of EPA, AA, and R (g/kg wet weight), and lowest and highest percentage values

For example, the lowest percentage of EPA is obtained with the lowest value of EPA (0.13) combined with the highest values for AA (0.39) and R (15), giving the lowest EPA percentage (0.8 %, Table 1). The calculated minimum- and maximum percentages for all of the three variables are shown in Table 1.

We add percentage distributions to the equation:

%AA (1.6-7.1)=- %EPA (0.8-4.2)+100 - %R (88.9-97.5)=- %EPA (0.8-4.2)+Z,

if Z=100 -%R (88.9-97.5). The highest value of Z is 100-88.9=11.1% and the lowest Z-value is 100-97.5=2.5%. With the lowest value of %EPA (=0.8), the lowest value of the right side of the equation is -0.8+2.5=1.7%, and the highest value is –4.2+11.1=6.9%. These values correspond with the lowest and highest values found for the distribution of %AA. The fact that %AA (1.6)+%EPA (0.8)+%R (97.6)=100% shows that these 3 values (in parentheses) are corresponding ones, that is: the lowest value for %AA corresponds to the lowest value of %EPA. Similarly, the sum of corresponding values for %AA (7.1%), %EPA (4.2), and %R (88.9) is also 100%, indicating that we have a positive association between %EPA and %AA. The appearance of the scatter between the extreme values will depend on the

particular distributions of %EPA, %AA, and %R. It is, however hard from these considerations to appreciate how close the scatterplot is to the regression line, and regression analyses seem necessary.

Alternatively, we may compare the presented calculations with a cake consisting 2 small pieces, A and B, and one large (C) piece, so that A+B+C=100%, like %"EPA"+%"AA"+"R"=100%.

The size of each of the 3 pieces should vary, but only within certain ranges, determined by the distributions of a, b, and r, representing corresponding absolute values from which the A, B, and R percentages are calculated (Figure 7).

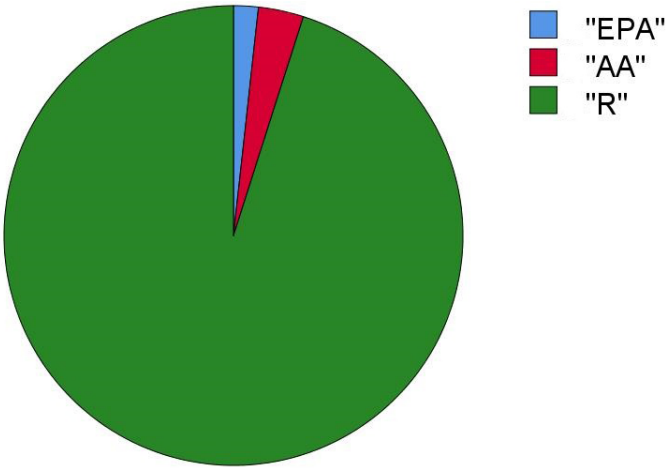

Figure 7: Pie chart to illustrate how percentages of "EPA", "AA" and "R" are related

If piece C progressively increases from the 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 A and B both decrease as C increases, 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 C, increase A and B, and cause a poorer scatter for the A vs. B association. Additionally, a narrowing of the distributions for a, b, or both will increase C, decrease A and 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 results which involve two small-amount fatty acids (EPA and AA), and one large third part (= sum of the remaining fatty acids, R).

Thus, we may get an idea of how %EPA and %AA will be related by considering the equation %AA=- %EPA+(100-%R), and knowing the distributions of percentage values of EPA, AA, and R. Additionally, we may intuitively conceive how the fatty acid distributions per se govern the association between percentages of fatty acids by making a pie chart of the fatty acid percentages.

Discussion

These results show that the relative abundances of EPA and AA in breast muscle lipids of chickens are positively associated. The finding seems reasonable since this couple of fatty acids has antagonistic metabolic actions, and we would expect biological regulatory mechanisms to ensure a positive correlation. Our calculations with random numbers demonstrate that a positive association between percentages of EPA and AA is completely explained by the particular ***distribution*** of the fatty acids, raising the question of whether there is a biological regulation of the concentration distribution of EPA and AA: a ***Distribution Dependent Regulation***, causing a positive association between their relative abundances.

Since the main purpose of this work was to explore how percentages of random numbers, sampled with the physiological distribution of AA and EPA are related, we will focus on the random number results below. However, we will first briefly comment on the observed differences between the diets subgroups concerning slopes of the % AA (ordinate) vs. % EPA (abscissa) regression lines: a steeper curve for subgroup A than for subgroup B. In this context we point out that chickens of group A were fed a low ALA/high LA diet, whereas group B birds were fed high ALA/low LA. Thus, in group A we would expect a high formation of AA from LA, but a low production of EPA from ALA as compared with group B. The slope differences are probably explained by this diet difference.

Comment on the Correlation between Percentages of Fatty Acids

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.

Distribution is the Crucial Point

From current knowledge of physiology, we would anticipate a positive association between the relative abundances of EPA and AA, due to the antagonistic actions of these fatty acids. The fact that we did observe a positive correlation raises the question of which type of regulatory mechanisms might be involved. The present analyses show that a positive relationship between relative abundances of "EPA" and "AA" can be obtained also with random numbers of variables with distributions similar to those of the real concentrations for EPA, AA, and amount of the remaining (R) fatty acids. Indeed, the regression lines for the % "EPA" vs. % "AA" relationship were very similar with random numbers and real values for the fatty acid concentrations, provided that the random numbers were sampled with the true distributions for EPA and AA. Initially this result seemed surprising, and made us wonder whether the positive correlation between the percentages was a type of spurious correlation between percentages of the same sum. However, the analyses with random numbers show that significant correlations between percentages of the same sum are not always obtained; the ***distribution*** of the variables is essential for the outcome. The finding that random number scatterplots of %"EPA" vs. %"AA" were quite similar to corresponding ones observed with the real values, as was verified by the equations for corresponding regression lines, strongly suggest that the positive association between relative abundances of EPA and AA is indeed caused by the particular ***distributions per se***. This conclusion is further supported by the fact that, with differing distributions of AA (EPA) in subgroup A and B, the outcomes with corresponding random numbers were very similar to the results with real values.

The histograms we made showed great variations in the *inter*-individual distributions of particular fatty acids. It seems reasonable to suggest that there are *intra*-individual variations as well, for example related to time, diet, and environment in general. Possibly, the magnitudes of the *inter*- and *intra*-individual variations may not be very different. Thus, the Distribution Dependent Regulation might be encountered both between and within subjects.

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. This situation was indeed encountered with EPA 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 mentioned previously, we may get some idea about the outcome by considering the distribution of percentages of the fatty acids, and the equation: %"AA"= - %"EPA"+ (100 – %"R"), and indicating distributions of the percentages in the equation. We were, however, surprised to see the strength of the ***Distribution Dependent Regulation***. That distribution is crucial for the outcome was demonstrated by the appreciable changes in the scatterplots, caused by even minor changes in distributions. In the present work, we show calculations and examples of scatterplots demonstrating the phenomenon that we name ***Distribution Dependent Regulation***. In a previous section of this article, we presented some practical approaches to understand our findings. Studies are currently in progress to provide a more comprehensive mathematical explanation.

Therefore, although the association between percentages of EPA and AA could have a non-biological explanation, our random number analyses may be interpreted otherwise. Thus, the ranges of the numbers that we used were not truly random ones since they were generated within the real concentration distributions of EPA, AA, and R (the remaining fatty acids). This restriction imposed upon our random numbers made us wonder whether the positive association found between % EPA and % AA could depend on the particular interval from which the random numbers were picked. We next reasoned that evolution might have chosen particular distributions of EPA and AA to ensure that fatty acids with antagonistic actions became positively associated, in order to ensure that their relative abundances will be balanced. We further hypothesized that, if distributions were essential, then we would expect that changes in the

distribution should disturb the relationship between % EPA and % AA, and so was indeed observed in our analyses. It would accordingly appear that % EPA and % AA must be positively associated as a consequence of their particular ***distributions***.

Our calculations with random numbers raise the *general* question of whether the concentration distribution per se of fatty acids does govern whether their relative amounts are positively or negatively associated, or not related at all. Furthermore, the results lead to the intriguing question of whether evolution might have "chosen" particular concentration ranges for each of the many types of fatty acids, to ensure that percentages of some of them must be negatively associated whereas others are positively correlated. From the present results, we are not able to conclude on this issue. However, if accepting that a negative (positive) relationship between percentages of fatty acids has any biological interest, it follows that also the Distribution Dependent Regulation should be of physiological interest. Further studies are required to elucidate whether this phenomenon should be considered as a correlation bias or as a novel, significant physiological regulatory mechanism. We are currently investigating whether correlations between percentages of other fatty acids may at least partly be explained by their distribution pattern; our preliminary analyses suggest that this may be the case.

It is tempting to speculate whether the mathematical rules governing the phenomenon of Distribution Dependent Regulation might also have relevance for other situations where association between relative abundances are studied, 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.

Do the Findings have *health implications?*

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 know have data to corroborate this hypothesis.

Our finding that distributions of EPA and AA were quite different in group A and B shows that diet can strongly regulate the tissue concentration of these fatty acids. However, in spite of this, in both diet groups we could demonstrate that the particular physiological concentration distributions of EPA and AA governed a positive association between their percentage amounts [14].

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.

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.

Limitations of the Study

Since this work was confined to studying the association between percentages EPA and AA, 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.

Conclusion

The present analyses show that percentages of EPA and AA are positively associated. This association seems to

be fully 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 EPA and AA. To our knowledge, this is the first report where a random number approach has been used to elucidate the relationship between relative abundances of EPA and AA. 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.

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