Studies to Explain Further, Why Percentages of Eicosanoid Precursor Fatty Acids Associate Positively in Chicken Muscle

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Abstract

Background: Polyunsaturated fatty acids with 20 or 22 carbon atoms are precursors of eicosanoids and docosanoids, which are important regulatory molecules in cell physiology. In breast muscle of chickens, we recently reported that percentages of these precursors were positively associated. Subsequently, we observed that the concentration *ranges* of the fatty acids seemed to cause the positive associations, e.g. between %EPA (eicosapentaenoic acid, 20:5: n3) and %AA (arachidonic acid, 20:4 n6).

Aim: To explain further correlations between relative amounts of eicosanoid and docosanoid precursors.

Methods: Typically, the precursors had low numbers and low variability, as compared with the predominant fatty acids, such as oleic acid (18:1 c9). We first present considerations concerning associations in general between relative amounts of three positive scale variables, two of which (A, B) having narrow ranges relative to the third one (C). Next, we show results of computer experiments to test the reasoning.

Results and Discussions: We made S = A + B + C, i.e. %A + %B + %C = 100. %A correlated positively with %B, whereas %A (%B) related negatively to %C. The particular *ranges* of A, B, and C seemed to explain these associations. We found that slope of %A (abscissa) vs. %B approached *B/A*. Furthermore, slope of %A (abscissa) vs. %C approached *–(1 +B/A),* and that of %B (abscissa) vs. %C was near *–(1+ A/B).* We also show equations of regression lines concerning associations between A (B, C) percentages of S, when ranges of A and B are narrow relative to C. Finally, we compare slope values obtained by the formulas, and by linear regression.

Conclusions: We suggest that *Intended Ranges* of eicosanoid precursor fatty acids might have arisen through evolutionary selection, thereby causing *Distribution Dependent Correlations*, mathematically. Possibly, this selection could improve the balance between eicosanoids (docosanoids).

Definitions and Abbreviations:

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

Distribution: graph showing the frequency distribution of a 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 "Distributions of A, B, and C were a - b, c - d, and e - f, respectively".

OA = Oleic Acid (18:1 c9); 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 = Docosa Hexaenoic Acid (22:6 n3); DGLA= Dihomo Gamma Linolenic Acid (20:3 n6); EDA = Eicosa Dienoic Acid (20:2 n6); ETA = Eicosa Trienoic acid (20:3 n3).

Keywords: *Fatty Acids; Eicosanoid Precursors; Relative Amounts; Correlation Rules; Ranges; Chicken Muscle*

Introduction

This work relates to body fatty acids, which are major diet constituents, and important factors in health and disease; polyunsaturated fatty acids with 20 or 22 carbon atoms are precursors of eicosanoids and docosanoids, which are significant regulatory molecules in cell physiology [1, 3]. AA, EPA, DPA, DHA, and DGLA are examples of such precursor fatty acids. Most organs and cell types produce eicosanoids, as catalyzed by cyclooxygenases, lipoxygenases, and epoxygenases [3].

EPA and AA represent two of the eicosanoid precursors, and these fatty acids seem to be metabolic antagonists [1 - 5]. EPA derived eicosanoids may decrease inflammatory diseases [6, 7], and may have a positive influence on coronary heart diseases [8, 9], and cancer [10]. However, a Cochrane Review of selected studies questioned the beneficial effects of long-chain n-3 fatty acids on all- cause and cardiovascular mortality [11].

We might expect positive effects of EPA if the fatty acid works to counteract effects of AA. LA, which is a major constituent in many plant oils, is the precursor of AA [1]. Under the catalysis of cyclooxygenase and lipoxygenase in tissues, AA may be transformed into various eicosanoids, i.e. prostacyclin, thromboxane, and leukotrienes [1, 4, 5]. Thromboxane A2 (TXA2) and leukotriene B4 (LTB4), synthesized from AA, have strong proinflammatory and prothrombotic properties [1, 2, 10], and may cause allergy [12]. Thus, an antagonism between EPA and AA could possibly explain positive health effects of EPA. In line with this reasoning, a decreased EPA/AA ratio in serum seems to be a risk factor for cancer death [10].

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 [3, 13]. Also, eicosatrienoic acid, i.e. 20:3 n6 (dihomo-gammalinolenic acid, DGLA) may give eicosanoids [3]. 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), and eicosadienoic acid (20:2 n6).

We should accordingly expect regulatory mechanisms ensuring a proper balance between the relative amounts of EPA and AA, and possibly between other eicosanoid (docosanoid) precursor fatty acids. One such mechanism could be that increased (decreased) relative amount of one of the precursor fatty acid would be accompanied by increased (decreased) percentages of many other precursors as well. In line with this reasoning, we previously reported strong positive correlations (Spearman's rho > 0.7) between relative amounts of many eicosanoid precursors, in breast muscle of chickens [14, 15].

% AA related however *negatively* to percentages of %OA and %ALA, which are predominant fatty acids in chicken muscle [16-19].

Typically, ranges of fatty acids that are precursors of eicosanoids (docosanoids) were narrow compared with ranges of the major-amount fatty acids, e.g. OA and ALA [20]. We recently suggested the concept of *Intended Ranges* pertaining to ranges suggested to arise through evolutionary selection [21]. Possibly, ranges of eicosanoid precursor fatty acids might be examples of such intended ranges.

When studying associations between relative amounts of eicosanoid (docosanoid) precursors, we previously reported great similarity when using true values of the precursor, and random numbers in lieu of the true values, however with the true ranges [19]. The outcomes were assessed using scatterplots and correlation coefficients. The aim of the present work was to explain in more detail associations between the precursor fatty acid percentages, using random numbers to replace the true values of the fatty acids. In particular, we raise the question of whether the

associations might approach *linearity*. If so, we aimed at finding equations of the regression lines, with particular focus on *slope values*.

Materials and Methods

Previously, we observed in muscle tissue of chickens that eicosanoid and docosanoid precursor fatty acids had *narrow ranges* (low variability) relative to ranges of the predominant fatty acids such as OA and ALA; the potential precursors were EPA, AA, DGLA, DPA, DHA, EDA, and ETA [20].

In computer experiments, we here used random numbers in lieu of the fatty acids, as reported previously [14-20]. To mimic the situation with eicosanoids, we made two of the variables (A and B) with narrow ranges, and a third (C) with broad range. Thus, C would represent sum of the remaining fatty acids, when omitting A and B. We generated uniformly distributed random numbers with true ranges. The outcome was, however, qualitatively the same if using random numbers with normal distribution (results not shown). Thus, A + B + C = S, where S is sum of the variables. Furthermore, each of the variables, and accordingly S, would have particular ranges, i.e. varying S – values for each of e.g. 200 "cases". We may express relative amounts of the variables as fractions, or percentages of S. The A-, B-, and C-*fractions* of S would be $Af = A/S$, $Bf = B/S$, and $Cf = C/S$. Hence, $Af + Bf + Cf = 1$, or e.g. $Bf = -Af + (1 - Cf)$. Alternatively, we may use percentages of S, i.e. $\%A = (A/S) \cdot 100$; $\%B = (B/S) \cdot 100$; and $\%C = (C/S) \cdot 100$. Thus, $\%A + \%B + \%C = 100$, or e.g. $\%B = -\%A + (100 - \%C)$. These formulas show dependency between the fractions (percentages).

Previously, we reported correlation coefficients (Spearman's rho), and scatterplots of e.g. %EPA (="%A") vs. %AA (= %"B"), as found in chicken muscle [14 - 22]. We also evaluated the physiological concentration distributions (g/kg wet weight) of eicosanoid (docosanoid) precursor fatty acids, using histograms. With surrogate random numbers of the precursors (named A and B), generated with their true ranges, we here carry out further computer experiments, as described in detail under Results and Discussions. We focus in particular upon how fractions (percentages) of S relate to the *range of C*. Thus, in the computer experiments, A and B represent two eicosanoid precursor fatty acids; C is sum of the remaining ones.

Using SPSS 28.0 for the analyses, and for making figures, we did several repeats of the analyses, with new sets of random numbers (n = 200 each time). The general outcome of the repeats was always the same, but corresponding correlation coefficients and scatterplots varied slightly. The significance level was set at p < 0.05. We present further details under Results and Discussions.

Results and Discussions

Associations between eicosanoid and docosanoid precursor fatty acids

We previously reported that relative amounts of EPA, AA, DGLA, DPA, DHA, EDA, and ETA correlated positively in chicken breast muscle [20]. Furthermore, we were able to reproduce the correlations, using random numbers in lieu of the true values, if the numbers had *true ranges* of the precursor fatty acids. Below, we try to clarify linearity and slope of associations between the precursor percentages.

Associations between A (B, C) ranges and A (B, C) fractions of S

If S = A + B + C, we previously [14 - 21] utilized the equation of a straight line $(y = ax + b)$, to explain correlations between %A, %B, and %C. Additionally, we considered the relationship between **S** and A (B, C) fractions of S. Below, we extend these analyses, focusing upon how **ranges** of the variables relate to the A (B, C) *fractions (percentages) of S*.

We define S to be the sum of three positive scale variables (A, B, C), making %A + %B + %C = 100. With ranges of the variables included (in

parentheses), the equation would be:$\%A(p-q) + \%B(r-s) + \%C(t-u) = 100$, or $\%B(r-s) = -\%A(p-q) + (100 - \%C(t-u)$

For simplicity in the presentation, below we omit indication of ranges, and mostly use A (B, C) *fractions* instead of percentages. Thus, *Af + Bf + Cf = 1*. The A-*fraction of S is Af = A/(A + B + C) = 1/[1 + (**B** + **C**)/**A**].* Similarly, the B fractions of S is *Bf = B/(A + B + C) = 1/[1 + (**A** + **C**)/**B**],* and the C-fraction is *Cf = C/(A + B + C) = 1/[1 + (**A** + **B**)/**C**].* We see that the three fractions of S have similar structures, and that *three ratios* govern the fractions, i.e. **R_1 = (B + C)/A; R_2 = (A + C)/B,** *and* **R_3 = (A + B)/C,** respectively**.** Thus, sum of two of the variables makes the numerators of the three ratios, and the remaining one is the denominator.

Furthermore, each of the fractions *Af = 1/[1 + (**B** + **C**)/**A**]; Bf = 1/[1 + (**A** + **C**)/**B**];* and *Cf = 1/[1 + (**A** + **B**)/**C**] should decrease* as the numerators of R_1 (R_2, R_3) increase, and *increase* as the denominators increase.

Since these fractions are functions of *three* variables, each of which with particular *ranges*, it could in general be hard to predict the combined influence of A, B, and C upon each of the fractions. However, in some situations we might predict how the fractions should respond as A (B, C) goes from lowest to highest value within the range. Below, we present one of these conditions, presumably relating to ranges of eicosanoid (docosanoid) precursor fatty acids (*vide infra*). We previously reported that ranges of these fatty acids were narrow relative to the large-amount fatty acids, e.g. OA and ALA, as observed in chicken muscle [19,20].

When reasoning about associations between ranges and A (B, C) fractions, a crucial point would be to clarify whether there is a *main variable*, and how each of the fractions should relate to this variable. As discussed below, we suggest that the main variable should have *broad range* relative to ranges of the other variables. *Furthermore, we would expect positive (negative) associations between fractions (percentages) of two of the three variables, if their relative amounts relate similarly (in opposite directions) to the main variable.*

Mimicking ranges of eicosanoids: Two of three variables (A, B) having narrow ranges relative to a third one (C)

We previously encountered variables in physiology with narrow ranges of some variables relative to others [14-26]. For example, in chicken breast muscle, the concentration of fatty acids that are precursors of eicosanoids (docosanoids) had narrow ranges as compared with other fatty acids [20]; the former ones had coefficients of variation about 10%, against 40 to 60% for other fatty acids.

Very narrow range of a variable would make this variable approaching a fixed number. Thus, with two of three variables approaching constants, we have *one main variable only*, implying that all fractions would depend mainly on this variable. To elucidate direction and strength of associations between fractions in this case, we start studying associations between fractions when two (A, B) of three variables are *constants*, and the third (C) has broad range. Below, we reason about linearity, slope, and equation concerning these associations.

LINEARITY and SLOPE of Af (abscissa) vs. Bf when ranges of A and B are very narrow relative to the C-range

To clarify the association between *Af and Bf* in this case, we consider *Af = 1/(1 + B/A + C/A),* and *Bf = 1/(1 + A/B + C/B).* Since A and B are close to constants, we may simplify to *Af = 1/(k + C/A),* and *Bf = 1/(t + C/B),* where *k = 1 + B/A,* and *t = 1 + A/B.* Accordingly, both fractions should decrease as C increases, implying that *Af should correlate positively with Bf.* Since Af and Bf depend mainly on C, we make C the abscissa variable when plotting C vs. Af (Bf).

We next raise the question of whether the Af vs. Bf association is *linear* in this particular case. If so, we should find a constant slope estimate, i.e. $\Delta Y/\Delta X$. When computing slope estimates of associations between e.g. Af

and Bf, it is crucial to know which one is the abscissa and the ordinate. *Below, we clarify by writing Af (abscissa) vs. Bf, Af (abscissa) vs. Cf, and Bf (abscissa) vs. Cf.*

To investigate linearity (slope) of the Af (abscissa) vs. Bf association, we raise the question of what happens to Bf (= Y), as Af (= X) increases, realizing that X as well as Y are mainly functions of C. For simplicity, we first consider A and B to be *constants*. If there is a linear relationship between X and Y, the equation of a straight line *(y = ax + b)* should apply. Thus, we should expect $\Delta Y/\Delta X = (Y_2 - Y_1)/(X_2 - X_1)$ to be constant. Thus, we find the C value (C_1) corresponding to X_1 and Y_1. $X_1 = A/(A + B + C_1)$; i.e. **$C_1 = (A - AX_1 - BX_1)/X_1$.** We next compute C_2 (corresponding to X_2 and Y_2) by adding ΔX to X_1. To simplify the presentation, below we use $\Delta X = 1$, however obtaining the same result if using just ΔX (not shown). Hence, $X_1 + 1 = A/(A + B + C_2)$, giving **$C_2 = (- B - AX_1 - BX_1)/(X_1 + 1)$.**

To find ΔY, we use C_1 and C_2, and compute Y_1 and Y_2. Thus, $Y_1 = B/(A+B+C_1)$; i.e.

$Y_1 = X_1 \cdot B/A.$ Similarly, $Y_2 = B/(A + B + C_2)$; i.e.**$Y_2 = (X_1 + 1) \cdot B/A.$** Accordingly, $\Delta Y = Y_2 - Y_1 = (X_1 + 1) \cdot B/A - X_1 \cdot B/A = B/A$, which is the change in Y per one-unit increase in X_1, i.e. $\Delta Y/\Delta X = (B/A)/1 = B/A$. Thus, *there should be a linear, positive association between Af (%A=abscissa) and Bf (%B), the slope being estimated by* **$\underline{\Delta Y/\Delta X = B/A.}$**

LINEARITY and SLOPE of Af (abscissa) vs. Cf, when ranges of A and B are very narrow relative to the C- range

If there is a linear relationship between Af (=X) and Cf (=Y), the equation of a straight line *(y = ax + b)* should apply to the relationship. Thus, we should find a constant slope value equal to $\Delta Y/\Delta X = (Y_2 - Y_1)/(X_2 - X_1)$. We accordingly need to find what happens to ΔY when increasing X_1 by ΔX, e.g. by one unit. Realizing that C governs both X and Y, we first find the C-values (C_1, C_2) that correspond to X_1 and $X_2 = X_1 + 1$. Below, we use A and B to denote the near to constant values of A and B.

Finding C_1 and C_2: By definition, $X_1 = A/(A + B + C_1)$, i.e. **$C_1 = (A - AX_1 - BX_1)/X_1$.** To find C_2, we add one X-unit to X_1. Thus, $(X_1 + 1) = X_1/(A + B + C_2)$, i.e. **$C_2 = (- B - AX_1 - BX_1)/(X_1 + 1)$.**

Computing Y_1 and Y_2: We use the above expressions of C_1 and C_2 to find the Y- values (Y_1 and Y_2) corresponding to X_1 and X_2. Thus, $Y_1 = C_1/(A + B + C_1)$;i.e. $Y_1 = [(A - AX_1 - BX_1)/X_1]/[A + B + (A - AX_1 - BX_1)/X_1]$. Simplifying this expression, we obtain

$Y_1 = (A - AX_1 - BX_1)/A.$

To find Y_2, we use C_2, i.e. $Y_2 = C_2/(A + B + C_2)$. Inserting the expression above, in lieu of C_2, we obtain: **$Y_2 = [(- B - AX_1 - BX_1)/(X_1 + 1)]/[A + B + (- B - AX_1 - BX_1)/(X_1 + 1)]$.** Simplifying this expression, we obtain **$Y_2 = (-B - AX_1 - BX_1)/A.$**

Accordingly, $\Delta Y = Y_2 - Y_1 = (-B - AX_1 - BX_1)/A - (A - AX_1 - BX_1)/A = (-B - A)/A$, i.e.

$\Delta Y = -(1 + B/A),$ which is the change in Y corresponding to a one-unit increase in X, i.e. **$\Delta Y/\Delta X = -(1 + B/A)/1 = -(1 + B/A).$** Thus, *Af (%A =(abscissa) should have a negative, linear relationship to CF (%C), with slope =* **$\underline{\Delta Y/\Delta X = -(1 + B/A).}$**

LINEARITY and SLOPE of Bf (abscissa) vs. Cf when ranges of A and B are very narrow

Using the approach above, we find that Bf (abscissa) should have a negative, linear association with Cf, the slope being estimated by **$\underline{\Delta Y/\Delta X = -(1 + A/B).}$**

Thus, if A, as well as B have very narrow ranges relative to C, then we have three slope estimates for the linear associations between relative

amounts of A (B, C). *1)* <u>*B/A*</u> *estimates slope of %A(abscissa) vs. %B; 2)* *– (1 + B/A) estimates slope of %A (abscissa) vs. %C; and 3)* *– (1 + A/B)* *estimates slope of %B (abscissa) vs. %C.*

We suggest that the slope estimates found above should apply also to conditions where the ranges of A and B are somewhat broadened. However, since we then would violate the requirement of having near constant values of A and B (relative to C), the scatterplots – and correlation coefficients - should be poorer, and the slope estimates should not work well, especially if increasing the A and/or B ranges appreciably (*vide infra*).

Suggested general rule, pertaining unit systems consisting of *three positive scale variables* (A, B, C, with S = A + B + C)*, where A and B have very narrow ranges relative to C:*

*%A (abscissa) should have a positive, linear association with %B, with slope approaching **B/A**. Furthermore, %A (abscissa) should have a negative, linear association with %C, with slope approaching – (1 + B/A), and %B (abscissa) should relate linearly and negatively to %C, with slope approaching – (1 + A/B).*

If using these formulas, we emphasize that *A and B* must be the two close-to-constant variables, and *C the broad-range variable*. The crucial point is to define the abscissa variable correctly.

Finding EQUATION of the positive, linear Af (abscissa) vs. Bf association when ranges of A and B are very narrow relative to the C-range

We next try to find the complete equation of the relationship between relative amounts of A and B, knowing that the formula of a straight line ($y = ax + b$) should apply. Above, we computed the slope value to approach **B/A**, if A and B have very narrow ranges relative to C. Thus, if $Y = Bf$ and $X = Af$, we have $Y = (B/A) \cdot X + b$. To find **b**, we use corresponding values of X and Y, e.g. their maximum or minimum values. Thus,

$b = Ymin – (B/A) \cdot Xmin,$ and $b = Ymax – (B/A) \cdot Xmax$. Accordingly, equation of the linear, positive X (Af) vs. Y (Bf) association should be:

$$\underline{Y = (B/A) \cdot X + Ymin – (B/A) \cdot Xmin} , or \underline{Y = (B/A) \cdot X + Ymax – (B/A) \cdot Xmax}$$

Example: A = 1, B = 2, C 1-10. We first use minimum and maximum values of X (= Af) and Y (= Bf). Thus, $Y = B/(A + B + C) = 1/[1 +(A + C)/B]$; $Ymin = 1/[1 +(A + Cmax)/B],$ i.e. $Ymin = 1/[1 +(1 + 10)/2] = 1/6.5 = 0.154 (15.4\%).$

Similarly, $X (=Af) = A/(A + B + C) = 1/[1 +(B + C)/A].$ Thus, $Xmin = 1/[1 +(2 + 10)/1] = 0.077 (7.7\%).$ Inserting the actual numbers into $Y = (B/A) \cdot X + Ymin – (B/A) \cdot Xmin,$ we obtain Y = (2/1) \cdot X + 0.154 – (2/1) \cdot 0.077 = 0, i.e. $Y = 2X.$ *Thus, Bf = 2Af, or* <u>*%B = 2·%A*</u>

We obtain the same result if using *maximum* values of X and Y to compute **b.** Thus,

$Ymax = 1/[1+ (A +Cmin)/B] = 1/[1 +(1+1)/2] = 0.5.$ Similarly, $X = Af = A/(A + B + C) = 1/[1 +(B + Cmin)/A].$ Xmax = 1/[1 + (2 + 1)/1] = 0.25. Using the equation above, i.e.

$Y = Af = (B/A) \cdot X + Ymax – (B/A) \cdot Xmax,$ we obtain Y = (2/1) \cdot X + 0.5 – 2·0.25= 2X. Thus, $Y = 2X, which$ may be written *Bf =2Af, or %B = 2·%A.*

Thus, in this particular case, **Af vs. Bf** shows a straight line with slope = 2. The extrapolated line should pass through (X, Y = 0, 0).

Finding EQUATION of the linear, negative <u>*Af*</u> *(abscissa) vs. Cf association*

As shown above, there should be a linear, negative association between Cf and Af (Bf). We define Cf = Y, and Af = X. Applying the equation of a straight line (y = ax + b), and the slope expression computed above, we have $Y = - (1 + B/A) \cdot X + b.$ We find **b** using corresponding values of X and Y, e.g. their maximum (minimum) values. Since there is an *inverse* relationship between X and Y, Ymax corresponds to Xmin,

Thus, $Ymax = -(1 + B/A) \cdot Xmin + b$, and $Ymin = -(1 + B/A) \cdot Xmax + b.$ We compute

b = $Ymax + (1 + B/A) \cdot Xmin.$ Inserting this b-value into $Y = - (1 + B/A) \cdot X + b,$ we obtain

$$\underline{Y = - (1 + B/A) \cdot X + Ymax + (1 + B/A) \cdot Xmin}$$

We next need to find Ymax and Xmin. By definition, $Cf (i.e. Y) = C/(A+ B + C) = 1/[1 + (A + B)/C].$ Using numbers of the example above, i.e. $A = 1; B = 2.0; C 1- 10,$ we obtain $Ymax = 1/[1 + (1 + 2)/10] = 1/1.3 = 0.77.$ Similarly, $Af (i.e. X) = A/(A + B + C) = 1/[1 + (B + C)/A].$ Thus, $Xmin = 1/[1 + (2 + 10)/1] = 0.077.$ The equation of X vs. Y in this case should be Y = -(1 +2/1) \cdot X + 0.77 + (1 + 2/1) \cdot 0.077 = 1, i.e. $Y = -3X + 1.$

Accordingly, the inverse, linear association between X (Af) and Y (Cf) should be $Y = -3X + 1.00$. Since Y = Cf, and X = Af , we may write $Cf = -3Af + 1.00 , or$ <u>*%C = -3·%A + 100.*</u>

Alternatively, we could use $Ymin = -(1 + B/A) \cdot Xmax + b, i.e. b = Ymin +(1 + B/A) \cdot Xmax$ Hence, the alternative, general formula would be

$$\underline{Y = - (1 + B/A) \cdot X + Ymin + (1 + B/A) \cdot Xmax}$$

Finding Ymin and Xmax: $Cf (i.e. Y) = C/(A+ B + C) = 1/[1 + (A + B)/C$. Thus, $Ymin = 1/[1 + (1 + 2)/1] = 0.25.$ $Af (i.e. X) = A/(A + B + C) = 1/[1 + (B + C)/A],$ i.e. $Xmax = 1/[1 + (2 + 1)/1] = 0.25.$ We insert the numbers into the general formula, i.e. $Y = - (1 + 2/1) \cdot X + 0.25 + (1 + 2/1) \cdot 0.25, i.e.$ $Y = -3X + 1.$ Thus, the equation of the negative Af vs. Cf association should be $Cf = -3 \cdot Af + 1.00 (or$ <u>*%C = -3·%A + 100).*</u>

Finding EQUATION of the linear, negative <u>*Bf*</u> *(abscissa) vs. Cf association*

The general equation is $Y = - (1 + A/B) \cdot X + b$, where $Y = Cf$ and $X = Bf$. We find **b** using corresponding values of X and Y, e.g. their maximum (minimum) values. Since there is an *inverse* relationship between X and Y, Ymax corresponds to Xmin.

Using the slope value found above, we have $Ymax = -(1 + A/B) \cdot Xmin + b$, and $Ymin = -(1 + A/B) \cdot Xmax + b.$ We next compute **b** = $Ymax + (1 + A/B) \cdot Xmin = Ymax + (1 + A/B) \cdot Xmin .$ Accordingly, the general formula would be

$$\underline{Y = - (1 + A/B) \cdot X + Ymax + (1 + A/B) \cdot Xmin}$$

We may alternatively use Xmax and Ymin, giving the equation

$$\underline{Y = - (1 + A/B) \cdot X + Ymin + (1 + A/B) \cdot Xmax}$$

With the example above, i.e. A = *1; B = 2.0; C 1- 10,* we find max (min) value of $Cf (=Y)$, and min (max) value of $Bf (=X)$. Since $Cf = C/(A + B + C) = 1/[1 + (A + B)/C],$ $Cf_{max} = 1/[1 + (1 + 2)/10] = 0.77,$ and $Cf_{min} = 1/[1 + (1 + 2)/1] = 0.25.$

Similarly, $Bf = B/(A + B + C) = 1/[1 + (A + C)/B];$ $Bf_{min} = 1/[1 + (1 + 10)/2] = 1/6.5 = 0.154,$ and $Bf_{max} = 1/[1 + (1 + 1)/2] = 0.5.$

Thus, $Cf = - (1 + A/B) \cdot Bf + Cf_{max} + (1 + A/B) \cdot Bf_{min} = -1.5 \cdot Bf + 0.77 + 1.5 \cdot 0.154,$ i.e.

$Cf = -1.5Bf + 1.00, or$ <u>*%C = -1.5·%B + 100.*</u> Alternatively, we may compute $Cf = -1.5 \cdot Bf + 0.25 + 1.5 \cdot 0.5,$ i.e. $Cf = -1.5Bf + 1.00.$

Computer Test: With *A = 1; B = 2.0; C 1- 10*, the A (B, C) - *fractions* of S are *Af = 1/(3 + C); Bf = 2/(3 +C);* and *Cf =C/(3 + C)= 1/(1 +3/C).* Thus, C is the only variable; Af and Bf should *decrease* as C runs from lowest to highest value, whereas Cf should *increase* (Fig. 1, upper panels). Accordingly, we should expect a strong *positive* association between %A and %B, whereas %C should relate *negatively* to %A and %B. The

outcome was as expected (Fig. 1, lower panels). There was a perfect positive %A vs %B association (rho = 1.000, p<0.01, n =200), with equation %B = 2·%B. %C correlated negatively with %A (%B), rho = -1.000 (-1.000), p<0.01 for both; n =200. Equations of the lines were *%C = -3·%A + 100, and %C = -1.5·%B + 100.* We previously explained the curvilinear associations, shown in the top panels [21].
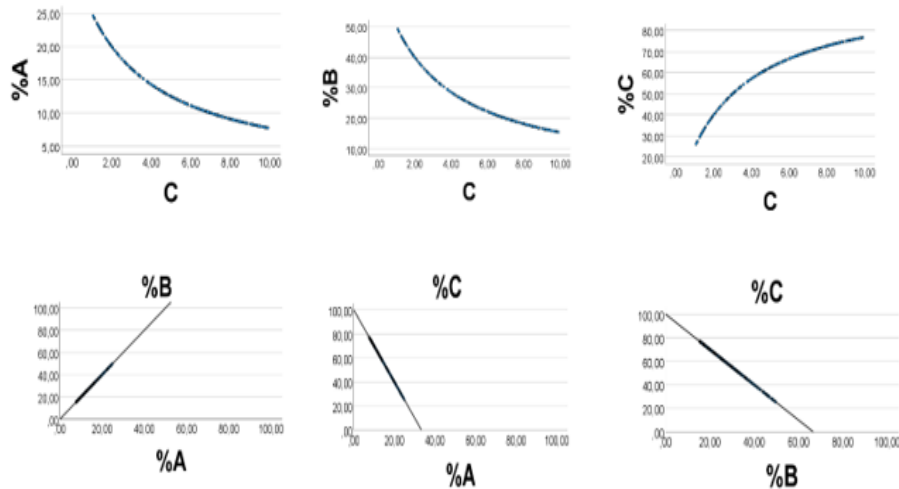
From the formulas above, slope of %A (abscissa) vs %B should be *B/A = 2/1 =* **2.0**. Slope of %A (abscissa) vs. %C should be *– (1 + B/A) = -(1+2/1) =* **-3**. Finally, slope of %B (abscissa) vs. %C should be *- (1+A/B) = - (1+1/2) =* **-1.5**. These slope values were equal to the corresponding ones made by the computer.

Making ranges of A, B, and C more similar

Above, we argued that associations between A (B, C) percentages of S should improve, if making the A (B, C) *ranges very different*. It follows that associations should be poorer if making the ranges more similar. This

outcome probably relates to poorer scatterplots of C vs. %A (%B, %C), if making A (B) deviate appreciably from constants, and narrowing the C- range.

Computer Test: To make A and B *deviate somewhat more* from the near-to-ideal example shown in Fig. 1, we broadened the ranges of A, B to be *A 1-2, B 3-5.* The scatterplots became poorer, as predicted (Fig. 2). C vs. %A (%B, %C): rho = -0.768 (-0.936, 0.976). %A correlated positively with %B, rho = 0.586; and %C related negatively to %A (%B), rho = -0.770 (-0.964), p<0.01 for all.
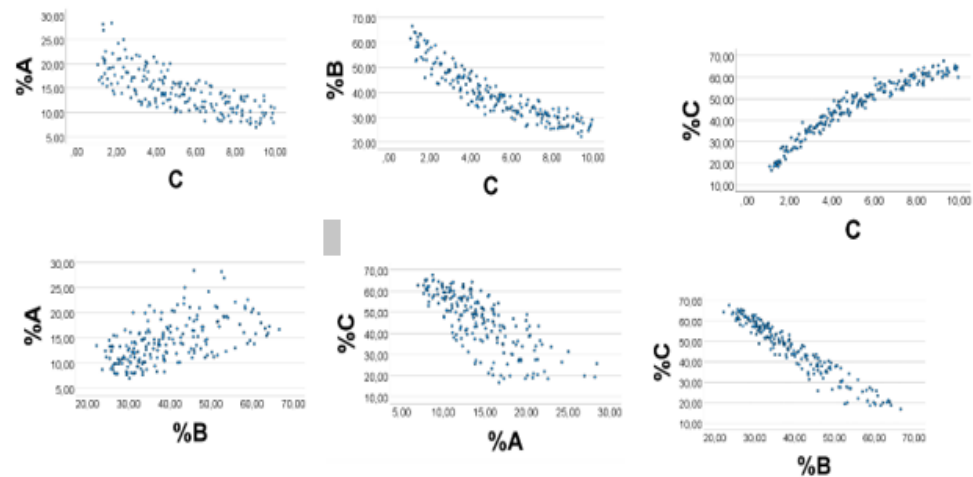
Equal ranges of A, B, and C

To substantiate further, that appreciable *differences in ranges* between A, B, and C govern the associations between their relative amounts, we now raise the question of how A (B, C) percentages of S should relate, if all of the three variables have **equal ranges**. Considering the formulas, we might in this case expect a negative association between *Af = 1/[1 + (B + C)/A]* and B (C), and a positive Af vs. A association. Furthermore, *Bf = 1/[1 + (A + C)/B]* should relate negatively to A and C, but positively to B. Finally, *Cf = 1/[1 + (A + B)/C]* should be negatively associated with A and B, but positively with C. Thus, when considering associations

between Af (Bf, Cf) and *one* of the three variables, the remaining two variables would disturb the associations. To obtain a positive association between e.g. Af (%A) and Bf (%B), both of them should preferably relate similarly to *all* of the A, B, and C variables. However, this requirement does not seem to exist, with *similar* ranges of the variables. To make a visual overview, we made scatterplots. The graphs show associations between fractions (percentages) and ascending values of A, B, and C (Fig.3). For example, for %A and %B, we see that these percentages respond in opposite directions as A and B increases, but similarly as C increases.
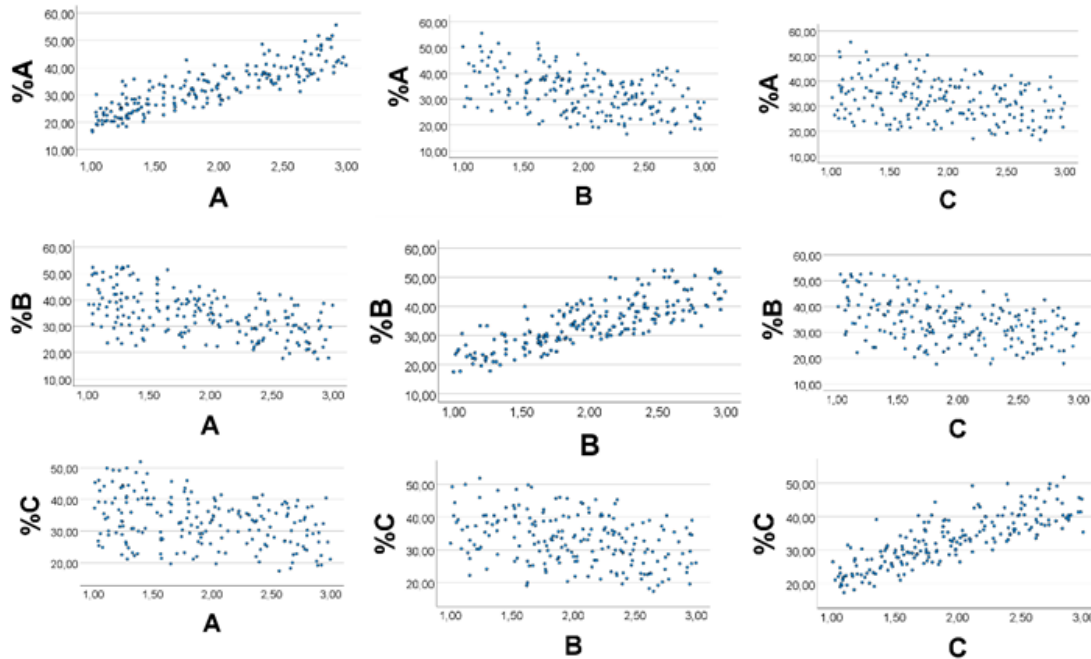

Figure 3: *Associations between A (B, C) variables and A (B, C) percentages of S. The figure refers to the equation A + B + C = S, see text. Upper panels: Scatterplots of %A vs. A (B, C). Middle panels: %B vs. A (B, C). Lower panels: %C vs. A (B, C). Range of A (B, C) was 1 - 3. Upper panel: %A vs. A (B, C); rho = 0.862 (-0.446, -0.283), p<0.01, n = 200 for all. Middle panel: %B vs. A (B, C); rho = -0.514 (0.814, -0.418), p<0.01, n = 200 for all. Lower panel: %C vs. A (B, C); rho = -0.314 (-0.362, 0.832), p<0.01, n = 200 for all.*

The scatterplots suggest that the *positive* associations were somewhat better than the negative ones, as supported by the correlation coefficients. In upper panels, for %A vs. A (B, C) we found rho **= 0.862** (-0.446, -0.283), p<0.01, n = 200 for all. In the middle panel, the correlation coefficients for %B vs. A (B, C) were -0.514 (**0.814**, -0.418), p<0.01, n = 200 for all. Finally, in lower panels, for %C vs. A (B, C), we found rho = -0.314 (-0.362, **0.832**), p<0.01, n = 200 for all.

Thus, Af and Bf respond in opposite directions as *two* of the variables (A, B) increase, but in the same direction as one of them (C) increases. We might, accordingly, at best expect a weak *negative* %A vs. %B association. The same type of reasoning goes for %A vs. %C, and for %B vs. %C, i.e. all associations between %A, %B, and %C should be weakly *negative,* in the current context. The computer test seemed to be in accordance with this reasoning (Fig. 4). %A vs. %B: rho = -0.568; %C vs. %A: rho = -0.421; %C vs. %B: rho = -0.458, p<0.01 for all, n = 200.
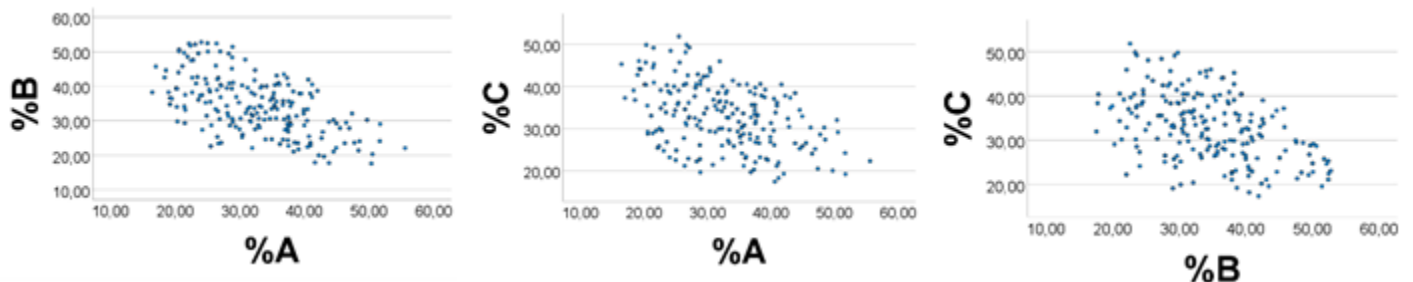

Figure 4: *Associations between A (B, C) percentages of S. The figure refers to the equation A + B + C = S, see text. . Range of A (B, C) was 1 - 3. %A vs. %B: rho = -0.568; %C vs. %A: rho = -0.421; %C vs. %A: rho = -0.458, p<0.01 for all, n = 200.*

We did additional computer experiments with many other ranges of A, B, and C, however always making A (B, C) ranges equal. The correlation outcomes turned out to be qualitatively similar to the corresponding ones presented in Fig. 4 (results not shown).

Further Experiments Concerning Effects on associations between A (B, C) percentages of S, if violating the requirement that A and B should be close to fixed numbers

In the above example, we used *constants* in lieu of variables for A and B. However, in physiology, we regularly encounter variables with particular *ranges*. Below, we present some examples to investigate equations of regression lines concerning associations between percentages of A, B, and C, if violating the above mathematical requirements that A and B should be very close to fixed numbers. We accordingly studied how increasing

the ranges towards higher values might influence associations between A (B, C) percentages of S. We mainly had our attention on slope values, since the aim of the present work was to elucidate associations between relative amounts of eicosanoids.

Increasing A and B by 10%, to make ranges A 1.0 - 1.1; B 2.0 - 2.2; C 1 - 10

With this broadening, the correlation pattern prevailed, and the points were still well scattered close to straight lines (Fig. 5). %A(abscissa) vs. %B: rho = 0.991; %A(abscissa) vs. %C: rho = -0.996; %B(abscissa) vs. %C: rho = -0.999; p<0.01 for all, n = 200. The computer - made equation of the linear relationships did not deviate much from those found by the formulas above. Equation of the regression lines were (SE in parentheses): *%B = 1.99 (0.01)·%A + 0.07 (0.02); %C = -2.99 (0.02)·%A + 99.9 (0.24); %C = -1.50 (0.004)·%B + 99.9 (0.12).*
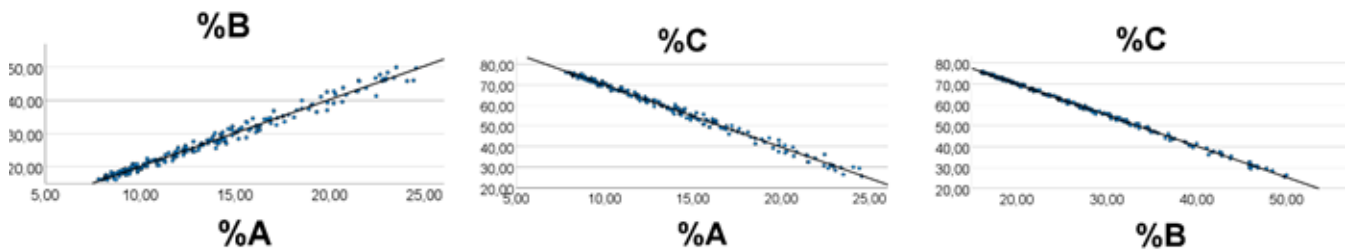

Figure 5: *Scatterplots of associations between A (B, C) percentages, with reference to the equation S = A + B + C, see text. Ranges were A 1.0-1.1; B = 2.0 – 2.2; C 1 – 10. %A vs. %B: rho = 0.991; %A vs. %C: rho = -0.996; %B vs. %C: rho = -0.999; p<0.01 for all, n = 200.*

Increasing A and B by 20%, to make ranges A 1.0 - 1.2; B 2.0 - 2.4; C 1 – 10

The correlation pattern prevailed, but the points were scattered at some distance from the regression lines (Fig. 6). %A vs. %B: rho = 0.972; %A

vs. %C: rho = -0.997; %B vs. %C: rho = -0.996; p<0.01 for all, n = 200. Equation of the regression lines were (SE in parentheses): *%B = 1.99 (0.04)·%A + 0.40 (0.54); %C = -2.99 (0.04)·%A + 99.6 (0.55); %C = -1.47 (0.01)·%B + 99.3 (0.26).*
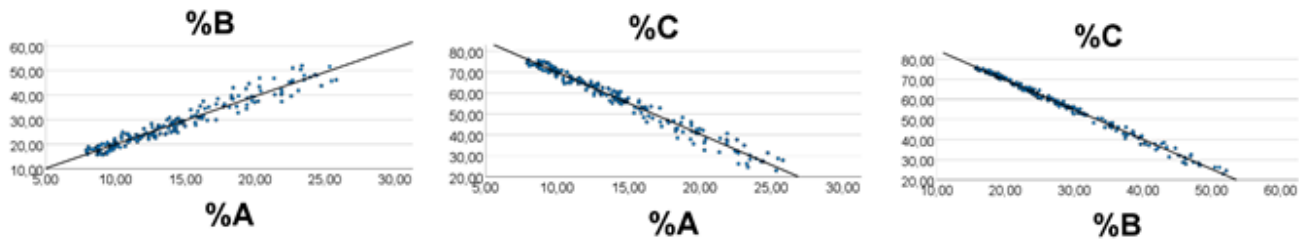

Figure 6: *Scatterplots of associations between A (B, C) percentage, with reference to the equation S = A + B + C, see text. Ranges were: A 1.0-1.2; B = 2.0 - 2.4; C 1 - 10. %A vs. %B: rho = 0.972; %A vs. %C: rho = -0.997; %B vs. %C: rho = -0.996; p<0.01 for all, n = 200.*

Increasing A and B by 50%, to make ranges A 1.0 - 1.5; B 2.0- 3.0; C 1 - 10

The correlation pattern prevailed also in this case, but the points were poorly scattered around the regression lines (Fig. 7). %A vs. %B: rho =

0.837; %A vs. %C: rho = -0.922; %B vs. %C: rho = -0.981; p<0.01 for all, n = 200. Equation of the regression lines were (SE in parentheses): *%B = 1.70 (0.08)·%A + 4.11(1.21); %C = -2.70 (0.08)·%A + 95.9 (1.21); %C = -1.42 (0.02)·%B + 97.4 (0.59).*
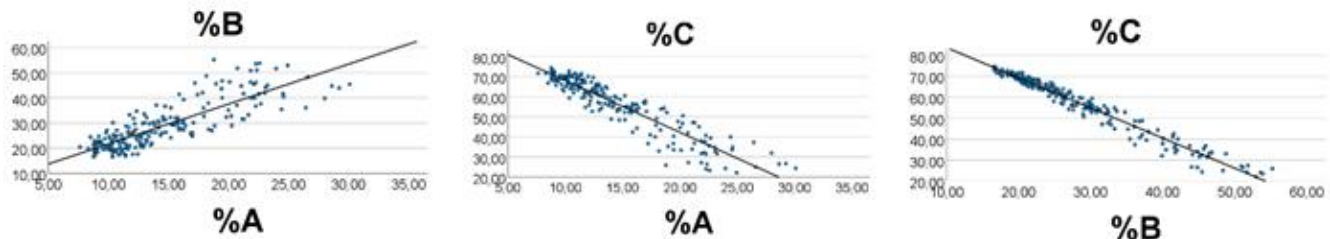

Figure 7: *Scatterplots of associations between A (B, C) percentages, with reference to the equation S = A + B + C, see text. Ranges were A 1.0 -1.5; B = 2.0 - 3.0; C 1 - 10. %A vs. %B: rho = 0.837; %A vs. %C: rho = -0.922; %B vs. %C: rho = -0.981; p<0.01 for all, n = 200.*

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Increasing A and B by 100%, to make ranges A 1.0 - 2.0; B 2.0 - 4.0; C 1 - 10

Even with this appreciable violation of the above requirement (i.e. A and B approaching constants), the *pattern* of the regression lines for associations between the A (B, C) percentages still prevailed, however with poor scatterplots (Fig. 8). %A vs. %B: rho = 0.611; %A vs. %C: rho = -0.823; %B vs. %C: rho = -0.933; p<0.01 for all, n = 200. Furthermore, computer-made formulas of regression lines deviated appreciably from those made manually using the above equations. Equation of the regression lines (made by the computer) were (SE in parentheses): *%B = 0.98 (0.11)·%A + 16.1(1.80); %C = -1.98 (0.11)·%A + 83.9 (1.80); %C = -1.13 (0.03)·%B + 93.8 (1.13).*
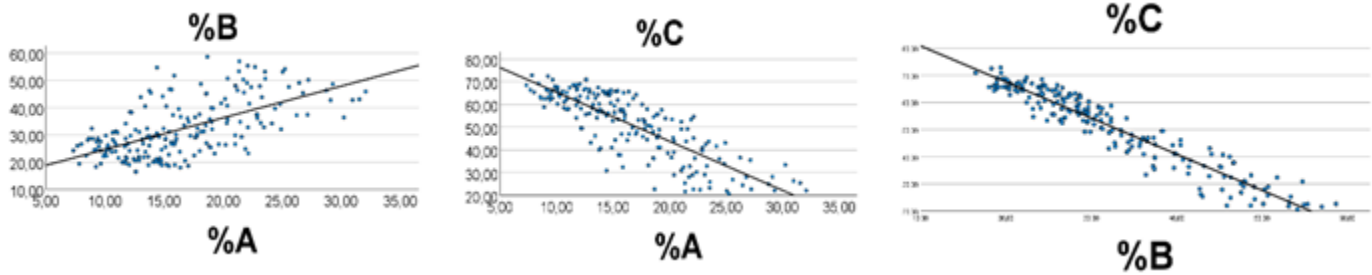

Figure 8: *Scatterplots of associations between A (B, C) percentages, with reference to the equation S = A + B + C, see text. Ranges were A 1.0 – 2.0; B = 2.0 - 4.0; C 1 - 10. %A vs. %B: rho = 0.611; %A vs. %C: rho = -0.823; %B vs. %C: rho = -0.933; p<0.01 for all, n = 200.*

It would appear, accordingly, that we may find the basic *patterns* concerning associations between A (B, C) percentages of S, in spite of strongly deviating from the demand of having A and B close to constants. It follows that the analyses presented in this article may explain why eicosanoid (docosanoid) precursor percentages correlate positively. The reason seems to be that their ranges are narrow as compared with ranges of the major-amount fatty acids, and accordingly also with total sum of the fatty acids, as we reported previously [18]. However, the equations presented above concerning regression lines between relative amounts of three variables do not apply, if strongly violating the above requirements.

Comparing slope estimate based upon 1) the B/A ratio; 2) linear regression with true values; and 3) linear regression using random numbers with true ranges

To clarify whether we might use the B/A ratio as a crude estimate of the slope of associations between eicosanoid precursor percentages, we compared values of the B/A ratio, with slope estimates obtained from regression lines made by the computer. We additionally compared slope values obtained by linear regression, and using either true (measured) values of the fatty acids, or random numbers having the true ranges. Ranges (g/kg, in parentheses) of the seven fatty acids under investigation were [20] EPA (0.13-0.24); AA (0.25-0.42); EDA (0.04-0.06); DPA (0.21-0.43); DGLA (0.06-0.11); DHA (0.11-0.32); and ETA (0.04-0.09). Explanation of abbreviations appear on Page 1. In Table 1, we present six of the altogether 21 pair combinations of these seven-eicosanoid precursor fatty acids (i.e. we show only 18 of the 63 slope estimates). In general, with all of the pairs, we found the pattern presented in Table 1. It turned out that the computer-made slope estimates based upon true values, and upon the substitute random numbers with true ranges, did not differ much (middle and right columns). However, the B/A ratio (Table 1, left column) seemed to give somewhat higher values.

Pair ("A" vs." B")	Slope, by B/A ratio	Slope, by true values	Slope, by random numbers
%EPA vs. %AA	1.8	1.2 (0.08)	1.4 (0.09)
% EDA vs %AA	6,2	5.6 (0.36)	5.3 (0.28)
%DPA vs %AA	1.0	0.8 (0.03)	0.7 (0.05)
%DGLA vs. %AA	3.9	3.2 (0.20)	2.8 (0.1|6)
%DHA vs %AA	1.6	0.9 (0.06)	0.8 (0.08)
%ETA vs. %AA	5.2	3.7 (0.36)	4.1(0.30)

Table 1: *Three slope estimates of the linear association between %AA and other eicosanoid (docosanoid) precursor percentages. The table refers to the general equation A + B + C = S, i.e. %A + %B + %C = 100, see text. Here, A and B represent eicosanoid (docosanoid) precursor fatty acids. C is sum of the remaining fatty acids, when omitting A and B. Left column: B/A ratio, computed as mean of B divided by mean of A (n = 200). Middle column: slope from equation of regression line, using true values of the precursor fatty acids [19]. Right column: slope from equation of regression line, using random numbers with true ranges of the precursors [19]. EPA = eicosapentaenoic acid (20:5 n3); AA = arachidonic acid (20:4 n6); EDA = eicosadienoic acid (20:2 n6); DPA = docosapentaenoic acid (22:5 n3); DGLA= dihomo-gamma-linolenic acid (20:3 n6); ETA = eicosatrienoic acid (20:3 n3).*

The denominator

Above, we showed that if A + B + C = S, then the slope of %A (abscissa) vs. %B would approach B/A, *if ranges of A and B were very narrow.* Thus, C did not influence the slope estimate in this particular condition. Additionally, we showed that the B/A ratio seemed to apply also when slightly broadening the A (B) ranges. Furthermore, we previously reported similar slope values for associations between eicosanoid precursor percentages, if using *true* values of the fatty acids, or substitute *random numbers*, however with true ranges. Below, we carry out a preliminary experiment to see if the B/A ratio may estimate slope of the association between percentages of such random number substitute values, if varying the C- range.

To represent two "random number fatty acids", we arbitrarily chose A = "EPA" (range 0.13 - 0.24), and B = "AA" (range 0.25 - 0.42), and examined whether a change in the range of C = S - A - B might influence the slope estimate, found by linear regression. Thus, we make *C sum of the remaining fatty acids when omitting "EPA" and "AA".* Mean "EPA" was computed as (0.13 + 0.24)/2 = 0.185, and mean "AA" = (0.25 +

0.42)/2 = 0.335. Hence, slope of %"EPA" (abscissa) vs. %"AA", calculated manually would be *B/A = 0.34/0.19 = 1.79*. The true range of C is near 3-15 [20]. By linear regression, and using range *3 - 15* for C, we found slope (SE in parenthesis) of %A (abscissa) vs. %B (i.e. %"EPA" vs. %"AA") to be *1.52 (0.06)*. We obtained similar slope values using *C 3 - 10*, and *C 3 - 20* , i.e. *slope values being 1.56 (0.08), and 1.57 (0.06)*, respectively. It would appear, accordingly, that the B/A ratio is useful to predict that there should be a *positive* association between %EPA and %AA. C is the major variable of the denominator when computing fractions (percentages) of S. However, this experiment suggests that the range of C, in a physiological context, probably does not have a major influence on slope *direction* of the %A vs. %B association. This outcome seems reasonable since the A and B fractions (percentages) of S should decrease as C runs from lowest to highest value, and would be largely unaffected by the C range. However, appreciable *narrowing* the C-range should disturb the %A vs. %B association (see below). The B/A ratio seemed in general to give slightly higher slope values than those obtained from equations of regression lines. This point probably is of minor importance in the current context, since we here focus mainly upon *direction* and strength of associations between relative amounts of eicosanoid precursor fatty acids.

Altering the range of just one of the A (B, C) variables

Above, we studied correlations between %A, %B, and %C when A = 1, B = 2, C 1 - 10, i.e. C was the only variable. We additionally investigated the influence of giving A, as well as B increased ranges, simultaneously. We now examine effects of altering *the range of either A, B, or C* on correlations between their relative amounts.

Giving range to either A or B

It turned out that also this violation of the above requirements weakened the associations between the A (B, C) percentages of S, as observed in scatterplots, correlation coefficients, and slope values (not shown). The explanation is that with *two main* variables (C and A, or C and B), the requirement above would not be fulfilled.

Increasing the C - range only, *while keeping A and B as fixed numbers*

We finally studied whether changes in the *C - range only* might influence scatterplots and correlations between A (B, C) percentages. However, in that case, all of the fractions, i.e. $Af = 1/[1 + (B + C)/A]$, $Bf = 1/[1 + (A + C)/B]$, and $Cf = 1/[1 + (A + B)/C]$ would still be dependent upon *one* variable only (C). We should not expect, accordingly, any change in the *slope* values.

Notably, when computing slope values using the formulas above, the C-variable disappeared from the original equations, implying that the *slope estimates* do not depend on this variable, under the current conditions. However, when computing percentages of S = A + B + C, all of the variables would make their contributions, when evaluating how associations between scatterplots should appear. We may illustrate the influence of the C-*range* upon the %A vs. %B (%C) scatterplots, or that of %B vs. %C, by comparing the outcomes when the C-range is very broad, against the situation with a very narrow range of C. As suggested above, we should expect the slope values to be equal in both cases, since C is *the only variable* when A, and B are approaching constants. Furthermore, when making scatterplots of associations between A (B, C) percentages, we should find all points exactly on lines, and with equal corresponding slope values, irrespective of the C- ranges. To test this reasoning, we compared scatterplots found with extremely different variabilities of C, i.e. *C 1 - 100,* and *C 1.00 - 1.01,* emphasizing that we did this this experiment just to illustrate a mathematical point, without any relationship to physiology.

The test showed (Fig. 9) that corresponding slope values were the same, irrespective of making C with very broad range (Fig. 9, upper panels) or very narrow range (Fig.9 , lower panels). For both of these widely differing C - ranges, we found equal corresponding slope values (Fig. upper panels compared with lower panels). Furthermore, slope values made by the computer were equal to those made by the formulas, i.e. *B/A =2.00 for %A (abscissa) vs. %B; - (1 + B/A) = -3.00 for %A (abscissa) vs. %C; and - (1 + A/B) = -1.50 for %B (abscissa) vs. %C. For all* associations shown in Fig. 9, we found rho = ± 1.000, p<0.01, n = 200. Additionally, we did not find any change in equations of the linear associations shown in Fig. 9 (not shown).
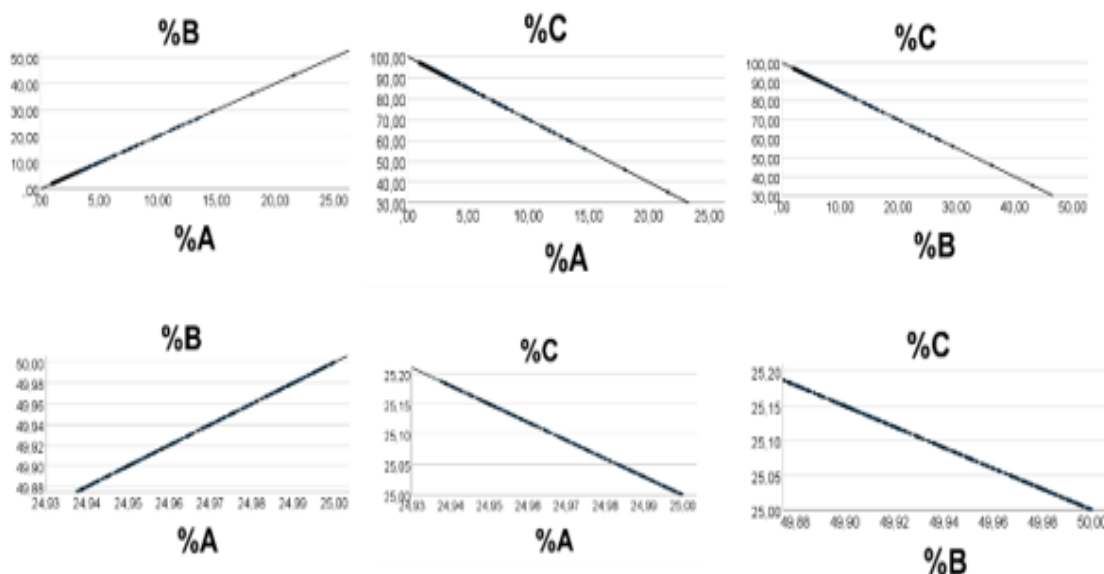

Figure 9: Scatterplots of associations between A (B, C) percentages, with reference to the equation S = A + B + C, see text. Upper panels: A = 1; B=2; C 1 - 100. Lower panels: A = 1; B=2; C 1 - 1.01. For all associations, rho = + 1.000, p<0.01, n = 200. In upper and lower panels; slope of %A vs. %B = 2; %A vs. %C: - 3; %B vs %C - 1.5. *Note scale differences in upper and lower panels.*

When inspecting closely the scatterplots in Fig. 9, we see that there is a difference between the upper and lower panels. In the upper panels, the points do not have an equal distribution on the lines; i.e. for %A vs. %B, the points assemble towards the lowest values. Furthermore, for %A vs. %C (%B vs. %C) the point density is highest at low values of %A (%B), corresponding to high %C values. In contrast to this picture, in the lower panels, there seems to be an equal point distribution on the regression lines.
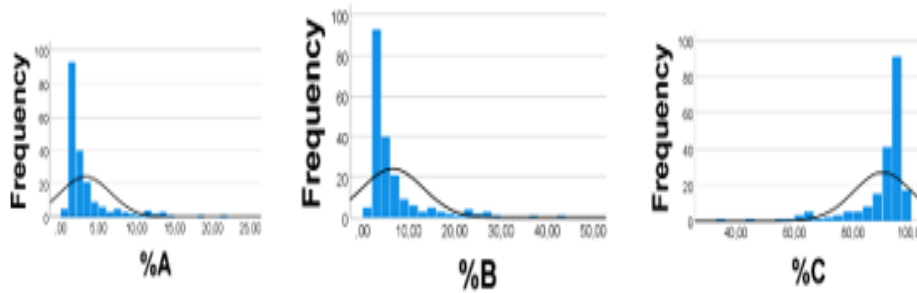

Figure 10: *Histograms of %A, %B, and %C, with reference to the equation S = A + B + C, see text. A = 1; B=2; C 1 - 100. Skewness 2.63 for %A and %B; -2.63 for %C.*

With broad C-range (1 - 100), the difference in frequency distribution of the points ("cases") is caused by high positive skewness of %A and %B (skewness 2.63 for both), and high negative skewness (-2.63) of the %C distribution (Fig. 10), SE of skewness was 0.17 for all.

When narrowing the C-range, the %A, %B, and %C histograms approached normal distributions, i.e. skewness of the %A, %B, and %C distributions were *-0.18, -0.18, and 0.18* (SE 0.17 for all), respectively (histograms not shown). We previously discussed how ranges relate to skewness [22].

In brief, the slope estimates found above seem to apply also if ranges of A and B are somewhat broadened. However, an extensive broadening of the A (B) ranges would strongly violate the requirement of having near - to - constant values of these variables. Accordingly, the scatterplots - and correlation coefficients - should then be poor, and the suggested slope estimates would not work properly. However, the association *pattern* prevailed in spite of making major increases in the ranges of A and B.

With the purpose of the present work, these analyses seem to support the view that *relative amounts of eicosanoid (docosanoid) precursor fatty acids become positively associated in chicken muscle, because of their narrow ranges as compared with range of the predominant fatty acids, and accordingly with range of the sum of all fatty acids.*

Thus, if all of the three variables are close to be fixed numbers, then *closeness to be fixed numbers* appears to govern the correlation outcome, i.e. relative amounts of the "closest – to – constant variables" should relate positively.

From this general reasoning, we might suggest this rule: *With three positive scale variables (A, B, C), where %A + %B + %C = 100, and **ranges** of A and B approach fixed numbers, we should expect %A to correlate positively with %B, and %A (%B) should relate negatively to %C.*

Computer Test #1: A and B with very narrow ranges relative to C, i.e. A 1.0 -1.1, B 3.0 - 3.3, and C 1 - 100. Here, C would be the main variable, and relate negatively to %A (%B), but positively to %C. Accordingly, we should expect a positive %A vs. %B association, and negative %A %B) vs %C associations. The outcome was as expected (Fig 11).
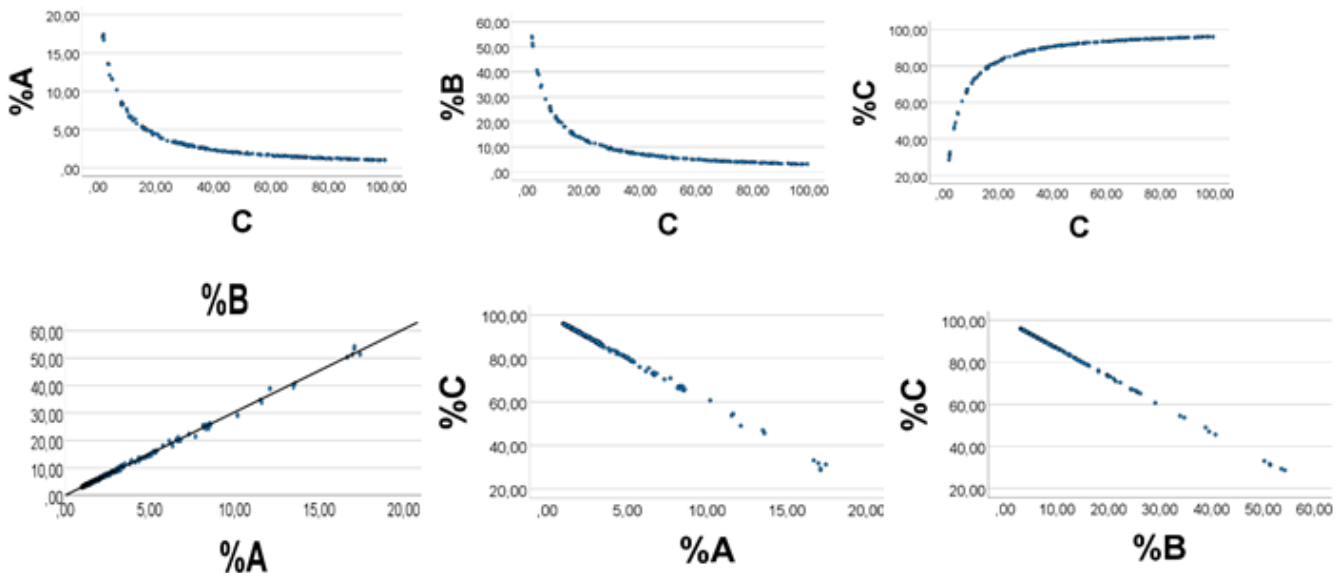

Figure 11: *Relationship between C and A (B, C) percentages of S (upper panels), and between %A , %B, and %C (lower panels). The figure relates to the equation %A + %B + %C =100, see text. We generated uniformly distributed random numbers (n =200) with ranges A 1.0 -1.1, B 3.0 - 3.3, and C 1 - 100. C vs. %A (%B, %C), rho = -0.998, -0.999, 0.999); %A vs. %B rho =0.997; %C vs. %A (%B), rho = -0.998(-1.000), p<0.01 for all.*

Due to the large difference in ranges between A (B) and C, we should expect large skewness of the A (B, C) percentages of S. As shown in Fig. 11, skewness of %A, %B, and %C were 2.64, 2.69, and -2.68, respectively

(SE 0.17 for all). Thus, %A and %B had strong positive skewness, and %C had strong negative skewness (Fig. 12). We previously explained how this skewness arises [22].
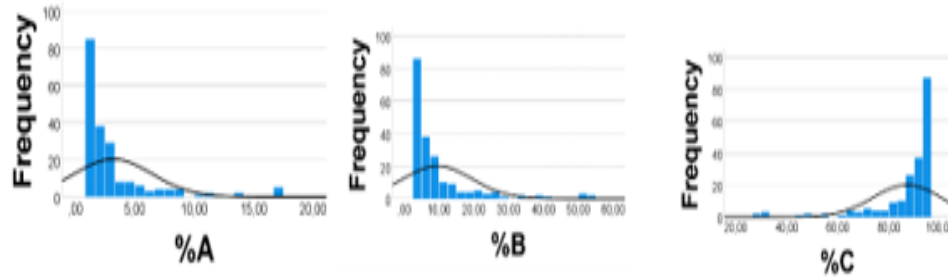

Figure 12: *Histogram of %A, %B and %C. The figure relates to the equation %A + %B + %C =100, see text. Skewness of %A, %B, and %C were 2.64, 2.69, and -2.68, respectively (SE 0.17 for all).*

To explain associations between A (B, C) percentages of S, we previously [14, 15, 20, 21] utilized the equation %A + %B + %C = 100, i.e. %B = -%A + (100 - %C). With very high %C values, as e.g. obtained when broadening the C - range towards higher values, this equation would approach %B = %A, suggesting a *positive* %A vs. %B association. The %A vs. %B association should be *positive*, since (100 - %C) > %A. This latter requirement follows the fact that the remaining value when calculating (100 - %C) would have to be divided between %A and %B. Since %A + %B = 100 - %C, (100 - %C) > %A and (100 - %C) > %B.

Rewriting the equation to %C = -%A + (100 - %B), we see that this equation approaches %C = -%A + 100, due to small %B values, i.e. %C should relate *negatively* to %A. Similarly, with low %A - values we may also do the approximation %C = -%B + 100, showing a negative %C vs. %B association as well. Quartiles of the distributions of %A, %B, and %C were *1.3, 2.0, 3.3; 4.0, 5.9, 10.1; 86.7, 92.1, 94.7,* respectively, i.e.

%C had high values relative to %A and %B, in support of the reasoning above.

To investigate whether the above reasoning applies for *widely differing conditions*, we carried out two additional experiments.

Computer Test #2: A 0.10 - 0.12; B 0.20 - 0.22; C 1- 10. The correlation outcome was as expected, i.e. C vs. %A (%B, %C): rho = -0.991 (-0.997, 0.997), p<0.01 for all, n =200. %A vs %B, rho = 0.989; %C vs. %A (%B), rho = -0.995 (-0.998), p<0.01, n =200. (Scatterplots not shown).

Computer Test #3. A 20.0 - 20.2; B 30.0 - 30.3; C 1-10. The correlation outcome was C vs. %A (%B, %C), shown in Fig.13, upper panels: rho = -0.998 (-0.999, 1.000), p<0.01 for all, n =200. Scatterplots of associations between A (B, C) percentages are shown in Fig. 13, lower panels: %A vs %B, rho = 0.995; %C vs. %A (%B), rho = -0.998 (-0.999), p<0.01, n =200.
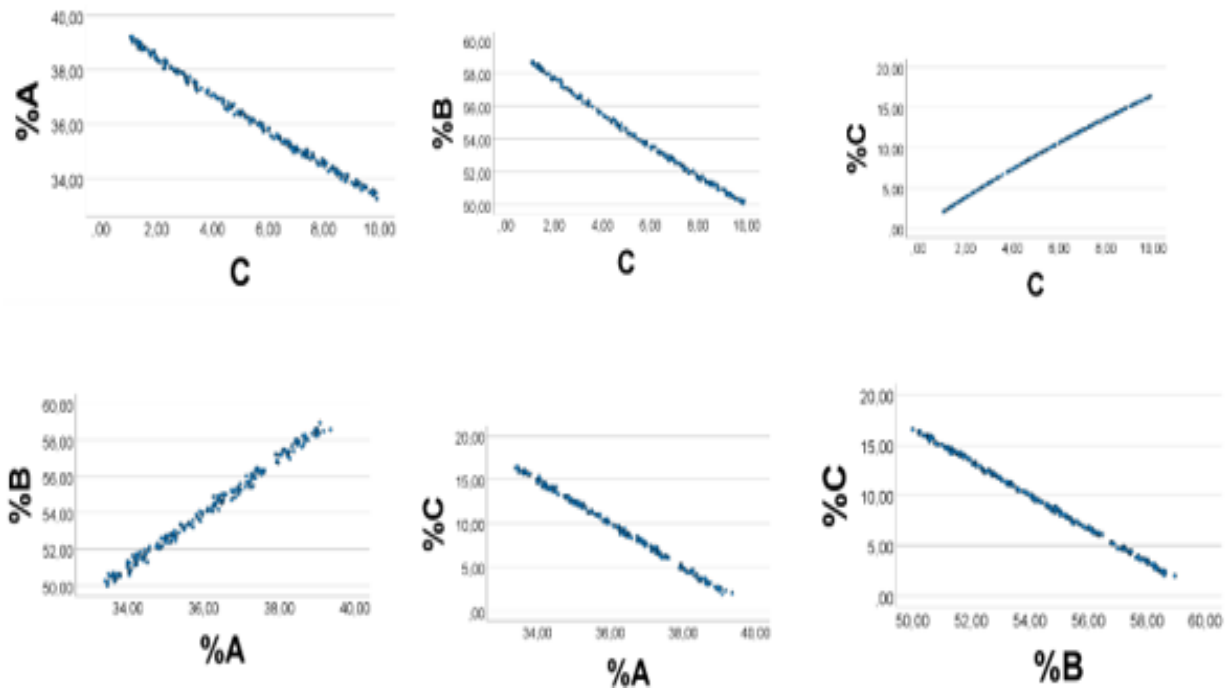

Figure 13: *Relationship between C and A (B, C) percentages of S (upper panels), and between %A, %B, and %C (lower panels). The figure relates to the equation %A + %B + %C =100, see text. We generated uniformly distributed random numbers (n =200) with the following ranges: A 20.0 - 20.2; B 30.0 - 30.3; C 1-10. All correlations were highly significant (rho >0.9, p<0.01).*

The figure illustrates that the association between **C** and A (B, C) percentages of S may explain correlations between %A, %B, and %C (lower panels). The points were near straight lines, because A and B had close to fixed numbers. Thus, for each C - value, there are few "disturbing" A and B numbers. In contrast to this, if we make scatterplots

of %A (%B, %C) vs. **A (B),** there are many values of C for each value of A or B, giving very poor scatterplots. Associations between **B** and %A (%B, %C) are shown in Fig. 14. Similar poor scatterplots were obtained for %A (%B, %C) vs. **A** (not shown).
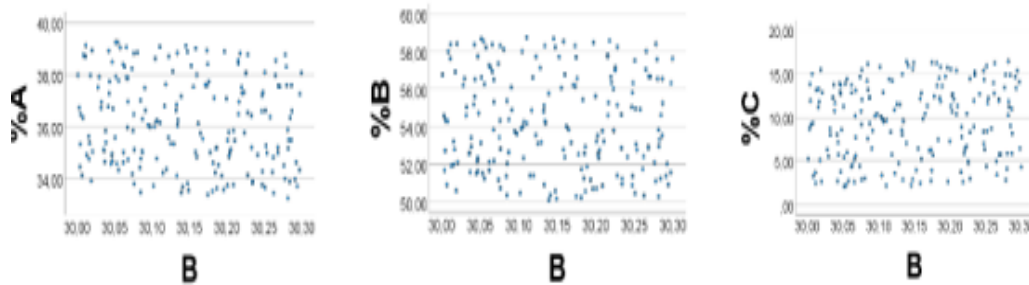

Figure 14: *Scatterplots of B vs. A (B, C) percentages of S. The figure relates to the equation %A + %B + %C = 100, see text. We generated uniformly distributed random numbers (n =200) with the following ranges: A 20.0 - 20.2; B 30.0 - 30.3; C 1-10.*

Limitations of the Study

This work deals with computer experiments. To evaluate the suggested concepts of *Intended Ranges* and *Distribution Dependent Correlations,* we need diet trials in various species, including man.

Conclusions

The present study explains in more detail the strong correlations between eicosanoid (docosanoid) precursor fatty acid percentages in chicken muscle. In particular, our results explain why the associations should be *positive and linear*. Thus, if A, as well as B represent two of the precursor fatty acids, and C the remaining ones, we have A + B + C = S. Then, slope of %A (abscissa) vs. %B would approach **B/A.** Furthermore, slope of %A (abscissa) vs. %C would be near **–(1+B/A),** and slope of %B (abscissa) vs. %C would be close to **–(1+A/B).**

The study supports the idea that *Intended Ranges* could make *Distribution Dependent Correlations*, mathematically. We suggest that such ranges might have arisen through evolutionary selection. Possibly, eicosanoid and docosanoid precursor fatty acids in chicken breast muscle are examples of intended ranges, which would make their relative amounts correlate positively.

Conflicts of Interest: None

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