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# Influence of Phospholipid Species on Membrane Fluidity: A Meta-analysis for a Novel Phospholipid Fluidity Index

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**Abstract** Generalized membrane lipid composition determinants of fluidity have been widely investigated, including phospholipid/cholesterol ratio and unsaturation index. Individual phospholipids differ in their physical characteristics, including their interaction with cholesterol and level of unsaturation, emphasizing the importance of examining their individual influence on membrane fluidity. Thus, the purpose of this study was to examine the dominant phospholipids of biological membranes (phosphatidylcholine, PC; phosphatidylethanolamine, PE; sphingomyelin, SM) through a meta-analysis to assess the validity of an inclusive phospholipid fluidity index (PFI = PC/(PE + SM)) as a determinant for membrane fluidity (expressed as polarization of fluorescent probe 1,6 diphenyl-1,3,5-hexatriene) in comparison to previous phospholipid ratios (PC/PE and PC/SM). The results demonstrate that all indices significantly predicted membrane fluidity at 25°C (based on 10–13 data points). In contrast, only PFI approached significance when predicting membrane fluidity at 37°C ($P = 0.10$ based on five points). As a result, PFI appears to be the only phospholipid index close to significantly predicting membrane fluidity at mammalian physiological temperature. Because this meta-analysis only assessed studies using mammalian membranes, future work should experimentally assess the validity of the PFI utilizing membranes from mammals and a variety of other species and tissues at their respective physiological temperatures.

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The structural properties of biological membranes can influence fundamental physiological processes that involve integral membrane proteins (McIntosh and Simon 2006). The microenvironment of membranes imparts a fluid like nature and alterations in membrane structure can alter fluidity and, in turn, impact the function of membrane proteins (van Meer et al. 2008). Membrane fluidity is in part dependent on the lipid composition. Specifically phospholipid species, their fatty acyl chain length and saturation, and cholesterol content can affect membrane fluidity (Sprong et al. 2001; van Meer and Vaz 2005; van Meer et al. 2008).

Membrane lipid composition determinants of fluidity have been widely investigated, including phospholipid/cholesterol ratio and unsaturation index (Cooper 1977; Owen et al. 1982; Shinitzky and Inbar 1976). In general, lower cholesterol content and higher unsaturation of phospholipid fatty acyl chains are associated with more fluid membranes. However, individual phospholipids differ in their physical characteristics, which include their ability to attract cholesterol as well as their levels of unsaturation, emphasizing the importance of examining their individual influence on membrane fluidity.

Various phospholipid species can exert an effect on membrane fluidity, independent of cholesterol content and unsaturation of their fatty acyl chains, through various physical parameters. Specifically, the size of the phosphate head group (Cullis and de Kruijff 1979) and/or the hydration status of the head group though their interaction with water (Crowe et al. 1987; Hazel and Williams 1990; Ladbrooke and Chapman 1969; M'Baye et al. 2008; van Meer and Vaz 2005) have been shown to alter membrane fluidity. The

combination of these physical parameters result in phospholipid species that either increase [phosphatidylcholine (PC), cardiolipin (CL)] or decrease [phosphatidylethanolamine (PE), sphingomyelin (SM)] fluidity.

The dominant phospholipids in general biological membranes are PC, SM, and PE, accounting for up to ~80% (Borochov et al. 1977; Escriba et al. 2008). As such, PC/PE and PC/SM ratios have often been used, but have not been statistically tested, as lipid determinants of membrane fluidity (Borochov et al. 1977; Mahler et al. 1988b; Owen et al. 1982; Treen et al. 1992). In addition, these ratios only utilize two of the three major phospholipids at a given time, and may not fairly represent the lipid determinant of membrane fluidity. To possibly improve upon these previous phospholipid ratios, it is important to find a more inclusive phospholipid determinant of membrane fluidity. Thus, the purpose of this study was to perform a meta-analysis of available literature data to assess the validity of an inclusive phospholipid fluidity index (PFI = PC/(PE + SM)) as a determinant for membrane fluidity in comparison to previous phospholipid ratios (PC/PE and PC/SM). It was hypothesized that all three phospholipid ratios would be positively correlated with membrane fluidity.

## Methods

### Literature Search

The search for literature was limited to studies published in English-language journals and were obtained from computer searches (Medline). A common measure of membrane fluidity utilizes a fluorescent probe, 1,6 diphenyl-1,3, 5-hexatriene (DPH) and its polarization ($DPH_p$) within the membrane. The polarization of this probe is inversely related to membrane fluidity i.e. an increase in $DPH_p$ indicates a decrease in membrane fluidity (Abel et al. 2001). Most studies commonly measure the $DPH_p$ at 25°C and/or 37°C, and because this membrane fluidity parameter may be affected by temperature (Fox and Delohery 1987), the influence of phospholipid species on membrane fluidity may differ between these temperatures. Thus, specific inclusion criteria were (1) research studies published in peer-reviewed journals, (2) use of biological, not synthetic, membranes, and (3) sufficient data to calculate changes in DPH polarization at either 25 or 37°C, and changes in at least one of 3 ratios: PC/SM, PC/PE and PC/(PE + SM) ratios.

### Calculations

Effects were determined as changes in $DPH_p$, PC/SM, PC/PE and PC/(PE + SM). The differences were calculated using the lowest phospholipid ratio as the baseline group (ie. lower PC/SM value subtracted from higher PC/SM values). The baseline group for $DPH_p$ were matched with the baseline groups of the phospholipid ratios from each study. By using the lower phospholipid ratios as baselines, in effect this removes the possible confounding effect of treatment/perturbation to membranes and focuses on the relationship between change in phospholipid ratios to $DPH_p$. Changes in $DPH_p$ were not converted to percent changes because all studies in this analysis utilized the same methods for determining $DPH_p$ resulting in consistent units. Changes in phospholipid ratios were also not converted to percent changes because data were presented as ratios eliminating any specific units used in the individual studies.

### Statistical Analysis

Changes in $DPH_p$ in response to changes in PC/SM, PC/PE, and PC/(PE + SM) ratios were tested by regression analysis. In regression analysis, the change in $DPH_p$ was used as a dependent variable and changes in PC/PE, PC/SM and PC/(PE + SM) were used as independent variables. The three regression equations are as follows:

$$\Delta DPH_p = b_1(\Delta PC/PE) + c_1 \quad (1)$$

$$\Delta DPH_p = b_2(\Delta PC/SM) + c_2 \quad (2)$$

$$\Delta DPH_p = b_3(\Delta PC/(PE + SM)) + c_3 \quad (3)$$

where $b$ values are slopes and $c$ values are $y$-intercepts. Regression analysis was done with $\Delta DPH_p$ measured at 25°C (13 points for ΔPC/PE (Alvarez et al. 2001; Mahler et al. 1988a; Owen et al. 1982; Popp-Snijders et al. 1986; Senault et al. 1990; van Blitterswijk et al. 1987) and ten points for ΔPC/SM and ΔPC/(PE + SM) (Alvarez et al. 2001; Mahler et al. 1988a; Owen et al. 1982; Popp-Snijders et al. 1986; van Blitterswijk et al. 1987) including human and rodent models) and 37°C (five points (Alvarez et al. 2001; Hitzemann and Johnson 1983; Owen et al. 1982) including human and rodent models).

## Results

### Literature Search

A total of eight studies met the initial criteria for inclusion (Abel et al. 2001; Alvarez et al. 2001; Hitzemann and Johnson 1983; Mahler et al. 1988a, b; Owen et al. 1982; Popp-Snijders et al. 1986; Senault et al. 1990; van Blitterswijk et al. 1987). The two studies conducted by Mahler et al. (1988a, 1988b) were no longer considered as part I and part II and were thus combined for the meta-analysis. One study was excluded from

**Table 1** Summary of study characteristics in seven studies assessing membrane fluidity and phospholipid composition

| Reference and group | Species | Source of membrane | Phospholipid measurement | PC/SM | PC/PE | PC/(PE + SM) | $DPH_p$ 25°C | $DPH_p$ 37°C |
|---|---|---|---|---|---|---|---|---|
| Owen et al. (1982) | | | | | | | | |
| 1 ($n = 25$) | Human | Erythrocytes | Phosphorus assay | 1.11 | 1.07 | 0.55 | 0.327 ± 0.005 | 0.287 ± 0.005 |
| 2 ($n = 30$) | Human | Erythrocytes | Phosphorus assay | 1.84 | 1.87 | 0.91 | 0.335 ± 0.008 | 0.304 ± 0.011 |
| Hitzemann and Johnson (1983) | | | | | | | | |
| 1 ($n = 8$) | Rodent | Synaptic membrane | Phosphorus assay | 2.23 | 1.70 | 1.69 | ND | 0.214 ± 0.004 |
| 2 ($n = 8$) | Rodent | Synaptic membrane | Phosphorus assay | 3.13 | 1.54 | 1.51 | ND | 0.230 ± 0.005 |
| 3 ($n = 8$) | Rodent | Synaptic membrane | Phosphorus assay | 2.94 | 1.19 | 1.12 | ND | 0.247 ± 0.005 |
| Popp-Snijders et al. (1986) | | | | | | | | |
| 1 ($n = 7$) | Human | Erythrocytes | Phosphorus assay | 1.17 | 1.00 | 0.54 | 0.346 ± 0.002 | ND |
| 2 ($n = 7$) | Human | Erythrocytes | Phosphorus assay | 1.10 | 1.01 | 0.53 | 0.342 ± 0.002 | ND |
| 3 ($n = 7$) | Human | Erythrocytes | Phosphorus assay | 1.16 | 1.02 | 0.54 | 0.344 ± 0.001 | ND |
| van Blitterswijk et al. (1987) | | | | | | | | |
| 1 ($n =$ ud) | Human | Erythrocytes | Phosphorus assay | 1.15 | 1.07 | 0.56 | 0.332 | ND |
| 2 ($n =$ ud) | Mouse | Erythrocytes | Phosphorus assay | 5.40 | 2.25 | 1.59 | 0.313 | ND |
| 3 ($n =$ ud)[a] | Rodent | Liver plasma membrane | Phosphorus assay | 1.30 | 1.58 | 0.71 | 0.322 | ND |
| 4 ($n =$ ud) | Rodent | Liver endomembrane | Phosphorus assay | 33.00 | 2.64 | 2.44 | 0.196 | ND |
| 5 ($n =$ ud) | Mouse | Plasma membrane | Phosphorus assay | 49.00 | 1.44 | 1.40 | 0.268 | ND |
| 6 ($n =$ ud)[a] | Mouse | Extracellular vesicles | Phosphorus assay | 4.33 | 1.15 | 0.91 | 0.334 | ND |
| Mahler et al. (1988a, b) | | | | | | | | |
| 1 ($n = 3$–6) | Rodent | Liver plasma membrane | Phosphorus assay | 2.23 | 1.14 | 0.76 | 0.340 | ND |
| 2 ($n = 3$–6) | Rodent | Liver plasma membrane | Phosphorus assay | 3.13 | 1.86 | 1.17 | 0.310 | ND |
| 3 ($n = 3$–6) | Rodent | Liver plasma membrane | Phosphorus assay | 2.90 | 1.72 | 1.09 | 0.250 | ND |
| Alvarez et al. (2001) | | | | | | | | |
| 1 ($n = 5$) | Rodent | Neutrophil membrane | TLC-FID | 5.79 | 0.33 | 0.31 | 0.303 ± 0.004 | 0.287 ± 0.003 |
| 2 ($n = 5$) | Rodent | Neutrophil membrane | TLC-FID | 1.57 | 0.36 | 0.30 | 0.293 ± 0.004 | 0.281 ± 0.004 |
| 3 ($n = 5$) | Rodent | Neutrophil membrane | TLC-FID | 1.59 | 0.31 | 0.26 | 0.284 ± 0.007 | 0.271 ± 0.006 |
| Senault et al. (1990) | | | | | | | | |
| 1 ($n = 6$) | Rodent | BAT mitochondria | Phosphorus assay | ND | 1.06 | ND | 0.163 | ND |
| 2 ($n = 8$) | Rodent | BAT mitochondria | Phosphorus assay | ND | 0.85 | ND | 0.169 | ND |
| 3 ($n = 6$) | Rodent | BAT mitochondria | Phosphorus assay | ND | 0.76 | ND | 0.157 | ND |
| 4 ($n = 8$) | Rodent | BAT mitochondria | Phosphorus assay | ND | 0.68 | ND | 0.164 | ND |

*ND* not detected in the study; *ud* undisclosed; *TLC-FID* thin layer chromatography–flame ionizing detection

[a] Groups were omitted from analysis because phospholipid data were obtained from another study and not measured by the original authors

the analysis because it did not specify at what temperature $DPH_p$ was measured (Abel et al. 2001). A summary of characteristics for the seven studies used in this analysis is given in Table 1. Six of seven studies measured $DPH_p$ at 25°C, all of which had sufficient data for ΔPC/PE ratios resulting in 13 points for regression analysis (Alvarez et al. 2001; Mahler et al. 1988a; Owen et al. 1982; Popp-Snijders et al. 1986; Senault et al. 1990; van Blitterswijk et al. 1987). Of the seven studies, 5 had sufficient data generating ten points for linear regression analysis with both ΔPC/SM and ΔPC/(PE + SM) (Alvarez et al. 2001; Mahler et al. 1988a; Owen et al. 1982; Popp-Snijders et al. 1986; van Blitterswijk et al. 1987). Two groups from one study were omitted from analysis because the phospholipid data presented were actually obtained from two different publications (van Blitterswijk et al. 1987). Of the seven studies, only three measured $DPH_p$ at 37°C resulting in five points for regression analysis with phospholipid ratios (Alvarez et al. 2001; Hitzemann and Johnson 1983; Owen et al. 1982). Of the five points for analysis at 37°C, four were obtained from studies using rodents (Alvarez et al. 2001; Hitzemann and Johnson 1983), and one point using humans (Owen et al. 1982).

## Linear Regression Analysis

The ΔPC/PE ratio of biological membranes was able to significantly predict $\Delta DPH_p$ at 25°C (Fig. 1a; $P = 0.015$, $r^2 = 0.42$, $b = -0.0005 \pm 0.0002$) but not at 37°C

(Fig. 2a; $P = 0.74$). Similarly, ΔPC/SM ratio approached significance in its ability to predict $\Delta DPH_p$ at 25°C (Fig. 1b; $P = 0.051$) but not at 37°C (Fig. 2b; $P = 0.92$). In contrast, the response of $\Delta DPH_p$ to changes in the novel phospholipid fluidity index (ΔPC/(PE + SM)) demonstrated that this ratio did significantly predict $DPH_p$ at 25°C (Fig. 1c; $P = 0.0062$, $r^2 = 0.62$, b = −0.068 ± 0.02) and was the only phospholipid index trending toward significance at 37°C (Fig. 2c; $P = 0.10$).

## Discussion

This study examined the relationship between phospholipid fluidity indices (PFI; two common and one novel) and membrane fluidity (expressed as $\Delta DPH_p$). The main findings of this study are (1) all phospholipid indices were significant predictors of membrane fluidity expressed as $\Delta DPH_p$ at 25°C and (2) only the novel PFI (ΔPC/(PE + SM)) trended toward significance when predicting changes in membrane fluidity expressed as $\Delta DPH_p$ at 37°C.

Membrane fluidity is an important parameter that can influence various membrane functions including signal transduction, ion transport and vesicle trafficking (Bookstein et al. 1997; Brown et al. 2003; Sengupta et al. 2007; Srivastava and Dash 2001). There are three main lipid composition factors of membranes, which include cholesterol content, unsaturation and chain length of phospholipid fatty acyl chains, and phospholipid species content (Borochov et al. 1977). Collectively, these factors result in each phospholipid species having unique abilities to alter membrane fluidity by how they interact and attract cholesterol and water, the chain length and level of unsaturation of their fatty acyl tails, and the size of their head group compared to these fatty acyl tails.

Independent of cholesterol affinity and unsaturation, phospholipid species can alter membrane fluidity via characteristics of their head group. As a result of the size of the phospholipid head group relative to the fatty acid chains, PC increases membrane fluidity as the choline head group is large, resulting in a relatively similar area of head group compared to fatty acyl chains (Cullis and Hope 1985). In contrast, PE reduces fluidity as ethanolamine is slightly smaller, resulting in a larger area of fatty acyl chains relative to head group (Cullis and Hope 1985). Importantly, the effect that PC and PE have on fluidity is not limited to shape, and can include factors such as hydration (Hazel and Williams 1990; Ladbrooke and Chapman 1969). Specifically, PC is suggested to be more hydrated than PE which negates tight packing of adjacent hydrophobic lipids (Hazel and Williams 1990). Moreover, PE is a hydrogen donor and can form hydrogen bonds with the anionic phosphate–oxygen of the adjacent phospholipid, thus restricting movement (Mahler et al. 1988a). Thus, it is plausible for PC/PE ratio to coincide positively

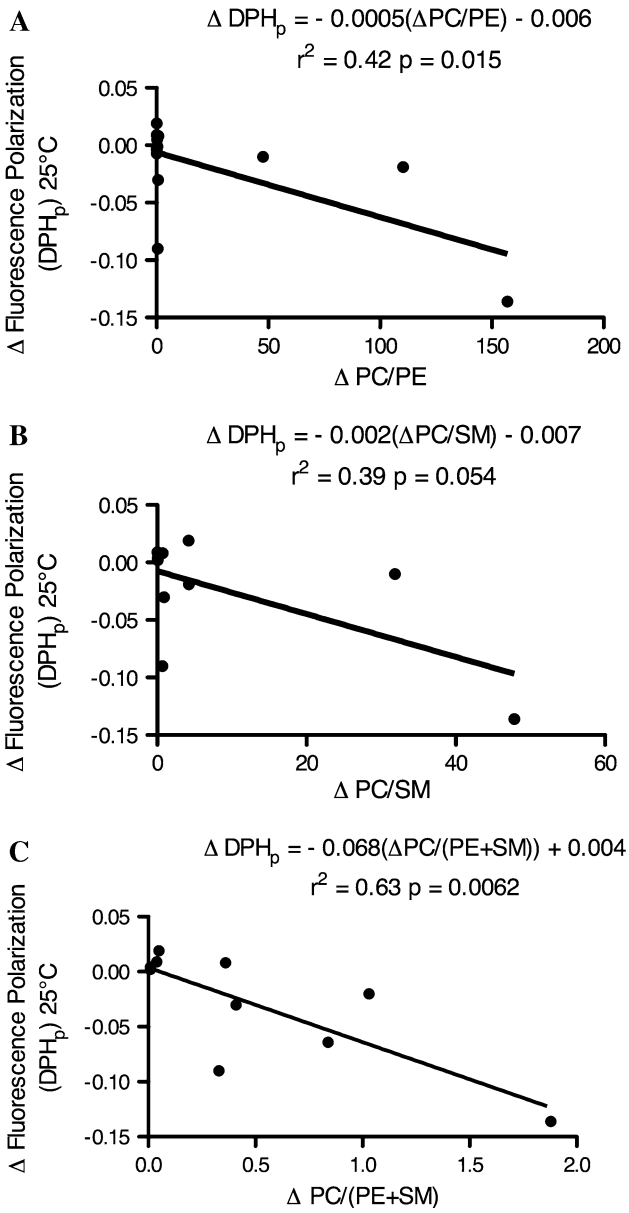


**Fig. 1** Linear regression analysis of phospholipid ratios **a** PC/PE, **b** PC/SM, and **c** the novel phospholipid fluidity index, PC/(PE + SM), on membrane fluidity expressed as $DPH_p$ at 25°C. *PC* phosphatidylcholine, *SM* sphingomyelin, *PE* phosphatidylethanolamine, *$DPH_p$* fluorescent probe 1,6 diphenyl-1,3,5-hexatriene. Of the seven total studies identified for this study, six were examined, generating 13 points for ΔPC/PE (Alvarez et al. 2001; Mahler et al. 1988a; Owen et al. 1982; Popp-Snijders et al. 1986; Senault et al. 1990; van Blitterswijk et al. 1987). Of the total seven studies identified for this study, five were examined generating ten points for ΔPC/SM and ΔPC/(PE + SM) (Alvarez et al. 2001; Mahler et al. 1988a; Owen et al. 1982; Popp-Snijders et al. 1986; van Blitterswijk et al. 1987)

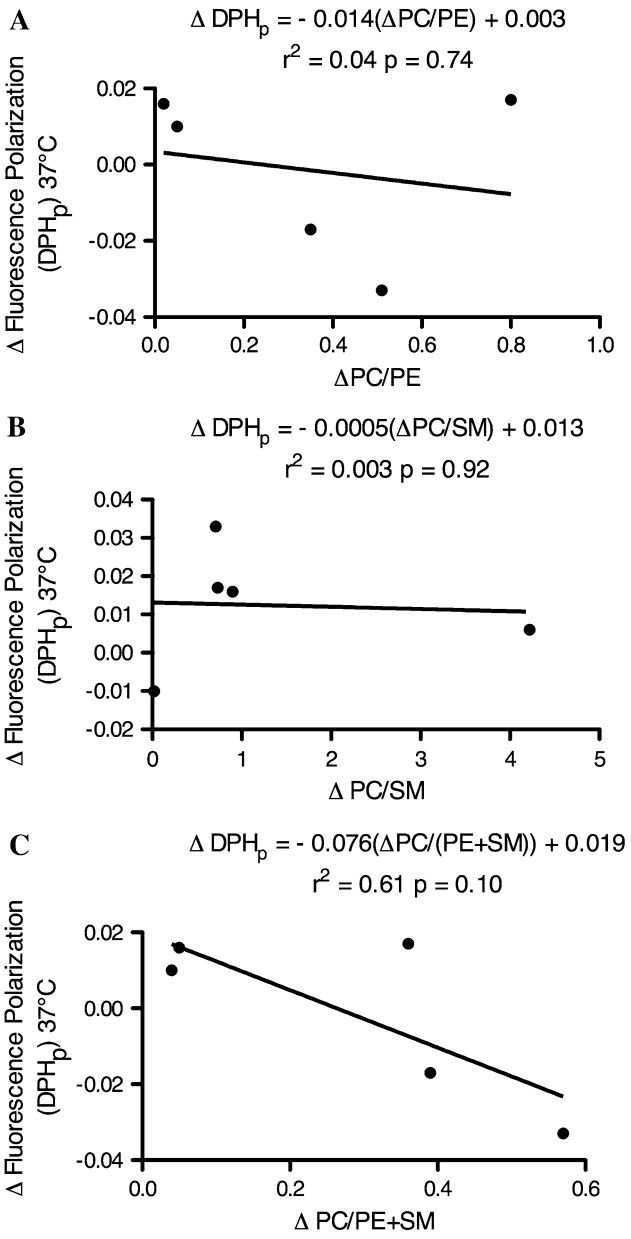


**Fig. 2** Linear regression analysis of phospholipid ratios **a** PC/PE, **b** PC/SM, and **c** the novel phospholipid fluidity index, PC/(PE + SM) on membrane fluidity expressed as $DPH_p$ at 37°C restricted to studies using rodents. *PC* phosphatidylcholine, *SM* sphingomyelin, *PE* phosphatidylethanolamine, $DPH_p$ fluorescent probe 1,6 diphenyl-1,3,5-hexatriene. Of the seven total studies identified for this study, three were examined, generating five points for all ratios (Alvarez et al. 2001; Hitzemann and Johnson 1983; Owen et al. 1982)

with membrane fluidity. This, however, was only seen at 25°C ($P = 0.015$, $r^2 = 0.42$) and not at 37°C ($P = 0.74$) in this meta-analysis.

SM is a major lipid in plasma membranes because of its high content in lipid rafts and caveolae (Koumanov et al. 2005; Smart et al. 1995). Although, PC and SM have the same phosphate head group (choline) it has been suggested that PC is more hydrated than SM (M'Baye et al. 2008) which would allow for SM to pack tighter than PC. It has been shown in synthetic membranes, that when membranes contained high amounts of SM (50–60%) it was paralleled with high $DPH_p$ values (~351–356) indicating a decrease in fluidity, when compared to membranes without SM (Sunshine and McNamee 1994). In addition, liposomes containing both PC and SM showed increases in $DPH_p$ as %SM increased with or without cholesterol (Cooper et al. 1977). Therefore, it is also plausible for PC/SM ratio to positively coincide with membrane fluidity, yet was only seen at 25°C ($P = 0.051$)

Given the predominant nature of PC, PE and SM in biological membranes, it is not surprising that collectively they would best represent a phospholipid determinant of membrane fluidity. When examining the commonly used PL indices, the results presented reveal instances in which extremely high values of PC/PE or PC/SM (30–200) resulted in a decreased calculated response of $\Delta DPH_p$ at 25°C (Fig. 1a: $b = 0.0005 \pm 0.0002$, Fig. 1b: $b = -0.0019 \pm 0.0001$, respectively). This may be due to the incomplete representation of the dominant membrane phospholipids in each of these ratios. Specifically, instances in which low PE and low SM cause high PC/PE and PC/SM values, respectively, can be best represented by combining both PE and SM to the ratio. This was very apparent at 37°C whereby the novel PFI was the only phospholipid index to correlate against $\Delta DPH_p$ ($b = -0.076 \pm 0.03$, $r^2 = 0.61$), despite only trending toward significance ($P = 0.10$). Given the lack of statistical significance of the effects of PFI on $DPH_p$ at a more physiologically relevant temperature of 37°C, it questions the sample size required to detect significance. With a desired power of 0.8, $\alpha = 0.05$, and $r^2 = 0.61$ (Fig. 2c), 15 data points are required to detect a significant correlation between ΔPFI and $\Delta DPH_p$ at 37°C. In contrast, ΔPC/PE and ΔPC/SM (desired power of 0.8, $\alpha = 0.05$, and $r^2 = 0.04$ and 0.004, respectively), 3,800 and 50,000 data points, respectively, are required to detect significance. Because both PC/PE and PC/SM require an extreme amount of data points to reach significance at 37°C, it may be suggested that their application to cells that are thermoregulated to function at 37°C is limited. Taken together, the results in this meta-analysis indicate that the novel PFI may be a more suitable phospholipid membrane fluidity determinant.

In summary, the present meta-analysis has revealed that the novel PFI was highly correlated with membrane fluidity expressed as $DPH_p$ at 25°C and, with more data points, may also show significance at 37°C when compared to previous untested ratios. In addition, ΔPFI generated the greatest response of $\Delta DPH_p$ at both 25 and 37°C, indicating that previous untested ratios underestimate the effect

phospholipid species have on membrane fluidity. Finally, ΔPFI accounted for the more variation in $\Delta DPH_p$ when compared to the previous untested ratios at both temperatures. Therefore, the novel PFI, in addition to other commonly used indices (e.g. UI, PL/C), may be a good determinant of membrane fluidity. Because this study only analyzed the membranes from mammalian cells, future studies should further assess the validity of the relationship between PFI and membrane fluidity utilizing membranes from both mammalian species and a variety of other species while measuring PFI and $DPH_p$. Furthermore, because membrane fluidity may affect protein function, future studies can assess the relationship between the novel PFI and specific membrane protein functions. In addition, various cellular membranes can differ in phospholipid composition such as the mitochondrial membrane shown to be high in cardiolipin and low in SM. The applicability of the novel PFI to these membranes may be limited and an alternate phospholipid ratio may be required.

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