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Else, P. (2020). The highly unnatural fatty acid profile of cells in culture. Faculty of Science, Medicine and Health - Papers: Part B. Retrieved from https://ro.uow.edu.au/smhpapers1/1156

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Publication Details

Else, P. (2020). The highly unnatural fatty acid profile of cells in culture. Progress in Lipid Research, 77 101017-1-101017-19.

This journal article is available at Research Online: https://ro.uow.edu.au/smhpapers1/1156

The Highly Unnatural Fatty Acid Profile of Cells in Culture

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Abstract

The fatty acid profile of cells in culture are unlike any natural cell with twice the monounsaturated (MUFA) and half the polyunsaturated fatty acids (PUFA) of natural cells (Mol%). This is not due to cell lines primarily being derived from cancers but due to limited access to lipid and inability to make PUFA *de novo* as vertebrate cells. Classic culture methods use media with 10% serum (the only exogenous source of lipid). Fetal bovine serum (FBS), the serum of choice has a low level of lipid and cholesterol compared to other sera and at 10% of media provides 2-3% of the fatty acid and cholesterol, 1% of the PUFA and 0.3% of the essential fatty acid linoleic acid (18:2*n*-6) available to cells in the body. Since vertebrate cell lines cannot make PUFA they synthesise MUFA, offsetting their PUFA deficit and reducing their fatty acid diversity. Stem and primary cells in culture appear to be similarly affected, with a rapid loss of their natural fatty acid compositions. The unnatural lipid composition of cells in culture has substantial implications for examining natural stems cell in culture, and for investigations of cellular mechanisms using cell lines based on the pervasive influence of fats.

Keywords

Cell line; Fatty acid; Polyunsaturated; Cancer cell; Stem cell; Peroxidation

Abbreviations

FBS, fetal bovine serum; GSH, glutathione; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; SAT, saturated fatty acids; *n-3*, omega-3 fatty acid; *n*-6, omega-6 fatty acid; 18:1*n* -9, oleic acid; 18:2*n*-6, linoleic acid; 18:3*n*-3, α-linolenic acid; 20:3*n*-9, Mead acid; 20:4*n*-6, arachidonic acid; 20:5*n*-3, eicosapentaenoic acid; 22:6*n* -3, docosahexaenoic acid.

Notes: Averaged Molecular Weights Used in Calculations are; Phospholipid 760; Fatty Acid 280; Cholesterol 387; Triglyceride 860; Protein content of cell lines assumed to be 130mgP/g of cell. SAT, MUFA and PUFA (unless otherwise stated) as used in this manuscript refer to the derivatized fatty acid esters formed from total lipid extracts of cells, tissue or phospholipid fractions (as indicated).

1. Introduction

Cells in culture are often used as surrogates for researching natural tissues, as vehicles for the expression and characterisation of proteins and in the manufacture of biopharmaceuticals. They also represent the future of individualised cell therapy. Yet the fatty acid profile of cells maintained in culture is unlike that of any natural cell. Cultured cells include cell lines, stem cells and primary cells. These cells are mostly derived from vertebrate tissues and are mainly from human sources (~70%) but when placed in culture their natural lipid profile is lost. The fatty acid profile of cells in culture reverts to one that is very low in polyunsaturated fatty acids (PUFA) and very high in monounsaturated (MUFA) fatty acids. The reason for this is that the lipid environment created by standard culture media is inadequate in its lipid concentration and composition to maintain the natural fatty acid composition of cells. This is exacerbated by the fact that vertebrate cells lack the ability to make their own polyunsaturated fatty acids (PUFA) [1, 2]. This short review examines this phenomenon exploring the amount and composition of lipid supplied to cells in culture and the resulting lipid composition of cultured cells compared to those in situ.

2. Lipids Supply for Cells

Vertebrate cells need to gain PUFA from their environment as they lack both the △12 and △15 desaturases needed to create the omega-6 (*n*-6) and omega-3 (*n*-3) series of PUFA respectively. Subsequently, two essential PUFA are required by vertebrates, these are linoleic (18:2*n*-6) and α-linolenic (α-18:3*n*-3) acid. These two PUFA can be used to synthesise longer, more unsaturated *n*-6 (e.g. 20:4*n*-6 arachidonic acid) and n-3 (e.g. 20:5*n*-3 eicosapentaenoic and 22:6*n*-3 docosahexaenoic acid) PUFA. For cells of the body, both essential and preformed longer-chained PUFA arrive in the diet and are absorbed across the gut and distributed into bodily fluids (lymph and blood). In the body the synthesis of the longer chained PUFA is facilitated by the liver [3] and any localised cells able to synthesise essential PUFA into more complex forms [4, 5]. However, the overall synthetic ability of turning essential PUFA into their longer, more unsaturated forms in humans is poor (especially for *n*-3 fats) with the diet suppling most complex PUFA preformed [6]. The one potentially prevalent PUFA that can be made in the body is Mead acid. Mead acid (20:3n-9) is normally association with essential fatty acid deficiency and is not made from a PUFA but from a MUFA, oleic acid

(18:1n-9) [7]. Mead acid typically appears in cells at higher levels (~5%) when the body is under extreme PUFA deficiency [1, 2] or in specialised tissues such as cartilage where it naturally occurs at higher levels [8]. In most tissues Mead acid appears to be an emergency PUFA produced under extreme conditions of PUFA deficiency.

For cells in culture, lipids including PUFA come from serum added to supplement media. Serum facilitates cell growth, attachment, spread and helps prevent apoptosis, it is not intended to be a balanced or adequate lipid supply. The main serum used in cell culture is fetal bovine serum (FBS). FBS has one-quarter the lipid content, one-third the MUFA, 11% of the PUFA and 8% of the normal level of linoleic acid [9, 10] compared to media made from human serum (see Table 1 for the relative fatty acid composition of sera and plasma of different vertebrates). When added at 10% of media volume, FBS provides 0.19 mM of total fatty acids and 0.04 mM of PUFA [9]. This compares to whole human plasma/serum with 7.9 mM of fatty acids and 3.5 mM of PUFA [9, 11], a 40 and 88-fold difference respectively. FBS also has a very low cholesterol concentration at 0.9 mM (or 0.09 mM at 10% FBS) versus 5 mM in human plasma [12, 13], a 54-fold difference in culture media. Subsequently, cells in culture are incubated in media with 2-3% of the fatty acid and cholesterol, 0.3% of the essential fatty acid linoleic acid, and 1% of the PUFA concentration of cells in situ.

3. Effect of Culture on Cell Lipid Profile

The effect of culture conditions is to produce cells that are very different to those of natural tissues in terms of their fatty acid profile. In humans and other mammals most tissues have between 30-50% of their total fatty acids as PUFA [14-24] whereas cell lines on average have 14% of their fatty acids as PUFA (a 2.5-fold decrease). This difference is clearly shown in Figure 1 that compares data for a variety of cell lines (Table 2) to the same tissues *in situ* (Table 3). Most of the difference in PUFA is offset by MUFA with 39% of total fatty acids being MUFA in cell lines compared to 21% in natural tissues (a 2-fold increase). Whereas the relative level of SAT in a wide variety of cell lines and natural tissues are quite similar at 45% in cell lines and 42% in natural tissues (Figure 1). Since vertebrate cells can synthesise MUFA and SAT, the reduction in PUFA in cultured cell lines appears to be resolved by using the only other unsaturated fat able to be synthesised i.e. MUFA.

Surprisingly most cell lines do not make Mead acid (20:3*n*-9). An examination of 53 different studies found 12 cell lines with a nominal level of Mead acid, six where from fish cell lines [25], two where from mice, a fibroblast (NIH3T3) and a liver (Hepa 1-6) cell line; [26]) and the remaining cell lines where from human sources, a liver (HepG2) [27], colorectal (Caco-2; [28]) and two leukocyte (Raj and THP-1; [29]) cell lines. Although most cell lines appear not to respond to culture conditions with Mead acid production, their high proportion of MUFA, notably oleic acid (18:1*n*-9), is a common characteristic of essential fatty acid deficiency [30, 31]. Therefore, cells that survive in culture adapt to the prevailing conditions of culture with increased MUFA production. This increase in MUFA synthesis of cells in culture is likely facilitated by their low PUFA levels since PUFA suppress many genes [32] at the transcriptional level that code for enzymes associated with MUFA synthesis e.g. stearoyl CoA desaturase-1. A striking example of adaptation to high MUFA levels is where four very different cell lines (HeLa , RSP-2, L and Huk-1) were maintained in serum-free synthetic media (no lipid present) for over ten years [33]. The cells that survived 10-years of continuous culture without serum lacked PUFA and possessed only MUFA and SAT, with no Mead acid. It shows how cell lines can readily adapt to extreme environments by using their inherent genetic instability that allows the clonal forms best suited to the prevailing conditions to proliferate. This ability to adapt has been shown in several recent studies measuring the heterogeneity [34] and genetic and transcriptional evolution of cell lines in response to environmental stimuli [35].

The conditions under which cells are currently cultured produce cells that have a highly restricted range of PUFA in their membranes when compared to the same cells in natural tissues. Figure 2 shows the relative level of each essential fatty acid in serum/plasma (natural cells) or media (cell lines) and an example of one major long-chain product (arachidonic acid or 20:4*n*-6 for the essential *n*-6 PUFA linoleic acid, and docosahexaenoic acid or 22:6*n*-3 for the essential *n*-3 PUFA α-linolenic acid) for a broad range of cell lines and natural cells. It shows how a low level of PUFA supply in cell lines restricts the level of each long chain PUFA able to be produced and incorporated into membrane phospholipids. In contrast the same natural tissues display an extensive compositional range in their long chain PUFA products. Subsequently, due to restrictions on PUFA supply cell lines cluster into tight compositional groupings with different cell lines being far more similar to one another than they are to the natural

tissues they are being used to represent. This reduction in lipid compositional range is analogous to results recently reported for protein diversity in cultured cells where an examination of eleven common cell lines showed a high degree of similarity in their expressed proteins, despite being from distinctly different tissues [36]. Overall, it would appear that the result of long-term culture under similar incubation conditions produces cells with similar composition, irrespective of their different origins.

4. Lipid Supplementation of Cell Lines

The long history of many cell lines being cultured in low lipid, low PUFA media might suggest that when supplemented with extra lipid these cells may not incorporate them. However, cell lines appear to readily incorporate lipids when made available to them in the media. In serum, free fatty acids are at very low concentrations in the nM-μM range [11, 37, 38] but in steady state with large reservoirs of lipid (7 mM) associated with lipoproteins and coupled to blood proteins [11]. Cells are known to readily take up free fatty acids, via diffusion and active transport from their surrounding media [39, 40]. For example, a mouse fibroblast cell line (L929) was found to rapidly absorb available free fatty acids, although triglycerides supplied the majority of external lipid (but at a slower rate of uptake) and *de novo* synthesis of SAT and MUFA supplied the majority of lipid [41]. L929 mouse fibroblasts did not take up phospholipids, whereas Chinese hamster V79 fibroblasts readily incorporated externally supplied phospholipids, as shown by the presence of radiolabel in membrane fractions and radiolabelled phospholipid lamellae in intracellular lipid droplets viewed in electron microscope autoradiographs [42]. Therefore, lipid uptake by cells in culture is likely to vary dependent upon factors such as cell type but also cell density, growth rate and media turnover.

Most studies that have supplemented lipids to cells in culture have used fatty acids in concentrations between 4 – 200 μM [43-47] with delivery methods varying from free fatty acids in ethanolic solutions, to fatty acids complexed with bovine serum albumin or FBS. Figure 3 shows the docosahexaenoic acid (DHA; 22:6*n*-3) composition of cell line membranes against the DHA concentration of the culture media. It shows how very different cell lines will readily incorporate DHA when made available. Cultured cells will readily take up PUFA when given access to them. This is presumably because SAT and MUFA can be synthesised and therefore their level dictated by the cell (i.e. potentially high), whereas PUFA cannot be synthesised *de novo* and therefore their level is dictated

by supply. For example, HT29 (human colon adenocarcinoma cells) supplemented with 50 μM 18:1*n*-9 increased membrane levels from 28 to 38%, an increase of 10% in total fatty acid concentration, or a 1.4-fold increase compared to the serum supplemented control condition. However, when supplemented with the same concentration (50 μM) of various PUFA, 18:2*n*-6 levels increased from 1-18%, 20:4*n*-6 from 6-25%, 20:5*n*-3 from 7 to 13% and 22:6*n*-3 from 2 to 12% [45]. These increases, between 6-21% for the four PUFA are not greatly dissimilar to that of oleic acid at 10% but these changes occurred against relatively low starting PUFA levels and therefore the relative changes were between 4 to 19-fold. When H35 (hepatoma) cells were supplemented (at 50 μM) with 12:0 and 14:0, two SATs present at very low levels in H35 cells, the relative level of these two fatty acids increased from 0.2 to 0.7% and from 1.5 to 4.7% respectively, 2 to 3-fold increases relative to their control condition. When the same cells were supplemented with 20:5*n*-3 and 22:6*n*-3 at 50 μM, PUFA levels increased from 0.7 to 7.3% and from 2.5 to 12.8% respectively, 5-10 fold increases relative to their control condition [46]. It would appear that cells in culture have a 'thirst' for fats, particularly PUFA, and have not lost their ability to incorporate fats into their membranes and lipid stores when available in the culture media.

5. Overall Lipid Composition and Lipid Level in Cell Lines

Although the fatty acid composition of cells in culture and those in natural tissue are quite different (Figure 1, Table 2 and 3), the composition of phospholipids and that of lipids generally appear to be quite similar. Table 4 compares the two most common measurements of lipid composition, i.e. phospholipids and total lipids of cells in culture and compares them to those of natural tissues. The comparison clearly shows that the differences in lipids are primarily limited to the fatty acids that reside under the head groups of the phospholipids, on the glycerol backbone of glycerides and under cholesterol for cholesteryl esters. Apart from differences due to the variation between cell types, and differences due to developmental [48] and growth [49] stages of cells, the overall picture is one of similarity in regard to gross lipid composition.

The much lower level of lipid in culture media might also be expected to influence the concentration of lipids in cultured cells. Balb3T3 (embryonic mouse fibroblasts) have a reported total lipid concentration of 42 mmol/kg of cell, a phospholipid concentration of 28 mmol/kg, a fatty acid concentration of 56 mmol/kg, and a cholesterol concentration

of 9.5 mmol/kg [13] (assuming 130g of protein per kilogram of cell for cell lines). In comparison, human liver tissue has a total lipid concentration of 74 mmol/kg, a phospholipid concentration of 18 mmol/kg [50], a fatty acid concentration of 33 mmol/kg [51] and a cholesterol concentration of 4.5 mmol/kg [52]. Human kidney has 19 mmol/kg [21] and rat tissues such as brain, heart and kidney have total fatty acid levels ranging from 33–79 mmol/kg [51]. The lipid concentrations of cancer cells *in situ* show a slightly lower level of phospholipids at 13.7 mmol/kg compared with normal liver at 16 mmol/kg [52] but higher cholesterol levels at 10 mmol/kg compared with 4.5 mmol/kg of normal liver tissue [52]. The higher cholesterol levels of many cell lines may reflect their cancer origins or higher concentration of cell when compared to a kilogram of tissue, however, the concentration of lipids in cell lines seems to be reasonably similar to that of normal tissue (also considering that cell line values are based on pure cell samples unlike those of tissues, as indicated by this lower phospholipid content). Therefore, the similar lipid concentration of cell lines and cells *in situ* suggests that lipid synthesis of MUFA and SAT is making up for what cannot be made from by the limited PUFA supply in culture media.

6. Stem and Primary Cells in Culture

6.1 Stem Cells

There is limited data available on the fatty acid composition of stem cells in culture. This is because most of the work in this area is more recent and tends to involve lipidomic studies with a focus on molecular species. However, data from mesenchymal stromal cells (multipotent stem cells) of human marrow origin show that the fatty acid composition of stem cells when in culture is similar to that of cell lines with 45% SAT, 38% MUFA and 16% PUFA [53]. Another recent study on mesenchymal stromal cells from human chorionic tissue supports this, showing reductions in PUFA and increases in MUFA associated with culture conditions [54]. This study analysed cell composition with each passage (P) during continuous culture. Under normal culture conditions in 10% FBS these stem cells rapidly decreased their PUFA levels from 30% (at isolation indicating normal tissue levels) to 18% at P1 (a level retained till the end of measurement at P8) with increases in MUFA and SAT levels [54]. Current formulations used for the culture of stem cell often avoid the use of serum preferring to employ synthetic media. One such chemically defined medium known as E8 contains only eight essential ingredients [55]. This formulation contains no lipid and based on the results of

the present analysis, stem cells in E8 will become increasingly devoid of PUFA and dominated by MUFA and SAT. Although based on few studies human stem cells appear to suffer the same deterioration in their lipid profiles as found in cell lines once in culture.

6.2 Primary Cell

Primary cells in culture (those isolated directly from normal tissues) also show decreases in their PUFA level over time [56-59]. These cells can be more resistant to change as some do not proliferate and some may undergo cellular senescence [58]. Therefore, decreases in PUFA in primary culture may also reflect PUFA turnover in cells if they are not proliferating but unable to restore PUFA from lipid supplied in the culture media. The overall conclusion that can be drawn from these studies is that irrespective of the source and type of cell, culture conditions produce similar changes in fatty acid composition due to the limited availability of PUFA in culture media. One valuable characteristic of cells however is their ability to respond rapidly to lipid availability. Most studies that supplement cell lines with lipid have used incubations lasting several days [43, 44, 47, 57, 60], yet two studies [45, 61] examined several different PUFA (18:2*n*-6, 20:4*n*-6, 20:5*n*-3 and 22:6*n*-3) at concentrations of 50 and 25 μM respectively, and both used short incubation times (24-36 hrs). These two studies also used very different cell lines (colorectal and macrophages) yet found that PUFA levels of total lipid [61] or phospholipid [45] fractions in all incubations doubled during the incubation. Therefore, it seems likely that cell lines, primary and stem cells in culture will rapidly remediate their lipid composition if lipid is available e.g. stem cells reintroduced back into the body.

7. Cancer and Cell Lines

Most cell lines were originally derived from various forms of cancers and this could conceivably account for their abnormal lipid composition. However, when the relative fatty acid composition of cell lines is compared to matched *in situ* cancers and normal tissues (Figure 4 and Table 5) it is apparent that this is not the case. In a wide variety of normal tissues and *in situ* cancers from the same tissues, although some smaller differences involving the slight upregulation of MUFA and down regulation of PUFA containing phospholipids are apparent [62], the overall fatty acid profile of cancer *in situ*

is far more similar to that of the tissue in which they reside. This suggest that the abnormal fatty acid profile of cell lines is not due to their cancer origins but due to conditions imposed by culture.

8. Consequences of Unnatural Lipid profile in Cultured Cells.

PUFA are highly influential in determining the physical and biochemical properties of cells [63-65]. PUFA can affect the fluidity, flexibility and permeability of membranes, force raft-forming lipids out of the lipid bulk phase to form rafts, influence signalling, gene expression, channel and transporter functions [66, 67]. Interestingly many of these effects have been studied on cells in culture. PUFA are also up to 1000x more likely to oxidise (peroxidise) than other fatty acids (e.g. cholesterol, SAT and MUFA) due to their content of bisallylic methylene (methylene between double bonds) with weaker hydrogen bond energies making them more likely to form radicals [68-70]. This also possess the problem for maintaining PUFA levels in added serum and culture media if not protected by antioxidants. Some of the most active molecules in the body (eicosanoids, resolvins, protectins, lipoxins) are derived from PUFA. Therefore, the lower PUFA levels of cells in culture would alter their oxidation state and peroxidation products derived from oxidation when in culture. This is likely to alter their function and responses compared to natural cells. The prevalent perspective of cells in culture is that they are under high oxidative stress [71] due to higher oxygen levels [72]. This is not surprising as culture commonly occurs in air/CO_2 mixes where solubilized oxygen is driven by a partial pressure of ~145mmHg rather than the 8-70mmHg more indicative of oxygen partial pressure at the tissue level [72] and where most oxygen is bound to proteins [73]. Cell lines also face the potential of transition metal ions (Fe^{2+} and Cu^{2+}) and generation of reactive oxygen species (ROS), plus the presence of hydrogen peroxide able to initiate peroxidation [71]. This perspective assumes that cells in culture have a similar PUFA composition to those of natural cells. The peroxidation index (PI) value for cell lines is ~40 and that for natural tissues is closer to 91 indicating that natural tissues are 2.3-fold more likely to undergo peroxidation than cell lines based on their different fatty acid compositions (see Tables 2 and 3; PI equation available in legend of Table 1, 2 and 5). Therefore, although the culture environment is pro-oxidative, cells in culture may not be as readily prone to peroxidation as natural cells.

9. Antioxidant Levels of Cells and in Culture Media

The most abundant soluble antioxidant produced by cells is glutathione (GSH). In mammals, plasma GSH level vary with body size with concentrations of 18–60 μM in mice [74], 20 μM in rat [75], 2.8 μM in humans [76] and 2 μM in calves [77]. This is presumably related to the higher rate of metabolism and higher levels of membrane unsaturated in smaller mammals [78]. Plasma GSH readily oxidises within hours at room temperature [75]. Therefore, the level of GSH in FBS would be expected to be at very low levels being derived from the fetus of a large mammal and being commonly heat inactivated before use. Cell lines produce GSH with HeLa cells maintaining GSH internal concentrations at 6.9 mmol/kg [79] and A549 cells (human lung tumour cells) at 9.0 mmol/kg in standard culture media [80]. This is similar to the concentration of GSH in whole rat liver at 6.5 mmol/kg [81] and within the range of rat tissues (per kg) generally at 1–10 mmoles [82]. Isolated rat hepatocytes in culture increase GSH levels 4-fold during their first 2–4 days in culture before falling back to ~6 mmol/kg of cell, a level 50% higher than that of isolated hepatocytes [83].

The major membrane antioxidant is vitamin E (α-tocopherol). Vitamin E is obtained preformed from the environment [84] and in mice vitamin E concentration of serum is 7–8 μM, in rats 15 μM [85] and in humans between 15–30 μM [86, 87]. Processed FBS has a low vitamin E concentration at 0.45 μM [84] or 0.045 μM at 10% of culture, a hundred times lower than the ~5 μM of vitamin E normally present in adult cow serum [88, 89]. Measurements of vitamin E concentration in cell lines are scarce but have been reported for L1210 (murine leukemia cell line) at 60 μM [90]. Freshly isolated rat hepatocytes have 26 μmoles of vitamin E per kg, yet in primary culture vitamin E levels fall to 4 μmol/kg [85]. Intact tissue levels in rats and mice maintain vitamin E concentrations between 6–28 μmol/kg [89, 91]. Therefore, although based on limited data, cell lines seem to be able to maintain vitamin E levels not dissimilar to those found in cells *in situ*.

Antioxidants are not normally added to most media formulations. A common additive in the culture of neural and stem cells used at 2% of total media or in serum-free culture is B27. At 2% of media volume B27 has 47 times the level of Vitamin E (2.1 μM) of 10% FBS (0.045 μM) and with 407 μM glutathione thousands of times that of normal culture

media (assuming 0.07 μM of reduced glutathione for media with 10% FBS) [92]. Therefore, cell lines maintain good levels of endogenous antioxidants that are similar to those of natural cells but are normally poorly protected by exogenous antioxidant levels in most culture media.

10. Interpreting the Effect of Lipids on Cellular Function using Cells in Culture

Considering both the specific and nonspecific effects that lipids can have on the functional characteristics of membrane proteins [93-95] it is surprising that the unnatural fatty acid composition of cultured cells is not well recognized. This lack of awareness may stem from the fact that there have been very few studies in this area. The only reviews that have noted this difference are those of Stoll and Spector [96] published 35 years ago and a more recent review by Lamaziere et al., [97]. Most studies in this area tend to restrict themselves to the effects of particular fats on specific functions (e.g. protein kinase C activation in MCF-7 cells [98]; antibody production in B9 hybridomas [99]; production of prostacyclin in cultured bovine aortic endothelial cells [100]; secretion of plasminogen activator inhibitor type I in HepG2 cells [101]). Consequently, the recognition that culture changes the fatty acid profile of cells has been largely lost.

The exceedingly low level of PUFA in cell lines however presents a unique opportunity to examine the effect of these important fats on cellular functions. This is particularly the case in cell lines that have exceedingly low levels of PUFA such as MDCK cells with 8% [96] and HepG2 cells with 3.9% [102] (verified in our own lab at 3.6% and 5.5% of total fatty acid of PUFA respectively when incubated in 10% FBS). Table 6 shows how the supplementation of cell lines with various fatty acids can both inhibit or stimulate a wide variety of cellular functions. These effects can be different to those associated with the immediate effects of fatty acid addition as shown for I_{Na+} [103] and Na^+/Ca^{2+} activity [104] in Table 6. Prolonged addition of fatty acids (i.e. their presence in culture media) being more likely to influence cell function via modification of membrane properties and/or level of protein expression. Many studies use cell lines (particularly those from humans) to express proteins (receptors, channels, transporters etc) in order to examine some functional characteristics and interpret these to those that occur in natural tissues. However, there is often an underling basic assumption that the lipid composition of cell

lines is similar to that of the original tissue. Since this is not the case for fatty acids then the results and conclusions draw may not fully represent the functional characteristics of expressed proteins if they are influence by the lipid composition of the cell. However, irrespective of some inherent problems, cells in culture remain one of the most important research tools available to biomedical researchers. The intention of this contribution is to improve this model by recognising the need to consider the fatty acid composition of cells in culture.

11. Conclusions and Perspectives

Culture conditions change the fatty acid profile of cells making them unlike natural cells with very low PUFA and high MUFA levels. The common practice of incubating cells in media with 10% sera (normally fetal bovine serum) results in cells incubated in 2-3% of the fatty acid and cholesterol concentration, 1% of the polyunsaturated fatty acid concentration and 0.3% of the concentration of the essential fatty acid linoleic acid compared to normal cells. Subsequently, the relative fatty acid profile of cells in culture show a 2.4-fold decrease in polyunsaturated fatty acid and a doubling in monounsaturated fatty acid (i.e. MUFA; mostly 18:1*n*-9), but similar levels of saturated fatty acid compared to normal cells. These changes are not due to cell lines being primarily derived from cancer cells as cancers *in situ* possess fatty acid profiles far more similar to those of tissues in which they reside. Overall, the consequence of culturing cells in similar media with low lipid content and low PUFA produces cells with similar lipid profiles that are distinctly different from cells *in situ*. These changes are not restricted to cell lines as stem and primary cell from vertebrates are similarly affected when in culture. Since the remodelling of stem cell lipids during differentiation include major increases in PUFA containing phospholipids [66] inadequate PUFA availability could impede studies in this area. Also, as the world looks towards future food formed from cells in culture (e.g. meat replacements [105]) the question as to their fatty acid profile compared to that of the natural foods they are replacing seems reasonable. Cell culture practice needs to address the lipid requirements of cells in culture. With the increasing use of chemically defined media the need for lipids must be considered. Since cells in culture readily take up lipid from their media, lipid additives need to be considered in order to establish lipid compositions that match those of natural tissues and ensure more natural responses of cells in culture.

Acknowledgements

I would like to thank Professors David Adams, Kylie Mansfield, Todd Mitchell and Mirella Dottori for useful comments on various versions of this manuscript.

Declaration of Interests

None association with this manuscript.

Funding

This research was facilitated by a small Continuity Grant provided by the Faculty of Science Medicine and Health at the University of Wollongong.

Table 1: Major Fatty Acids as a Relative Percentage in Plasma and Serum of Vertebrates

Species Source	Type	Saturated Fatty Acids (SAT)			Monounsaturated Fatty Acids (MUFA)		Polyunsaturated Fatty Acids (PUFA)										∑SATS	∑MUFA	∑PUFA	PI	Reference
						n-9		*n-6*				*n-3*									
		Other	***16:0***	***18:0***	***Other***	***18:1***	***Other***	***18:2***	***20:2***	***20:3***	***20:4***	***18:3***	***18:4***	***20:5***	***22:5***	***22:6***					
Bovine^{+S}	Fetal	3.1	26.3	14.5	11.9	21.0		3.6		1.8	4.4		6.8	1.0	2.6	3.3	43.9	32.9	23.5	72	[44]
Bovine^{+S}	Fetal	6.1	22.7	12.2	12.8	20.4	6.8	5.5	1.0		3.6	0.4		0.7		1.8	46.0	33.2	19.8	40	[28])
Bovine^{+S}	Fetal	1.4	21.9	12.4	6.8	27.7	6.3	6.2			9.6	0.3				3.5	35.7	34.6	25.9	66	[96]
Bovine^{+S}	Fetal	4.1	27.3	12.6	12.9	19.5		5.6		2.0	7.7		1.8	0.8	1.9	3.0	44.0	32.4	22.8	64	[46]
Bovine^{+S}	Fetal	10.6	24.1	10.9	13.8	18.5	1.5	4.7	0.3	1.7	8.2	0.5		0.6	1.9	2.8	45.6	32.3	22.2	61	[106]
AVE	**Fetal**	**5.1* ±1.6**	**24.5 ±1.0**	**12.5 ±0.6**	**11.6*** ±1.2**	**21.4* ±1.6**	**2.9* ±1.5**	**5.1*** ±0.5**	**0.3 ±0.2**	**1.1 ±0.5**	**6.7 ±1.2**	**0.2 ±0.1**	**1.7 ±1.3**	**0.6 ±0.2**	**1.3* ±0.5**	**2.9 ±0.3**	**43.0 ±1.9**	**33.1*** ±0.4**	**22.8** ±1.0**	**61 ±5**	
Mouse^{+S}	Adult	3.9	18.2	13.8	6.8	13.6	0.3	18.1	0.1	1.6	17.6	0.3		0.1	0.1	5.4	35.9	20.4	43.6	104	[107]
Rat^{*P}	Adult	1.4	25.2	17.3	3.6	6.7	0.3	18.4		0.7	21.1	0.2		0.1	0.5	3.3	43.9	10.3	44.6	103	[108]
Rabbit^{*S}	Adult	1.0	18.5	23.9	2.8	12.2		24.4	0.9		10.5	3.7		0.4		1.7	43.4	15.0	41.6	74	[109]
Cat^{+S}	Adult		10.7	14.8		20.6		38.6			7.7						25.5	27.2	46.3	62	[110]
Cheetah^{+S}	Adult	3.7	31.9	49.0	1.0	4.1		6.8	0.2		1.2	0.2		0.1			84.6	5.1	8.5	11.5	[111]
Dog^{+P}	Adult	1.0	13.3	21.2	4.0	14.2	1.1	23.8		0.9	13.4	0.4		2.3	2.0	2.3	35.5	18.2	46.2	98.8	[112]
Pig^{+S}	Adult		16.9	13.9	2.5	28.4		20.9			10.6	0.4				0.5	30.8	30.9	32.4	56	[96]
Human^{*S}	East Asian		29.9	13.6		10.9	0.81	20.9		2.6	8.9	0.3		1.0	0.8	3.0	43.5	10.9	38.3	78	[113]
Human^{+P}	Caucasian	1.5	23.4	7.0	2.6	21.8	1.4	33.1	0.2		5.6	1.1		0.6	0.3	1.3	31.9	24.4	43.6	66	[11]
Cow^{+S}	Calf	0.3	15.2	14.2	2.4	16.9	0.6	46.4			3.6	1.1				0.4	30.5	18.8	50.5	60	[96]
Horse^{+P}	Adult	3.1	21.3	21.3	2.2	10.8	0.5	33.5		0.7	1.2	1.0	0.2			0.8	45.7	13.0	37.9	46	[114]
Elephant^{+P}	Adult	3.2	22.8	10.9	1.0	16.0		25.3	0.4		8.0	5.5					36.9	17.0	39.2	61	[115]
Chicken^{+P}	Adult	1.2	22.3	15.8	2.4	19.0	2.1	24.8	0.3	1.4	9.0	0.6		0.2	0.4	0.4	39.3	21.4	39.2	67	[116]
AVE	**Adult**	**1.6 ±0.4**	**20.7 ±1.7**	**18.2 ±2.9**	**2.4 ±0.5**	**15.0 ±1.8**	**0.6 ±0.2**	**25.8 ±2.8**	**0.2 ±0.1**	**0.6 ±0.1**	**9.1 ±1.6**	**1.1 ±0.5**	**0.0 ±0.0**	**0.4 ±0.2**	**0.3 ±0.2**	**1.5 ±0.5**	**40.6 ±4.1**	**17.9 ±2.0**	**39.4 ±2.9**	**68 ±7**	

Fatty acid expressed as mole % recalculated were necessary from original data. ∑ includes all fatty acids listed in original paper. Other refers to fatty acids not identified within fatty acid classifications and when across fatty acid groups other were proportioned according to relative amount of SAT, MUFA and PUFA in serum/plasma. Compositional data from either total fatty acid^{+}, total phospholipid* or not identified. * $p<0.05$, ** $p<0.01$, *** $p<0.001$ differences between fetal versus other sera. Note FBS is sometimes called fetal calf serum even though a calf is a bovine in the first year of its life postnatally. PI is the peroxidation index calculated as PI=∑1*(% di-PUFA)+2*(% tri-PUFA)+3*(% tetra-PUFA)+4*(% Penta-PUFA)+5*(% Hexa-PUFA). Statistical analysis compares average for FBS against average for other vertebrate plasma and sera with * $p<0.05$, ** $p<0.01$, *** $p<0.001$.

Table 2: Major Fatty Acids of Cells Lines

Tissue	Species	Detail	Saturated Fatty Acids (SAT)			Mono-unsaturated FA's (MUFA)		Polyunsaturated Fatty Acids (PUFA)									∑SATS	∑MUFA	∑PUFA	PI	References
							n-9		*n-6*				*n-3*								
			Other	*16:0*	*18:0*	*Other*	*18:1*	*Other*	*18:2*	*20:2*	*20:3*	*20:4*	*18:3*	*20:5*	*22:5*	*22:6*					
LIVER	Rat*	H35 Hepatoma	3.0	15.0	16.7	20.9	36	0.1	1.2	1.2	0.4	2.3		0.5	1.2	1.5	34.7	56.9	8.4	24.7	[44]
	Rat*	H35 Reuber Hepatoma	5.7	18.6	16.3	16.1	28.3	2.1	1.9	0.9	0.7	4.2		0.7	1.9	2.7	40.6	44.4	15.1	47.0	[46]
	Human+	HepG2 Carcinoma	6.4	37.0	7.1	15.6	31.5		1.5			0.5		0.4	0.2	0.3	50.5	47.1	2.9	6.9	[101]
KIDNEY	Dog*	MDCK Proximal Tubule	3.9	13.8	16.4	10.5	45.3	1.1	2.0			4.2				1.0	34.1	55.8	8.3	21.8	[96]
	Monkey*	CV-1 Epithelia	11.1	24.3	14.5	17.1	23.0		2.5			3.7				1.5	45.4	39.1	7.7	21.1	[117]
BRAIN & NEURAL	Mouse*	HT-22 Hippocampal	1.9	23.3	14.8	19.5	23.2	0.6	1.6			8.5	0.4	0.6	1.9	2.2	42.0	42.7	15.2	48.9	[43]
	Mouse*	SN56 Septal Neurons	4.6	25.9	12.6	18.4	23.2		2.5			8.3		0.4	1.4	2.4	43.1	41.7	15.0	47.4	[43]
	Human*	SH-SY5Y Neuroblastoma		37.1	18.9		23.9		2.8			11.1				6.1	56.0	23.9	20.0	66.6	[22]
	Human+	CHP-212 Neuroblastoma	4.0	27.7	15.3	4.3	26.6	3.6	4.6		0.5	4.4	1.4	0.9	1.6	1.6	47.0	30.9	18.6	50.5	[60]
LUNG	Human*	A549 Alveolar Epithelia	4.6	34.2	15.6	10.1	25.9		2.8			5.4				1.5	54.4	36.0	9.7	26.5	[47]
	Human*	IMR90 Fetal Lung Myofibroblasts		19.8	17.2	4.6	30.3	0.9	3.0	0.3	2.2	8.1	0.8		2.0	3.9	35.4	34.1	21.2	63.8	[118]
	Human*	16HBE Bronchial Epithelia	40.6♣			40.4		0.3	2.1			6.5		0.6	2.4	3.0	40.6	40.4	14.9	49.8	[119]
BLOOD CELLS	Mouse+	L1210 B-Lymphocyte	1.9	16.5	22.4	4.0	34.4	1.9	2.8	0.1	1.2	6.2	1.1	0.9	1.4	1.7	40.8	38.4	17.3	49.3	[90]
	Mouse+	WEHI-3 Macrophage	2.2	25.9	19.5	4.2	37.6		3.3			5.3				0.4	47.2	41.8	9.0	21.2	[61]
	Mouse+	J774A.1 Macrophage	1.4	33.0	10.9	11.5	24.3		3.6			9.5				2.9	45.7	35.8	16.0	46.6	[61]
	Human+	Jurkat T-Lymphocyte	8.0	31.0	23.8		19.1	3.0				11.2				4.0	64.8	18.9	16.2	55.4	[120]
	Human+	Raji B-Lymphocyte (CD19)	4.4	24.9	20.3	15.0	20.1	1.8	3.9		1.2	4.4	0.3	0.6	1.7	2.8	49.6	35.1	16.7	47.9	[29]
	Human+	THP-1 Macrophage	5.3	28.8	16.4	15.6	22.0	1.7	1.5		1.0	3.5	0.1	0.5	1.4	2.9	50.5	37.6	12.6	40.4	[29]
GUT	Human+	Caco-2 TC7 Colorectal Epithelium	5.7	17.4	11.2	17.6	29.7	2.1	3.9			2.9	0.1	1.0		0.6	34.3	47.3	9.2	24.0	[28]
	Human+	SW-620 Colon Carcinoma	3.3	28.3	15.6	5.6	24.3	2.2	4.7		1.3	6.0	0.8	1.0	1.4	3.3	47.2	29.9	22.4	65.5	[60]
	Human*	HT29 Colonic Adenocarcinoma	4.5	24.9	9.1	17.3	28.1		2.2			5.9		0.7		2.3	38.5	45.4	11.1	34.2	[45]
OTHER																					
Larynx	Human+	Hep2 Larynx	4.9	23.2	10.2	19.3	21.1	1.6	3.8	6.1	1.2	3.0			1.4	4.2	38.3	40.4	21.3	58.8	[121]
Cervix	Human*	HeLa	8.3	48.0	9.4	4.0	7.6		1.4		12.2	2.2					65.7	11.6	15.8	32.4	[122]
Pancreas	Rat+	AR42J	4.1	29.8	15.7	3.7	24.8		3.2			3.7	3.4	0.5		1.6	49.6	28.5	12.4	30.8	[123]
Breast	Human*	MCF-7	4.8	13.0	4.2	24.8	38.8		1.6	0.1	0.4	1.9			0.7	1.6	22.0	63.6	6.3	19.0	[98]
		AVERAGE (Cell Lines)	**4.2**** ±0.5	**25.9** ±1.6	**14.8** ±0.9	**12.8***** ±1.8	**26.0***** ±1.9	**0.9*** ±0.2	**2.6***** ±0.2	**0.4** ±0.3	**0.9** ±0.5	**5.3***** ±0.6	**0.3** ±0.2	**0.4** ±0.1	**0.8** ±0.2	**2.2*** ±0.3	**44.7** ±1.9	**38.7***** ±2.3	**13.7**** ±1.0	**40.0***** ±3.3	

All values are relative mole percentage (original data has been converted to mol % where required). Other includes all other fatty acids listed in the original paper. Original analysis of either total fatty acid+, total phospholipid* or not identified, ♣ Includes both 16:0 and 18:0 as well as other SATs. Cell lines were cultured with 10% FBS without lipid supplementation. PI is the peroxidation index calculated as PI=∑1*(% di-PUFA)+2*(% tri-PUFA)+3*(% tetra-PUFA)+4*(% Penta-PUFA)+5*(% Hexa-PUFA). Statistical analysis compares average values of cell lines (Table 2) to those in natural tissues (Table 3) with * $p<0.05$, ** $p<0.01$, *** $p<0.001$.

Table 3: Major Fatty Acids of Natural Tissues

Tissue	Species	Detail	Saturated Fatty Acids (SAT)			Mono-unsaturated FA's (MUFA)		Polyunsaturated Fatty Acids (PUFA)									ΣSATS	ΣMUFA	ΣPUFA	PI	References
							n-9		*n-6*				*n-3*								
			Other	***16:0***	***18:0***	***Other***	***18:1***	***Other***	***18:2***	***20:2***	***20:3***	***20:4***	***18:3***	***20:5***	***22:5***	***22:6***					
LIVER	Mouse*		0.3	17.0	16.2	5.9	9.1	2.4	10.4		2.2	23.2		0.2	0.5	11.8	33.5	15.0	50.7	152.2	[15]
	Rat*	Hepatocytes (Isolated)	0.6	19.3	19.1	1.0	10.3		17.0			23.5				9.5	39.0	11.3	50.0	135.0	[124]
	Rat*		0.6	14.3	22.9	5.1	3.8	0.5	9.9		0.8	32.7	0.1	0.2	0.8	6.9	37.8	8.9	52.5	151.1	[15]
	Human*		5.8	16.4	10.2	5.2	9.4	2.0	2.0			21.6	1.2	5.1	6.4	14.8	32.4	14.6	53.1	197.2	[16]
KIDNEY	Mouse*			12.9	15.8	8.7	7.8	2.2	9.5		2.0	23.3			1.0	16.0	29.0	16.5	54.6	177.7	[15]
	Rat*		0.2	18.8	18.5	9.6	6.8	1.3	8.6		0.6	32.9			0.2	2.0	37.9	16.4	45.6	123.2	[15]
	Dog +	Brush Border	4.6	30.0	12.2	3.1	12.7		11.5			23.8				0.5	48.6	15.8	35.8	85.4	[125]
	Human	Infant	4.5	21.6	13.8	4.9	16.5	1.0	18.3	0.6	1.6	14.8	0.3	0.3	0.4	1.3	39.9	21.4	38.6	79.4	[126]
	Human	Adult	1.1	33.7	12.8	3.2	16.3	2.7	7.8	0.2	0.3	8.9		1.2	1.0	1.4	47.5	19.6	23.5	57.4	[21]
BRAIN & NEURAL	Mouse*	Brain	0.1	21.9	15.8	10.3	16.5	3.5	0.5		0.4	10.5	0.1	0.3	0.2	17.7	37.8	29.2	33.2	134.3	[15]
	Rat*	Brain	0.3	16.9	13.6	10.0	17.0	5.7	0.5		0.3	13.7	0	0.3	0.1	19.3	30.8	29.0	39.9	158.4	[15]
	Human*	Cerebellum		33.3	18.9		22.9		0.7			8.8				15.4	52.2	22.9	24.9	104.1	[22]
	Human	Mitochondria		23.9	22.5		24.5	5.1	0.3			4.3			2.5	9.9	46.4	24.5	22.1	72.7	[109]
LUNG	Rat+		2.5	32.4	7.9	8.0	18.7	3.3	15.2		0.4	8.7	0.7		0.7	0.6	42.8	26.7	27.8	52.9	[127]
	Human+		1.6	30.6	9.6		27.1	2.5	6.8			12.0	2.0	0.9	3.0	3.9	41.8	27.1	31.1	89.1	[17]
BLOOD CELL	Rat+	Red Blood Cells	2.5	28.0	13.1	4.6	8.3	1.6	8.0	0.3	0.7	24.6	0.1	0.6	2.4	4.1	43.6	12.9	42.4	121.4	[108]
	Human	Red Blood Cells		29.6	17.1	1.6	19.2	2.1	13.3			13.4	0.3	0.5		2.9	46.7	20.8	32.5	74.8	[128]
	Human+	$CD4^{+}$ T-Lymphocytes	4.1	27.2	26.9	5.2	7.9	3.2	6.9		1.5	12.8	0.4	0.1	1.3	2.7	58.2	13.1	28.9	77.2	[29]
	Human+	$CD19^{+}$ B-Lymphocytes	5.5	31.8	30.5	5.1	7.1	2.7	4.7		0.9	8.2	0.5	0.1	0.7	2.0	67.8	12.2	19.8	53.0	[29]
	Human+	$CD14^{+}$ Monocytes	2.4	20.4	24.2	3.5	14.5	5.0	6.6		1.4	16.4	0.2	0.1	2.4	3.1	47	18	35.2	99.3	[29]
GUT	Human+	Colorectal Full-Thickness	2.1	25.3	9.4	5.7	32.4	0.7	12.1	0.5	1.1	5.1		2.3		2.8	36.8	38.1	24.6	55.4	[129]
OTHER																					
Skin	Human+	Basal Layer	8.0	21.1	18.0	3.1	15.8		20.9	0.6	3.4	7.8				1.2	47.1	18.9	33.9	57.7	[130]
Aorta Thoracic	Human	Tunica Media	6.6	24.3	19.8	2.8	19.8		18.2			8.5					50.7	22.6	26.7	43.7	[131]
Cervix	Human*	Epithelia		19.7	30.9		11.9		11.4	13.2		13.1					50.6	11.9	37.7	63.9	[132]
Pancreas	Rat+		4.1	26.6	6.7	3.6	23.7		25.1			5.8					37.4	27.3	30.9	42.5	[133]
Adipose	Rat+		1.1	24.9	3.7	8.3	35.5		20.0			0.3	2.4		0.1	0.2	31.5	43.8	23.0	27.1	[108]
Breast	Human*	Glandular tissue	2.6	24.9	4.0	7.0	40.8	1.2	16.9		0.2	0.4		0.3		0.6	31.5	47.8	19.6	26.8	[134]
Heart	Human*	Biopsy	2.9	16.9	14.1	9.8	7.6	1.0	19.3		0.7	21.3	0.2	0.5	1.4	4.5	33.9	17.4	48.9	117.7	[23]
Skeletal Muscle	Human*	Vastus lateralis	2.1	25.8	13.4	1.0	12.2		29.9		1.2	9.9	0.4	1.0	1.2	1.9	41.3	13.2	45.5	81.1	[135]
Skeletal Muscle	Human*	Quadriceps		15.7	12.2	2.2	8.6	1.4	33.0			16.6				2.3	27.9	10.8	53.3	97.1	[136]
		AVERAGE (Tissues)	**2.2** ±0.4	**23.5** ±1.1	**15.8** ±1.3	**4.6** ±0.6	**16.2** ±1.7	**1.7** ±0.3	**12.2** ±1.6	**0.5** ±0.4	**0.7** ±0.2	**14.2** ±1.6	**0.3** ±0.1	**0.5** ±0.2	**0.9** ±0.2	**5.3** ±1.1	**42.2** ±1.7	**21.1** ±1.7	**35.2** ±2.2	**90.9** ±8.4	

See Table 2 legend.

Table 4: Total Phospholipids and Lipids of Cells in Culture and Tissues

		Unit	Glycerol phospholipids					Sphingo-lipids	Cholesterol Forms		Glycerides		Free Fatty Acids	Unident-ified / Other	% Neutral Lipids	% Polar Lipids	References/units
			PC	PE	PS PS+PI♠	PI	Other	SM	C	CE	TG	DG	FFA				
PHOSPHOLIPIDS																	
Cell Lines	K562 (Human proerythrocytes)	Mol%	58.3	24.6	5.2	6.5		5.8								100	[137]
	HL60 (Human promyeloblast)	Mol%	52.8	21.7	3.9	11.7		11.1								100	[137]
	C1300 N18 (Mouse neuroblastoma)	Wt %	57.0	18.0	5.5	11.5	0.09	7.9								100	[138]
	NF1T (Human Schwann Cell)	Mol%	50.0	24.6	3.9	12.5		9.0								100	[139]
	MDCK (Dog kidney epithelia)	Wt %	42.8	23.2	7.6	8.8	17.7									100	[96]
	HepG2 (Human)[PM]	Wt%	42.3	27.8	7.0	4.4	12.4	6.1								102	[140]
	EA.hy926 (Human endothelia)		55.1	14.0	3.1	7.0		23.0								100	[141]
	3T3 (Mouse Fibroblast)	Wt%	47.6	23.9	7.6	8.5	12.4									100	[96]
	HT29 (Human colon) in culture	Mol%	46.7	22.3	12.3	8.3	1.6	6.4								98	[142]
	HT29 (Human colon) in tumour in nu/nu Mice	Mol%	43.7	35.2	6.1	8.1	2.8	4.0								100	[142]
		Average	**49 .6** ± 1.9	**23.5** ± 1.8	**6.2** ± 0.8	**8.7** ± 0.8	**4.7** ± 2.2	**7.3** ± 2.1								100	
Tissues	Mouse Brain	P%	45.7	16.7	11.8	14.0		11.5								100	[143]
	Rat Brain	P%	36.8	36.4	11.8	3.1	4.9	5.7								100	[144]
	Mouse Kidney	P%	33.4	22.2	14.5	14.1		15.3								100	[143]
	Mouse Liver	P%	45.7	16.7	11.8	14.0		11.5								100	[143]
	Mouse Heart	P%	38.1	22.5	12.5	12.1		14.1								100	[143]
	Calf Heart	P%	42.8	26.6	3.4	3.4	13.6	9.1								99	[144]
	Colonic Cells of nu/nu Mice	Mol%	51.3	19.7	2.9	8.2		14.8								97	[142]
		Average	**42.0** ± 2.3	**23.0** ± 2.6	**9.8** ± 1.8	**9.8** ± 1.9	**2.6** ± 1.9	**11.7** ± 1.3								99	
TOTAL LIPIDS																	
Cell Lines	HeLa (Human Cervical Cells)	Wt%	26.6	11.6	3.5♠		11.5	15.8	12.1		18.7				30.8	69.0	[49]
	L-M (Mouse Fibroblast)	Wt%	38.8	23.3	5.1♠		8.0	7.6	12.7	0.4	1.8		1.6	0.5	17.0	82.8	[145]
	L-929 (Mouse Adipose)	Wt%	36.8	18.7	8.6♠		9.1	7.3	9.5	0.4	6.5		1.0	2.0	19.4	80.5	[49]
	3T3 (Mouse Fibroblast)	Wtl%	37.6	16.7	10.2♠		3.0	8.1	14.8	3.3	2.2		3.0	1.0	24.3	75.6	[13]
	PC-3 (Human Prostate)	Mol%	51.1	13.3	5.5	1.0	1.1	6.9	19.3	0.2		1.0		0.7	21.2	78.9	[146]
	EPC (Carp Epithelial Papiloma)	Wt%	21.8	22.0	7.1	6.4	3.9	8.5							29.9	70.1	[147]
	EPC (Carp Epithelial Papiloma)	Wt%	23.7	20.9	7.7	6.0	4.7	8.7							28.0	72.0	[147]

	DHA (20μM) supplemented																
Primary Culture	Trout Keratinocytes - Initial	-	14.2	10.1	4.5	1.6	1.0	4.9	16.7	5.6	38.0		3.4		63.7	36.3	[48]
	Trout Keratinocytes - 14 day	-	30.3	13.6	7.6	5.0	1.6	4.6	24.6	6.3	3.4		3.0		37.2	62.7	[48]
	Trout Keratinocytes - 4 month	-	31.2	19.4	7.2	5.4	2.5	3.7	23.3	2.4	2.7		2.1		30.5	69.4	[48]
	Human Endothelia	Wt%	26.0	18.1	18.3♠			9.2	14.6	6.7	2.6		4.6		28.5	71.5	[148]
	Human Fibroblast	Wt%	40.7	12.6	4.0	5.8	4.8	9.6	10.0		8.6			0.4	19.0	79.0	[149]
		Average	**31.6** **± 2.8**	**16.7** ± 1.2	**7.4** **± 1.3**	**5.1** ± 1.0	**4.7** ± 1.0	**7.9** ± 0.9	**15.8** **± 1.7**	**3.2** ± 1.0	**9.5** **± 4.3**	0.1 ± 0.0	**1.6** ± 0.5	**0.4** ± 0.2	**29.1** **± 3.5**	**70.7** ± 3.4	
Tissues	Rat Liver	Wt%	55.2	19.9		4.4	4.5	2.1	5.4	1.5	6.7		0.32		13.9	86.1	[150]
	Rat Heart	Wt%	38.6	33.4		3.7	12.3	1.76	4.1	0.22	3.8	0.65		1.54	10.3	89.7	[150]
	Rat Brain	Wt%	26.9	20.5	9.1	3.2	9.6		30.3	0.3	0.3				69.3	29.1	[151]
	Human Liver	Wt %	18.2	4.9			0.4	1.2			75.3	0.1			24.7	75.4	[50]
	Human Atria (microsomal)	Wt%	24.5	16.8	4.1	6.0	8.0	6.9	5.5	2.8	20.6		4.8		33.7	66.3	[152]
	Human Ventricle (microsomal)	Wt%	34.4	19.8	2.6	6.5	10.5	5.4	5.2	5.2	6.9		3.4		20.7	79.2	[152]
	Human Pineal Gland	Wt%	35.4	17.5	7.6	6.6	1.9	10.4	12.4	1.4	4.1	2.1			20.0	79.4	[153]
	Human Pancreas	Wt%	17.5	8.6	3.1	3.0	1.9	4.4	2.8	1.4	47.4		9.8		61.4	38.5	[154]
		Average	**31.3** ± 4.4	**17.7** ± 3.1	**5.3** ± 1.3	**4.8** ± 0.6	**6.1** ± 1.6	**4.6** ± 1.3	**9.4** ± 3.7	**1.8** ± 0.6	**20.6** ± 9.5	**0.4** ± 0.3	**2.3** ± 1.3	**0.2** ± 0.2	**31.8** ± 7.8	**68.0** ± 7.9	
	Rat Erythrocytes	Wt %	24.1				1.4	2.0	5.7	16.0	48.7	0.5	1.7		72.6	27.5	[150]
	Rat Plasma	Wt %	32.0	20.8	3.1	3.4	0.9	8.2	30.2			0.4		1.0	31.6	68.4	[150]

Values are in Mol (mole), Wt (weight) and P (phosphorus) %. Compositional analysis is for either total of phospholipids or lipids. Other phospholipids include any unidentified phospholipids such as diphospatidylglycerol (cardiolipin), phosphatidic acid, phosphatidylglycerol and any lysophospholipids identified without head group. Average ± SEM. ♠ PI + PS.

Table 5: Major Fatty Acids of Cancer Cell Lines, '*in vivo*' Cancers and Tissues

Tissue Type	Detail	Saturated Fatty Acids (SAT)			Mono-unsaturated FA's (MUFA)		Polyunsaturated Fatty Acids (PUFA)									∑SATS	∑MUFA	∑PUFA	PI	References
						n-9		*n-6*				*n-3*								
		Other	***16:0***	***18:0***	***Other***	***18:1***	***Other***	***18:2***	***20:2***	***20:3***	***20:4***	***18:3***	***20:5***	***22:5***	***22:6***					
LIVER																				
Cell Line (rat) *	H35 Hepatoma	4.1	20.9	23.5	18.1	27.9	0.1	1.1	1.0		1.8		0.1	0.6	0.8	48.5	46.0	5.5	14.5	[44]
Cell Line **+**	Hep2G	6.4	37.0	7.1	15.6	31.5		1.5			0.5		0.4	0.2	0.3	50.5	47.1	2.9	2.9	[101]
Tissue (rat)*	Normal	0.6	14.3	22.9	5.1	3.8	0.5	9.9		0.8	32.7	0.1	0.2	0.8	6.9	37.8	8.9	52.5	151.1	[15]
Cancer	Rat (cell 7288CTC implant)	0.5	14.6	22.8	5.0	31.6		11.9			13.6					37.9	36.6	25.5	52.7	[155]
BRAIN																				
Cell Line*	HT22 Hippocampal (SV40 Immortalised)	1.9	23.3	14.8	19.5	23.2	0.6	1.6			8.5	0.4	0.6	1.9	2.2	42.0	42.7	15.2	48.9	[43]
Tissue*	Control Subjects (Normal)		26.4	22.7	2.5	23.0	4.8	0.8		0.8	8.9			0.3	8.3	49.1	25.5	23.9	86.2	[156]
Tissue*	Grey Matter (Normal)	10.5	27.1	23.6	0.5	18.4		1.6			6.6	0.8			10.9	61.2	18.9	19.9	77.5	[157]
Cancer*	Glioma		31.0	15.4	4.6	23.6	3.3	3.4		1.7	10.0		0.1	1.3	3.9	46.4	28.2	23.7	71.8	[156]
Cancer*	Meningioma	8.4	25.5	16.7	0.4	27.5		5.2			12.3	1.2			2.2	50.6	27.9	20.9	55.5	[157]
LUNG																				
Cell line*	A549 Alveolar Epithelia	37.5			41.3		0.3	2.0		0.9	6.1		0.9	0.6	2.9	37.5	41.3	13.7	43.8	[119]
Tissue**+**	Normal	1.6	30.6	9.6		27.1	2.5	6.8			12.0	2.0	0.9	3.0	3.9	41.8	27.1	31.1	89.1	[17]
Cancer**+**	Pulmonary Carcinoma	1.1	20.2	12.1		33.7	2.9	4.4			15.4	3.1	1.5	2.2	3.4	33.4	33.7	32.9	97.5	[17]
BREAST																				
Cell Line*	MCF-7 Adenocarcinoma	4.8	13.0	4.2	24.8	38.8		1.6	0.1	0.4	1.9			0.7	1.6	22.0	63.6	6.3	19.0	[98]
Tissue**+**	Normal (Adjacent)	2.6	24.9	4.0	7.0	40.8	1.2	16.9		0.2	0.4		0.3		0.6	31.5	47.8	19.6	26.3	[134]
Cancer**+**	Not Defined	2.3	24.7	6.2	6.6	38.7	0.7	15.2		1.0	2.0		0.4		1.3	33.2	45.3	20.6	33.4	[134]
COLON																				
Cell Line (Human)**+**	HT29 Adenocarcinoma	5.3	26.2	8.6	18.1	26.9		2.1			5.2		0.6		1.9	40.1	45.0	9.8	29.6	[45]
Cell Line (Human)**+**	Caco 2 Adenocarcinoma	7.8	21.3	17.0	12.4	20.4		2.3			6.5		1.4		1.1	45.8	32.8	11.2	32.5	[158]
Tissue**+**	Normal (Adjacent)	2.1	25.3	9.4	5.7	32.4	0.7	12.1	0.5	1.1	5.1		2.3		2.8	36.8	38.1	24.6	55.4	[129]
Cancer**+**	Adenocarcinoma	2.1	26.4	14.4	2.7	26.3	1.6	15.8		1.5	7.2		0.3		1.8	42.9	29.0	28.2	55.4	[129]
STEM CELLS																				
Human**+**	MSCs (bone marrow)	0.7	21.4	22.4	2.7	35.2		1.8		1.6	12.4					44.5	37.9	15.8	42.2	[53]

All values are relative mole percentage. Tissue and cancer are from human in vivo samples (except for some liver values). ∑ includes all fatty acids listed in original paper. Composition analysis of either total fatty acid⁺ or total phospholipid*. Other refers to those fatty acids not listed in the table plus those not identified or those summed rather than listed in the original source paper within each category. Cell cultures with 10% FBA and no lipid supplementation. Adjacent refers to normal healthy tissue next to cancer. PI is the peroxidation index calculated as PI=∑1*(% di-PUFA)+2*(% tri-PUFA)+3*(% tetra-PUFA)+4*(% Penta-PUFA)+5*(% Hexa-PUFA)

Table 6: Changes in Cell Function Following Fatty Acid Supplementation

Function/ Characteristic	Cell Type	Species and Organ	% Change with Fatty Acid Supplementation									Reference & Supplementation Concentration
			18:0	18:1*n*-9	18:2*n*-6	18:3*n*-3	18: *n*-3	20:3*n*-9	20:4*n*-6	20:5*n*-3	22:6*n*-3	
Cell Oxidant Production	HT29	Human Colon		6%	35%				94%	40%	429%	[45] 50μM
Mitochondrial Potential	HT29	Human Colon		-11%	-18%				-5%	0	11%	[45] 50μM
Mitochondrial Potential	Neuro-2A	Mouse Brain	0	-27%	-24%							[159] 70-140μM
Oxidisability (TBARs)	NCTC 2544	Human Skin	7%	4%	41%	105%			149%			[160] 50μM
Apolipoprotein A	HepG2	Human Liver	15%		12%	3%	7%			5%	11%	[161] 50μM
Apolipoprotein B	HepG2	Human Liver	14%		15%	26%	29%			31%	41%	[161] 50μM
Plasminogen Activator Inhibitor Type 1 (PAI-1)	HepG2	Human Liver		15%	44%							[101] 35μM
Chymase	C2	Canine Mast Cells			-16%	-13%						[162] 14μM
Tryptase	C2	Canine Mast Cells			41%	-42%						[162] 14μM
Histamine Release	C2	Canine Mast Cells			15%	-22%						[162] 14μM
PGE_2 Production	C2	Canine Mast Cells			-6%	-19%						[162] 14μM
PGE_2 Production	Endothelia	Human Umbilical (HUVEC) in Primary Culture							13%	-46%	-29%	[100] 10μM
Cell Proliferation	Raji	Human B-Lymphocyte							82%	27%		[163] 25μM
Interlukin-10 (IL-10)	Raji	Human B-Lymphocyte							-58%	-70%		[163] 25μM
Tumour necrosis factor (TNF-α)	Raji	Human B-Lymphocyte							-30%	-41%		[163] 25μM
Interferon-gamma (IFN-γ)	Raji	Human B-Lymphocyte							-24%	-29%		[163] 25μM
Oncogene HER2/neu mRNA	MCF-7	Human Breast			-33%					60%		[164] 17μM
Stearoyl-CoA Desaturase Activity	3T3-L1	Mouse Adipocytes							-60%			[165] 300μM
Protein Kinase C (PKC) IGF-1 Stimulated	MCF-7	Human Breast			67%	56%						[98] 107μM
Mitogenic Response IGF-1 Stimulated	MCF-7	Human Breast			-19%	-30%						[98] 107μM
E-cadherin	MCF-7	Human Breast						87%		25%		[166] 50μM
E-cadherin	HRT-18	Human Colon						-69%		81%		[166] 50μM
Ca^+ Release Thrombin Stimulated	Endothelia	Human in Primary Culture						-49%				[56] 100μM
Alkaline Phosphatase	MC3T3-E1	Mouse Osteoblasts						-39%				[167] 10μM
Sodium Channel Flux (I_{Na})	hBSMCs	Human Bronchial Smooth Muscle	-8%	-7%					-47%	-64%	-74%	[103] 10μM in media not supplemented
Sodium Channel mRNA	hBSMCs	Human Bronchial Smooth Muscle							-55%			[103] 30μM
Na^+ /Ca^{2+} Exchanger	HEK293t	Human Kidney	-6%						-73%	-89%	-97%	[104] 10μM in media not supplemented
Na^+ Current	HEK293t	Human Kidney	1%	9%	41%	60%				58%	53%	[168] 5μM
K^+ Intracellular	Neuro-2A	Mouse Brain	3%	8%	2%							[159] 70-140μM
K^+ Permeability	Neuro-2A	Mouse Brain		-29%	-24%							[159] 70-140μM
Na^+ Permeability	Neuro-2A	Mouse Brain		106%	117%							[159] 70-140μM
Na^+, K^+-ATPase Activity	Neuro-2A	Mouse Brain	-9%	-4%	-8%							[159] 70-140μM

Percent difference is relative to standard culture conditions (at varying supplemented fatty acid concentrations as indicated in reference column).

Figure Legends

Figure 1: The relative percentage of saturated (SAT), monounsaturated (MUFA) and polyunsaturated (PUFA) fatty acids in natural tissues and cell lines derived from mammals (human, monkey, dog, rat and mice). Organs of origin include; liver, kidney, brain, lung, blood cells, gut, pancreas, cervix, breast gland, as well as skeletal muscle, adipose, skin and aorta for tissues and larynx for cell lines. Data is taken from Tables 2 and 3. Horizontal line represent mean values, N = 30 for tissues and 25 for cell lines.

Figure 2: Relationship between levels of precursor fatty acids (linoleic and α-linolenic acid) and one long-chain product in membrane phospholipids of cells and tissues of mammals. (A) show the relationship between the *n*-6 essential fatty acid, linoleic acid (18:2*n*-6) and its product arachidonic acid (20:4*n*-6). (B) shows the relationship between the *n*-3 essential fatty acid, α-linolenic acid (18:3*n*-3) and its product docosahexaenoic acid (22:6*n*-3). Original organs used for both cell lines and tissues include brain, blood cells, liver, kidney and lung. The 21 different cell lines (in closed symbols) were examined mainly from humans with a few rodent values. The 28 natural tissues (open symbols) were from species including rodents, pig, sheep, human, cattle and horse. Note: in cases where 18:3*n*-3 levels fell below the detection level a nominal value of 0.1 was assigned. References for cell lines include [29, 43, 44, 46, 47, 60, 61, 90, 96, 102, 117-119, 169, 170] and for tissues [15-17, 21, 29, 43, 108, 125, 127, 128, 171].

Figure 3: Membrane docosahexaenoic acid (DHA) phospholipid composition plotted against the concentration of DHA in cell culture media cross five different cell lines (SN56 Neuronal, HT22 Hippocampal, HT29 colonic; H35 liver, A549 lung) from four different studies. Values from [43, 45-47]. A DHA concentration value of 6 μM was used for 10% FBS culture media based on values from [9, 169, 170].

Figure 4: The relative percentage of saturated (SAT), monounsaturated (MUFA) and polyunsaturated (PUFA) fatty acids in liver, brain, lung, breast and colon, in natural tissues and *in situ* cancers from the same tissues compared against cell lines derived from the same five tissues. Most values were from human sources except liver that contains mainly rat tissues (and one human cell line HepG2). Values (and references) are from Table 5.

Figure 1

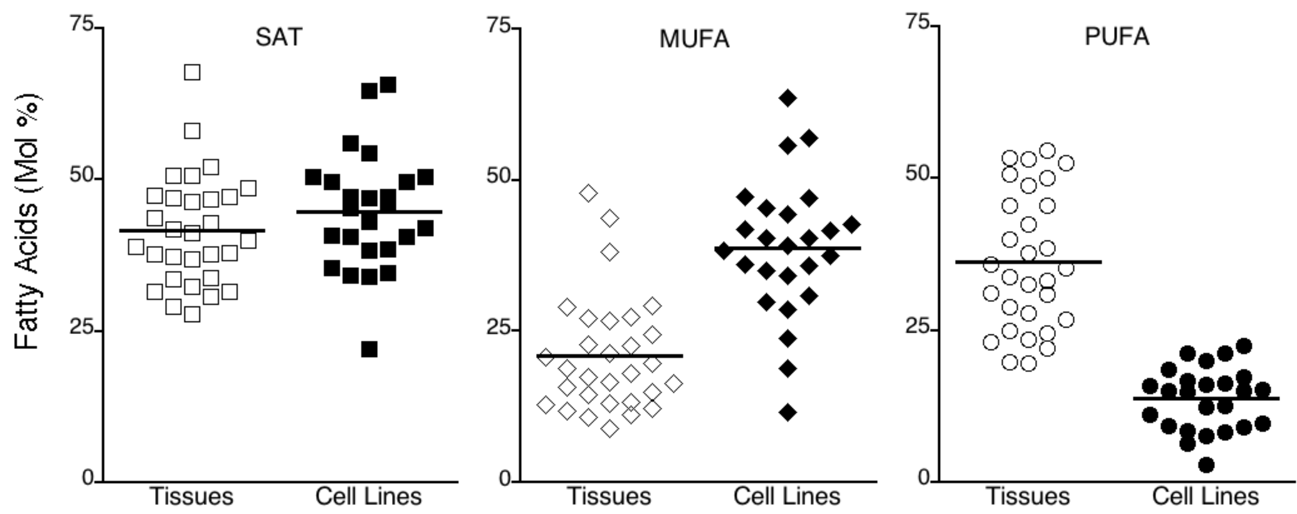


Figure 2

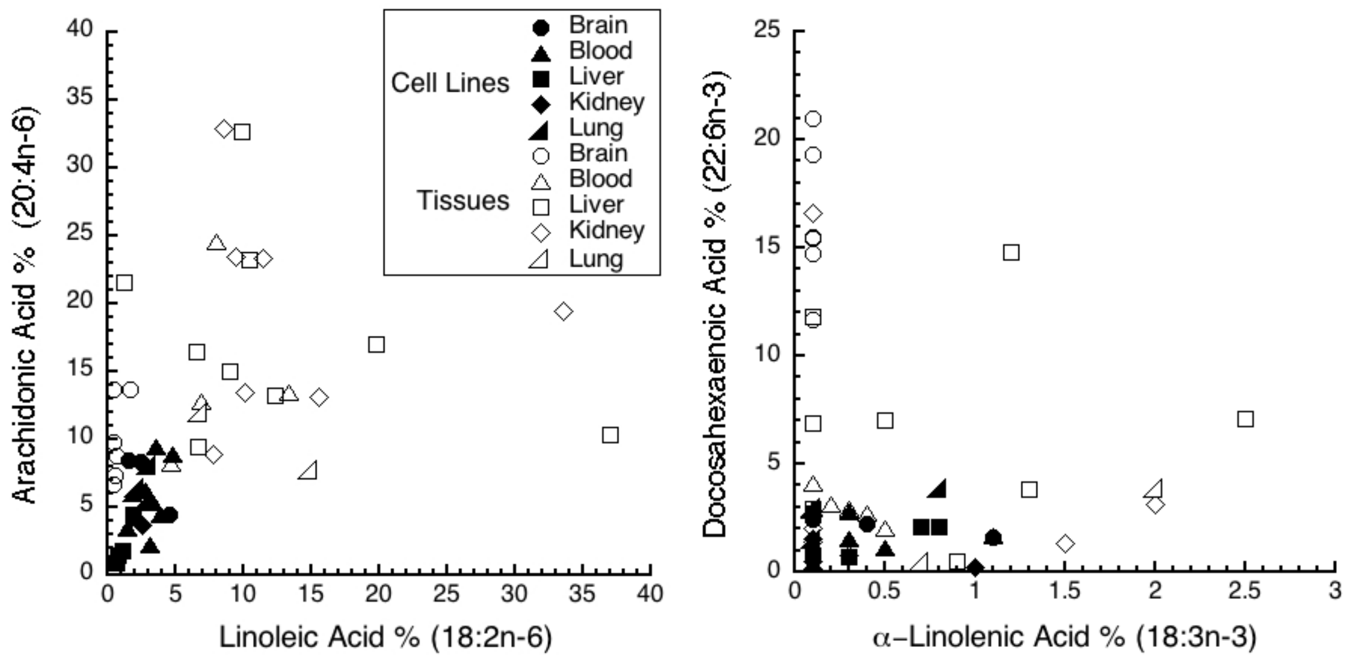


Figure 3

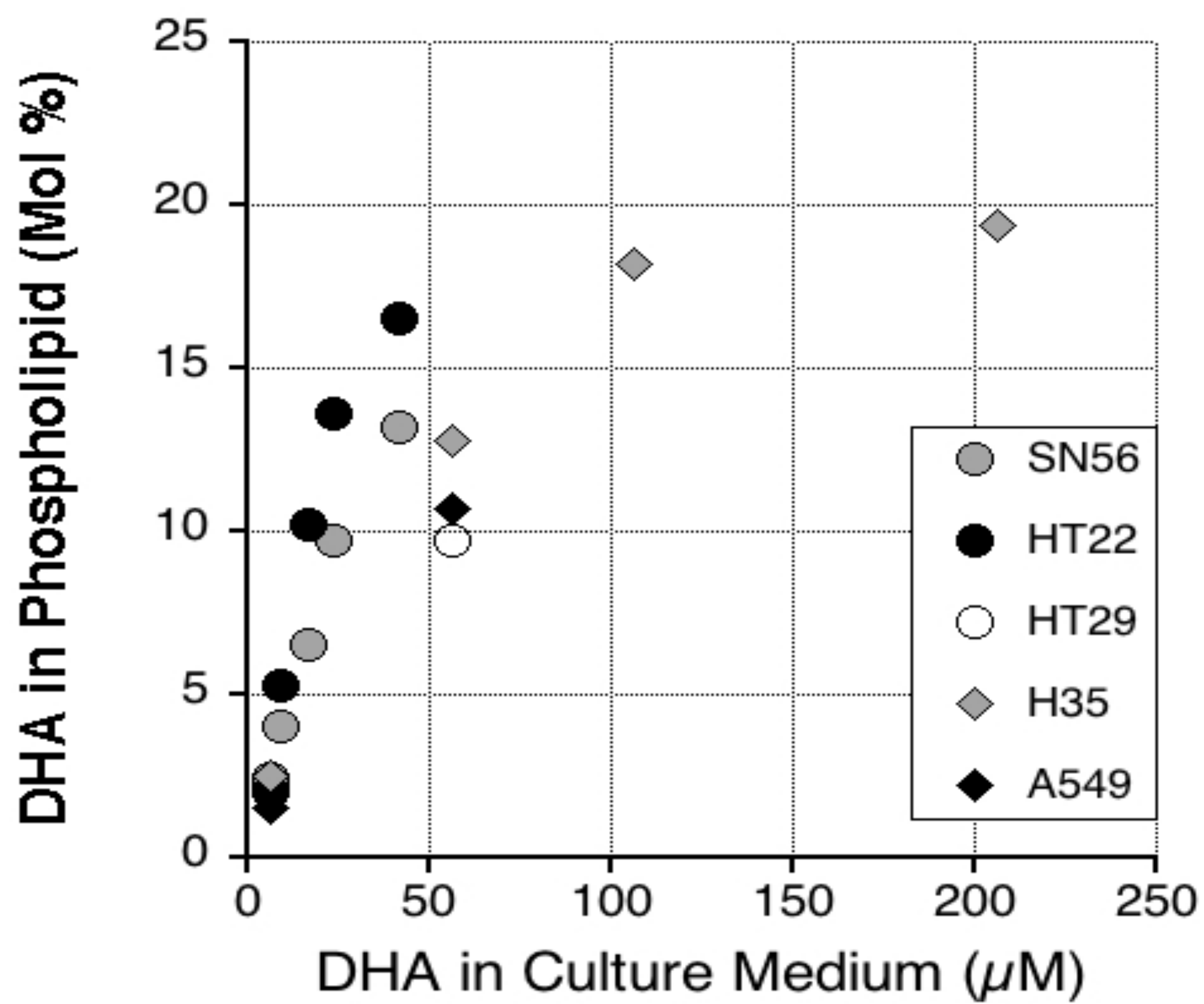
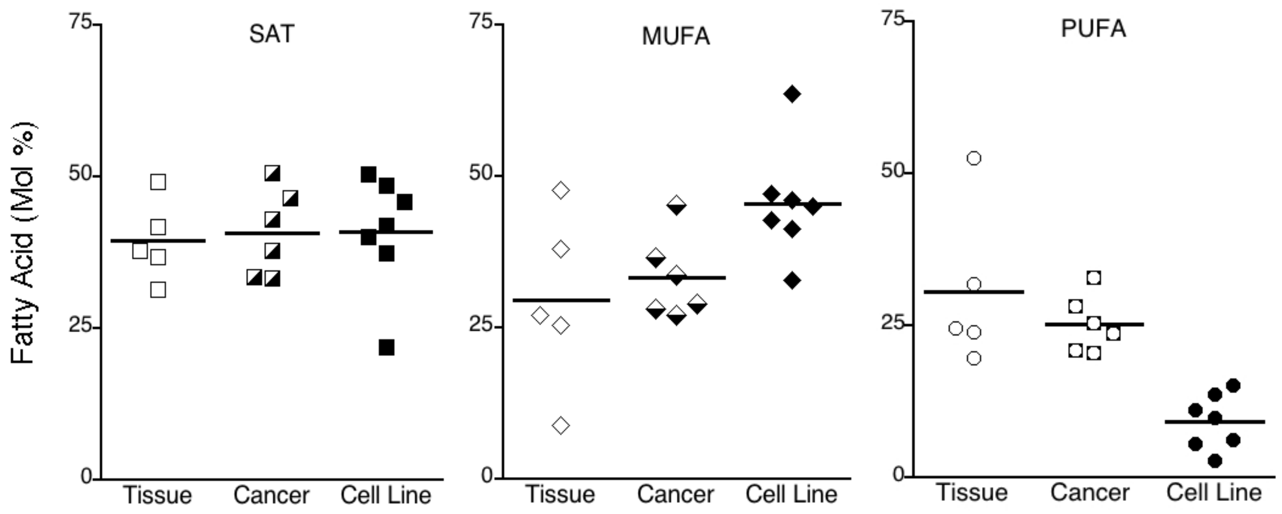


Figure 4



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