Relative and Absolute Quantitation of Metabolites and Lipids using LC/MS/MS on the TSQ Quantum Discovery MAX

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Dedication

For current and future UT graduate students who want to learn LC/MS/MS

Abstract

Two biological systems were studied using LC/ESI/MS/MS on a triple quadrupole operated in SRM (selected reaction monitoring) scan mode. The first bacterium system is aquatic and microscopic in size known as *Roseobacter.* The second mammalian system is terrestrial and large in size relative to humans known as Holstein cows.

Roseobacter is a clade of marine bacteria abundant in the ocean. Roseophages are viruses that infect *Roseobacter* and cause viral lysis. *Sulfitobacter* sp. 2047 was isolated and infected with Roseophages, and the fold change in the metabolic pool relative to a control was studied at discrete time points. The absolute concentration of glutamate and glutamine in the infected and control was determined at each time point using an external calibration curve. Flux analysis through the addition of ^{13}C-acetate at early and late post infection was compared to the control.

Holstein cows are a breed of cattle known to be the world's highest producers of milk. Twelve Holstein dairy cows were selected, and samples of blood and milk were taken at different weeks of lactation. The fold change in the phospholipid pool relative to the first week of lactation was studied from early, mid, and late lactation. The absolute concentration of lipids at each week of lactation was determined using isotope dilution mass spectrometry with the exception of GPC (glycerophosphocholine) where an external calibration curve was used due commercial unavailability of an isotope-labeled standard.

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Introduction

The TSQ Quantum Discovery MAX is a triple quadrupole capable of performing quantitative H-SRM (Highly-Selected Reaction Monitoring) scans. The additional selectivity is owed to the hyperquads, which have a hyperbolic profile compared to traditional quadrupoles that use round rods. The increase in selectivity decreases the chance of a false positive. The SRM scan results in two confirmation points of parent ion and product ion. When used in conjunction with liquid chromatography an additional confirmation point of retention time is obtained. These three confirmation points further decreases the chance of a false positive. Another compound would need to have the same parent ion, product ion, and retention time to result in a false positive.

Chapter 1 reports the metabolomics of an isolated *Roseobacter, Sulfitobacter* sp. 2047, in response to a Roseophage viral infection. Metabolomics is the simultaneous study of a large number of individual metabolites to provide a wholistic understanding of metabolic pathways. To achieve this goal, discrete time points are chosen strategically to monitor the change in the metabolic pool. Extraction solvent quenches the metabolism and provides a snapshot of the metabolic pools at each time point. However, the metabolic pool concentrations alone do not provide the complete story of how the virus changes the metabolism of the host. A difference in pool concentration can be the result of faster assembly of the metabolite or slower disassembly. A flux analysis is needed to complement the pool concentrations. The appearance of labeled metabolite in the pool determines the rate of assembly, and the disappearance of unlabeled metabolite determines the rate of disassembly.

Chapter 2 reports the lipidomics of twelve Holstein dairy cows after calving. Lipidomics is similar to metabolomics except the subject of study is lipids. The time points were chosen to be in week intervals extending to late lactation. Plasma and milk samples were collected to correlate the differences between the two. The idea is that the lipids in the milk are taken from the bloodstream. An increase in concentration of a lipid in the milk can be correlated to a decrease in concentration of the lipid in plasma. Isolating the week of lactation when a lipid is low in the plasma can provide insight on when supplementation of this lipid should be provided to the cow.

Chapter 1

Metabolic Response of a Roseobacter to Phage Infection: Insights into the Influence of Viral Lysis on Ocean Biogeochemistry

Material for Abstract, Introduction, Roseobacter Biology, Roseophage, and Molecular Tools and Marine Virus Communities taken from:

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Abstract

Viruses contribute significantly to the mortality of marine microbes and consequently alter species composition and influence biogeochemistry. While it is well established that host cells provide metabolic resources for virus replication, the extent to which the infection process reshapes host metabolism at a global level and the subsequent effect of this alteration on the quantity and quality of material released into the environment following viral lysis is less understood. To address this fundamental gap in knowledge, a LC/MS/MS metabolomics approach was used to quantify global intracellular metabolite changes in cultures and extracellular lysate profiles of phage infected *Roseobacter*. In contrast to a simple model in which all extracellular compounds were increased in concentration due to lysis, < 30% of the measured compounds displayed increased concentrations. The measurable extracellular metabolic profiles were significantly altered, including enrichments in amino acids involved in stress responses and acyl-CoAs. We hypothesize that phage infection leads to a cellular stoichiometric imbalance of C:N due to the redirection of host metabolism towards the production of more nitrogen rich metabolites, needed to support new virion production. Intracellular concentrations for 83 core metabolites were also measured over an infection cycle and by the end of the experiment, the majority (80%) were significantly elevated in infected populations. Experiments that monitor metabolite turnover using ^{13}C-acetate provide evidence that phage-infected populations have increased metabolic activity and directly scavenge metabolites released from lysed siblings, which offsets their increased need for nitrogen.

Background and Significance

Introduction

It is estimated that one billion tons of carbon are released in the world's oceans each day as a result of virus lysis.[1] The released dissolved organic matter (DOM) is a major contributor to marine microbial activity[2], stimulating both primary and secondary productivity[3-5]. Through their lytic activities, viruses influence the flow of carbon, nitrogen and other nutrients in the marine environments.[1, 4, 6-10] While previous studies have revealed that viral lysates can be rich in free and combined amino acids[3] and may be an important source of labile organic nitrogen,[11] we know relatively little of the distinct chemical character of viral-derived DOM. Furthermore, the extent to which viral redirection of host metabolism alters cellular constituents released as a result of lysis remains an open question.

Although the majority of viruses in aquatic systems are thought to be phage,[1, 12] relatively few environmentally relevant marine phage-host systems have been described. Bacteria belonging to the *Roseobacter* lineage are abundant in marine systems and carry out critical biogeochemical transformations.[13-17] Their abundance and amenability to cultivation make *Roseobacter* a tractable model for investigating the influence of bacteriophage on both host metabolism and DOM release.[13, 14]

Although there have been numerous studies of the composition of biologically produced DOM in marine systems,[3-5, 10, 18, 19] most have focused on characterization of the material bulk properties, such as C:N or the size distribution of the particulate matter. Only recently has the technology necessary to allow identification of specific compounds within DOM become available.[20, 21] However, the compositional heterogeneity of DOM makes it difficult to extract and detect specific metabolites from seawater. To address this concern, we have implemented methods that identify and characterize metabolites in marine bacteria and their culture filtrates and can be extended to studies of natural marine systems. Specifically, targeted liquid chromatography-

tandem mass spectrometry (LC-MS/MS) based metabolomics[20, 21] were used to monitor both metabolite concentrations and turnover rates in these systems. Herein we report a study of a roseobacter-roseophage system that probed the effect of viral infection on composition of both the intra- and extracellular water-soluble, small molecule metabolites, thus giving insight into both the impact on ocean biogeochemistry and host metabolism. These results provide support for the hypothesis that virally infected cells are physiologically distinct from their uninfected counterparts.

Roseobacter Biology

The most abundant and biogeochemically important organisms in the world's oceans are bacterioplankton. The application of molecular tools to the study of picoplankton has enriched the understanding of the diversity of picoplankton, which in turn, developed an image of the distribution of picoplankton members in the last 20 years.[22, 23] The target of this experiment is the *Roseobacter* lineage, which is one of the major marine bacterial lineages identified in independent cultivation surveys. Members of this lineage are classified as "model marine heterotrophs" and are studied to extrapolate conclusions for other marine heterotrophs. This lineage is considered as a model for the following three reasons. Firstly, they are abundant in the world's oceans. Secondly, they are easily cultured and maintained in the lab. Thirdly, they carry out relevant biogeochemical processes. Their abundance in the ocean is demonstrated by molecular-based experiments targeting the 16S rRNA genes, which shows that the Roseobacter clade typically comprise ~20% of coastal and ~15% of mixed layer ocean of the total bacterioplankton community.[15, 17, 22, 24] In conclusion, cultivated roseobacters serve as "model marine heterotrophs" to improve the understanding of marine bacterial ecology and physiology.

Roseobacters have been linked to carbon and nutrient cycling in both culture and field-based studies. The results from tracer studies determined that roseobacters are among the most active members in the bacterioplankton community in coastal and open ocean environments.[25-28] Isolated lab cultures and natural roseobacter populations have the capability of converting various aliphatic compounds containing carbon, nitrogen, and sulfur, in addition to, aromatic

compounds abundant in coastal environments.[29-33] Cultured roseobacter in the Buchan lab provides evidence that Fe is scavenged to produce structurally diverse siderophores, thus making roseobacter a reservoir of the element Fe, which was previously unrecognized. In addition to scavenging Fe, roseobacters appear to play a role in processes that affect sulfur cycling. Cultivated roseobacters are capable of converting inorganic sulfur and osmolyte dimethylsulfionio-propionate (DMSP). The algal DMSP is either cleaved to form dimethylsulfide (DMS) or demethylated to form methanethiol (MeSH). DMS readily fluxes to the atmosphere owing to its volatility where it affects cloud condensation nuclei and backscatters solar radiation.[34] MeSH is likely to reside around the surface of ocean water where it is utilized by marine bacteria. Therefore, the cleavage of DMSP, resulting in DMS formation, has important consequences on global climate regulation, while the demethylation of DMSP, resulting in MeSH, is importation in supplying carbon and sulfur to surface ocean marine bacteria. Further evidence for the metabolic potential of roseobacters stems from the availability of ~40 roseobacter genome sequences which results in diverse metabolic functions of the *Roseobacte*r lineage. In conclusion, the abundance, activity, and diversity of the Roseobacter clade suggest that relatively high growth and proliferation rates would occur in high-nutrient niches. The dense population of roseobacter in the high-nutrient niche could in turn support high Roseophage viral loads and activity.[1]

Roseophage

The importance of roseobacters in the biogeochemical cycling of elements has been recognized but, to date, there is a limited understanding of the phage that infects them. Roseophages are tailed dsDNA viruses with modest genome sizes of ~50-75 kb that infect roseobacter. Information on latency and burst size for roseophages is limited, but the information available suggests that these phages have the potential for high productivity. There are 9 roseophages described in the literature, while two are members of the *Siphoviridae* family, and the remaining seven belong to the *Podoviridae* family. A roseophage in the *Siphoviridae* family, the roseophage RDJL□1, infects *Roseobacter denitrificans* and has an estimated latency period of ~80 min and a burst size of ~200.[35] The characterized members in the *Podoviridae* family belong to the T7-like or N4-like genera. Two N4-like roseophages, DSS3□2 and EE36□1, were

isolated from the Chesapeake Bay area and have latency periods between 2-3 h and burst sizes that range from 350-1500.[36] These burst sizes are in disagreement with the average of 24 for bacterioplankton.[37] This can be explained because latency and burst size depend on the physiological status of the host and have been found to be shorter and larger, respectively, in cultured studies than in natural environments.[38, 39] Regardless of the conflicting data, the data provides a step forward in the understanding of the potential of roseophage to cause viral lysis and change host metabolism, especially in high-nutrient niches where the host would be expected to have a higher metabolically active dense population.

The N4-like group has become the model for studies of roseophage biology. The abundance of N4-like roseophage is greater in coastal environments compared to open ocean.[36] The sequences can be found in metagenome databases, and there is high sequence conservation between the two characterized N4-like roseophages, DSS3□2 and EE36□1, of the *Podoviridae* family. Although the conservation in sequence is high, both phages infect different host. Conservation in sequence is a poor predictor of preferred host as >85% of all ORFs share 80-94% sequence identity.[36] The correlation between the abundance of phage and host is yet to be determined, but the available data suggests a greater abundance of N4-like roseophages in habitats favored by their specific hosts.

Molecular Tools and Marine Virus Communities

Genetic diversity is a function of the total abundance of different virus types (richness), the numerical distribution of viruses within and across groups (evenness), and the differences in genetics within and across groups (relatedness). The comparisons of whole genomes have proven that conserved genes exist among different viral genomes and can be used as phylogenetic markers.[40] These genes have been used in diversity studies, and viruses that infect primary producers are targeted. The genes of interest are typically the cyanophage g20[41] and the *Phycodnaviridae* DNA polymerase genes.[42] The majority of roseophages have not been isolated in lab cultures as was determined by direct studies that sequenced viral fractions from seawater. The diversity of viral metagenomes from the fractions confirmed previous hypotheses that

roseophages exist as a diverse community, and previously unrecognized viral entities were identified.[43,44] To date much of the efforts to catalog the genetic diversity of marine viral communities have focused on richness and relatedness, while evenness, or specific quantification,[45-47] has received less attention.

Merits of Liquid Chromatography-Mass Spectrometry

Liquid chromatography (LC) separates compounds from one another resulting in a confirmation point of retention time (RT) and separates compounds from ion suppression zones reducing ion suppression of target compounds when using Electrospray Ionization (ESI). Direct infusion would result in all compounds and anything in the matrix present at the same time during ionization, in addition to, manually infusing each sample which is impractical for a high volume of samples. An autosampler can automate injections using flow injection analysis (FIA), but will still result in ion suppression. LC before ESI reduces ion suppression and produces the confirmation point of RT reducing false positives.

Mass spectrometry (MS) detection applies a mass filter that can separate compounds based on their difference in *m/z* removing the requirement of baseline resolution of chromatographic peaks when using UV-Vis as a detector. However, in some cases MS requires baseline resolution when the isotope of a compound passes through the mass filter of another compound. It would be almost impossible to know if an impurity co-elutes with a target compound when a shoulder is not present and erroneous quantitation would result using UV-Vis whereas MS can mass filter the impurity. The ability of MS to mass filter impurities also saves method development time as baseline resolution is not required and when switching from standards to samples the introduction of impurities does not require further method development to achieve baseline resolution of sample impurities.[48]

The detection limits of MS is generally lower than UV-Vis, because UV-Vis uses one wavelength for all compounds in the analysis even though each compound has its own optimum lamba max and mobile phases have absorbance that results in background that changes when gradients are used. MS/MS detection in Selected Reaction Monitoring (SRM) scan mode uses an optimized Collision Energy (CE) for each compound, and the background of mobile phases are mass filtered out of the chromatogram. The advantage of MS detection is clear, but MS has its own challenge of needing an ion whereas UV-Vis needs a chromophore. The ion is needed because neutrals are not affected by electric fields and cannot be guided to the detector nor mass filtered. Many different ion sources utilizing different ionization mechanisms have been developed to circumvent this problem, and derivatization of target compounds to increase ionization efficiency has been used.[49]

LC-MS results in two confirmation points of RT and *m/z* to identify a compound. More confirmation points and higher selectivity is obtained when performing MS/MS. The SRM scan mode on a triple quadrupole results in three confirmation points of RT, parent ion, and product ion. Further confirmation points are obtained by monitoring the same parent ion at other product ions to reduce false positives.[50]

Triple Stage Quadrupole (TSQ) Quantum Discovery MAX

ESI

The choice of an ion source should consider flow rate, molecular weight, polarity, thermal stability, dynamic range, and resistance to ion suppression.[51] An Electrospray Ionization (ESI) source is suitable for flow rates of 1 mL/min, molecular weights greater than 100,000 amu and multiple charging[52] allows a mass analyzer with lower mass range, polar compounds, heat labile compounds, and has a linear range of 3 to 5 orders of magnitude though ESI is not resistant to ion suppression.[53] ESI is stable and robust,[54] performing the same throughout the duration of an experiment. The merit of a large linear range in a multicomponent analysis is the ability to dilute the highest concentration compound to be in the linear range while still having the lowest concentrated compound in the linear range.

The theory of ESI for positive mode is that positive ions are preformed[55] in the solution controlled by the pH of the mobile phase, and then a positive charge is placed on the surface of the solvent using a spray voltage typically between 3 to 5 kV. A small droplet is dried with nitrogen gas, and the positive charges are repelled from each other moving to the surface of the droplet.[56] When the droplet is too small for the positive charges to move away from each other, a critical point is reached known as Rayleigh stability limit where electrostatic repulsions are greater than surface tension, and the droplet divides into small droplets. The smaller droplets are further dried with nitrogen, and the Rayleigh stability limit is reached again and so on until the positive ion is ejected from the droplet into gas phase.

ESI is a surface competition ionization mechanism, and when many ions are present not all ions will make it to the surface and be split into smaller droplets according to the charge residue process involving the formation of a Taylor Cone.[57] Ion suppression exist because as the droplet dries the more volatile organics dry faster than water. The more hydrophobic an ion is the more affinity it will have with the surface and the hydrophilic ions will be suppressed. ESI works best

with high solvent volatility, optimized pH of the solvent system but less than 10 mM of salt when using a buffer, and with an initial small droplet meaning a small ID of fused silica tubing.

The ESI probe of the Ion Max ion source is at a fixed 60˚ spray angle for best sensitivity and stability but can adjust side-to-side, front-to-back, and up-and-down for best signal. Other ESI ion sources use a fixed 90˚ or 0˚ spray angle for the ESI probe. The 90˚ spray angle is not best for sensitivity as gas phase ions are not vacuumed inside the ion transfer tube (ITT), and 0˚ spray angle is not best for stability as contamination of the inside of the MS results in an unstable environment for ion transmission. The 60˚ spray angle is the compromise.

Mass Analysis

The choice of a mass analyzer should consider mass range and mass accuracy.[58] A quadrupole is four rods evenly spaced at 90˚ to each other. Rods opposite of each other are connected electrically sharing the same amplitude and sign of DC voltage while in the same phase of AC voltage. The voltages applied to the different rod pairs are equal in amplitude but opposite in signs, the DC voltage and AC voltage are 180˚ out of phase. A quadrupole is a mass analyzer when a specific DC/AC voltage is set allowing the transmission of a set *m/z*, and a quadrupole is an ion transmission device (ITD) when only RF voltage is applied allowing the transmission of all ions.

In SRM scan mode Q1 and Q3 are mass analyzers and Q2 is an ITD. A parent ion is monitored using Q1, and a product ion is monitored using Q3. This results in MS/MS, and isomers of the same RT can be mass filtered if they have a unique product ion. Ions from the ESI source are guided into the ion transfer tube by a decreasing pressure gradient, and ion guides focus and transmit all ions into Q1. In Q1 the parent ion has oscillations that result in a stable trajectory and is transmitted to Q2. Other *m/z* are unstable because they have oscillations that result in a trajectory in which they collide with a quadrupole rod thus becoming neutralized and are pumped out by vacuum or miss the quadrupole rods and are ejected from Q1. The parent ion collides

with argon gas in Q2, and a product ion is transmitted to Q3. The product ion achieves stable oscillations in Q3, and is transmitted to the detector.

Q1 and Q3 have a large 12 mm ID hyperbolic profile that provides high ion transmission and good spectrum peak shape.[59] These quadrupoles are often referred to as hyperquads because the rods are not round in shape but hyperbolic.[54] The hyperquads are more selective without a loss in sensitivity at the same FWHM (full width at half max) because the theory expects a hyperbolic profile and round rods just approximate using the ratio of ID to spacing of the round rods to lessen field faults.[60] Inferior round rod quadrupoles may be more selective than hyperquads at a lower set FWHM but at a loss of sensitivity. A mass spectrometrist can achieve greater selectivity with inferior round rod technology by understanding the mass spectrometry parameters that affect selectivity. The mass range of Q1 and Q3 is between 30 to 1500 m/z. Q2 has a smaller ID and square profile because an ITD does not require large stable oscillations. Q2 is bent 90˚ to take the ion source out of the line of sight with the detector. This reduces noise because neutrals and photons do not take the 90˚ turn and interact with the detector.[61]

High voltage is applied to Q2 in between SRM scans to eliminate cross talk.[54] Crosstalk is when the product ion from the previous SRM scan remains in Q2 during the next SRM scan. Crosstalk is only an issue when the next SRM scan shares the same product ion as previous SRM scans and the compound elutes at the same RT. This is common for classes of compounds such as phosphatidylcholine as all share the same phosphocholine head group as a product ion and elute at similar RT in high throughput methods.[62] The product ion in Q2 from the previous scan can pass through Q3 and interact with the detector. If two SRM transitions share the same product ion crosstalk may not be observed if other SRM transitions are scanned between the two allowing Q2 to purge. Using high voltage to avoid crosstalk contributes to the pause time between SRM scans and is why SRM scans have a higher duty cycle than a full scan, but are more quantitative as interferences are removed for the next scan in addition to removing interferences through an additional mass analyzer.

Detector

The choice of a detector should consider dynamic range, sensitivity, and discrimination. The detector is an electron multiplier, and in positive mode a product ion is attracted to the conversion dynode by applying -15kV. The product ion strikes the surface of the conversion dynode, and one or more secondary particles of negative ions or electrons are produced. The secondary particles are now repelled by the -15kV on the conversion dynode, and the concave shape of the conversion dynode focuses the particles into the acceleration voltage of the electron multiplier of about +1kV. The conversion dynode and electron multiplier are off axis in respect to Q3 to reduce background noise from neutrals. The secondary particles strike the electron multiplier causing electrons to be ejected, and because of the funnel shape of the electron multiplier the ejected electrons do not travel far until striking the electron multiplier resulting in a cascade of electrons or gain. The result is a measurable current that is converted to voltage before an analog-to-digital converter is used to display a number.[63] Mass discrimination exists with electron multipliers because one product ion of a larger mass striking the conversion dynode will eject more secondary particles than one product ion of a smaller mass resulting in a stronger signal.[64]

The gain of an electron multiplier is the measure of how many electrons are generated from a single ion. During the first few months the gain from an electron multiplier is not stable and requires a gain procedure to reset a typical gain of 10^5 to avoid a higher gain that would result in saturation or a lower gain that would prevent low limits of detection.[65] External calibration curves without an internal standard should be acquired in a similar timeframe during this time when the electron multiplier is new, and a gain procedure should not be conducted mid experiment. The gain procedure uses direct infusion of the polytyrosine hexamer. The ion optics are placed out of focus so that the ion beam is lost; then they are slowly refocused until the electron multiplier detects one ion at 1000 ions/sec. Then the acceleration voltage of the electron multiplier is adjusted to achieve a gain. The faster the acceleration of secondary particles into the electron multiplier, the more ejected electrons will result from the impact. Any calibrant can be used for the gain procedure but it is important to use the same calibrant because of mass discrimination. Equivalent gains from a low mass and high mass calibrant would result in higher

and lower acceleration voltages of the electron multiplier, respectively. High ion transmission and high gain ages the electron multiplier faster as only a finite amount of electrons exist to be ejected, and once a gain of 10^5 cannot be achieved the electron multiplier needs to be replaced.

High Performance Liquid Chromatography and Tandem Mass Spectrometry Method Transfer Routine

Using an established method developed in another lab requires optimization of parameters. Many times the parameters are not reported, mistyped, or are for a different mass analyzer, ion source, and column. Differences exist from one instrument to another even when parameters are provided for the same mass analyzer and ion source but different brand. Furthermore, differences exist across brands from the beginning when they are new and as they age. Chromatographic differences in retention time and peak shape can exist from column to column of same geometric parameters, stationary phase, brand, and all of these can also change as the column ages.[66]

Optimization of Chromatography

Chromatography differences in retention times and peak shapes arise from differences in matrix, gradient delay volume, dead volume, loop size, pump proportioning, flow rate, and re-equilibration. The pressure limitations of the LC pump and carryover of an autosampler need also be considered. In general, the chemical reactivity and pH stability of lines, rotor, columns, pump seals, and etc. should be considered before using a sample solvent, wash solvent, or mobile phase. PEEK and stainless steel are common for lines while titanium is overall more inert[67] but expensive.

Matrix Effect

Sample matrix is everything surrounding one molecule of target compound in the sample. This includes particulates, salts, sample solvent, pH, other target or non-target compounds, and even other molecules of the target compound. Sample preparation steps of centrifugation and solid

phase extraction (SPE) are common to remove matrix. Centrifugation helps remove particulates, although use of a guard column is also preferred as band broadening and pressure increase is insignificant at installment. However, the pressure will slowly increase as the guard column is contaminated. The same steady increase in pressure is observed as the frit of a column entrance is contaminated, but a guard column can be replaced whereas a column must be flushed in reverse flow or replaced at a higher cost. SPE removes interfering compounds and salts that also cause ion suppression of ESI.[68] Sample solvent that is not identical to the initial mobile phase composition will change chromatography as the initial mobile phase composition is a combination of mobile phase and sample solvent.[69] Temperature changes of the column may result from endothermic and exothermic reactions with the mobile phase and the mixing of sample temperature. Precipitation may occur if the eluent strength of the sample solvent is stronger than the mobile phase or at a different in pH. The effect depends on sample solvent, injection loop size, and flow rate.

If a buffer is used as a mobile phase, the buffer capacity may need to be increased if a split peak is observed. The protonation of analytes and stationary phase controls retention.[70] Increasing the buffer capacity through the addition of more salt will cause ion suppression of ESI, and require testing the solubility of buffer in the highest organic percentage.[71] The steepness of slope to re-equilibrate, and the column may also need to be altered, therefore first trying alternatives like buffering or adjusting the pH of the sample, injecting smaller loop sizes, and clarify the buffer preparation procedure is preferred.

It is common for the preparation of buffers to not be reported, and adjusting for target pH in aqueous solution by adding organic solvent rather than adjusting for target pH in the final aqueous-organic mixture will result in a different pH affecting buffer capacity.[72] The reason is a different pH scale due to a unique autoprotolysis constant (K_w) at every solvent composition and temperature in addition to a change in buffer pK_a.[72] Preparation of a buffer by weights and volumes is the clearest way to report a buffer preparation, but is not used in chromatography literature.[73] The pH controls the protonation of analytes, and ions formed by ESI are preformed

in the mobile phase resulting in a different response at a different pH. The consequences of using a different pH outside of the range of column stability are cleaving the stationary phase resulting in column bleed or dissolving the support particles. The pH stability range for polymer support particles is larger than silica though both are typically tested at a low temperature, and methods that use higher column oven temperatures will shorten the pH stability range. Buffers greater than 10 mM are not recommended for ESI[74] because of ion suppression, and some buffers even at low concentrations, such as phosphate buffers, result in significant ion suppression and should be replaced with an ESI compatible buffer.[75, 76]

Gradient Delay Volume

Gradient delay volume is the volume that exists after the proportioning valves of the pump and the inlet of the column. This volume causes a delay in the time a gradient is received by the column from when it is entered in the instrument method. The gradient delay volume is often not reported and is an important variable for gradient methods on different LC pumps.

To maintain separation, the start of the gradient should be start earlier by the extra gradient delay volume or the start of the gradient should be delayed by the less gradient delay volume. This may not be possible as pre run conditions in the line are from re-equilibration resulting in an initial isocratic. If the gradient starts before the isocratic can clear the lines than lowering gradient delay volume by bypassing the thermal equilibrating tubing is an option though this allows mobile phase to enter the column at room temperature. Shorter analysis times are achieved by starting re-equilibration sooner accounting for gradient delay volume leaving one column and post column tubing volume as a safeguard to prevent a rapid change in gradient while samples are still in the system.

The determination of gradient delay volume is traditionally achieved by spiking methanol with ethyl paraben, purging the lines, flushing the mixing chambers with water, then running 100% spiked methanol to a UV-Vis detector at 254 nm.[77] The difference of the time it takes for

detection of ethyl paraben and the start of the run multiplied by the flow rate is the total gradient delay volume. To get the gradient delay volume of the pump the dead volume of tubing and detector are subtracted. An alternative to approximate the gradient delay volume is to use an acidic mobile phase and pH paper.

Dead Volume

Dead volume, also known as void volume, in the rotor slots, pre column tubing and post column tubing affects RT and band broadening. In addition to the dead volume of tubing, factors such as bottoming out loops and tubing and cutting tubing at a 90˚ angle contribute dead volume. The dead volume in rotor slots may be slightly different from the slots in another rotor and it is best not to change the rotor in mid analysis as the rotor slot used to fill the loop is the same slot used to inject sample onto the column which adds to the total amount of sample injected onto the column in addition to the loop. The dead volume in stator ports contribute to loop injection, and the dead volume of another stator port will be different but more of a consequence would be the difference in port depths. The ferrule of a stainless steel loop is swaged permanently setting the length of a nib which may not bottom out on another stator adding dead volume which adds to sample loop injection. For this reason when the loop is removed the ends of the loop must be placed back in the original ports because the port depths vary within a stator.

Loop Size Injections

Loop size injections may need to be made larger if the concentration of sample is too low for detection[78] or if broader peaks are desired for duty cycle considerations and smaller if the sample is limited or sample matrix affects chromatography.

Full loop injections are the most precise because the loop is overfilled which removes error from syringe precision to meter sample into the loop, uptake of a sample, and from sample loss during transfer to loop. Full loop is used when the sample amount is in excess as the formula for determining the needed sample amount is 3 times the nominal loop volume plus dead volume and some excess sample.

Partial loop injections are less precise than full loop because the sample is metered into the loop by the syringe motor, and like all motors the performance degrades with age until replacement. Partial loop uses less sample than full loop as the formula is the requested injection volume plus 22 µL. Partial loop allows the flexibility of injecting variable amounts and is useful in determining the optimum injection volume before switching to full loop for quantitation.[79] Partial loop injects mobile phase that remains in the loop onto the column adding gradient delay volume as the loop is back flushed. This will cause slightly different chromatography when switching to full loop because the gradient delay volume is missing resulting in lower retention times. To minimize this effect only use a loop large enough for maximum injection volume.

No waste injections are the least precise because of syringe precision uptake, metering, and sample loss during transfer to loop. The sample loss is decreased using a slower loop loading speed but any loss affects precision as only the requested injection volume is taken from the sample. No waste injection is used when the sample is limited and diluting the sample, lowering dead volume of transfer tube, using smaller autosampler vial or insert, or commanding the auto sampler needle to find bottom is not an option. The chromatography is affected as air and flush solvent is injected onto the column in addition to the sample. Matching the flush solvent to the mobile phase will lessen the effect.

Laminar flow in microfluidics[80] and the differences in nominal loop volume determine how much sample is needed for full loop injection and the sample restrictions of partial loop injection.[66] A 10 µL sample volume can occupy 20 µL of tubing volume due to laminar flow, and loops are ±20% of the nominal volume.[81] The full loop formula uses 3 times the injection volume to account for laminar flow and actual loop volume.[82] The injection volume for partial loop cannot be more than half of 80% the nominal loop volume. The 80% assumes the shortest loop possible, and the half prevents overfilling the loop losing sample because of laminar flow. Since loops vary ±20% of the nominal volume, switching the current inline loop with a loop of same nominal value would deliver different sample volume to the column, and results could not be compared when using full loop.

Pump Proportioning and Flow Rates of LC Pump

The pump proportioning and flow rates of LC pumps should be investigated if chromatography is significantly different. Pump proportioning is port dependent and switching ports may resolve the issue. Ethyl paraben and a UV-Vis detector are used to determine if two ports are proportioning correctly at a given flow rate.[77] A flow rate test at a given flow rate is done using one port and a 10 mL volumetric flask Differences in pump proportioning or flow rate may explain why the chromatography is different or if the pump is within specifications then one must question if the pump the LC method was developed with was out of specifications. Product data supplied with the pump will have a certificate certifying a proportioning and flow rate test, but one should test the flow and proportioning at the flow and gradient of the LC method. The proportioning and flow rate is less precise at low flow rates[83] as the relative error is higher, and the proportioning is less reproducible at steep gradients due to gradient distortion[84] meaning steep gradients at low flow rates are the least reproducible. The flow rate for an isocratic is different if the mobile phase is hand-mixed or online mixed by the pump because the volumes of water and organic solvents are not additive.[66] This means the flow rate changes during a gradient throughout the analysis.

Re-equilibration

Re-equilibration of a column ensures that each sequential injection of a sample experiences the same initial column conditions as the sample before. This is accomplished by running the initial mobile phase composition at the end of a chromatographic run. The duration of the re-equilibration for typical packed columns is a time that allows for 10-30 column void volumes which is dependent on flow rate and column void volume. The differences in matrices of samples, such as salts, may change the column void volumes required for re-equilibration. To shorten analysis times, lower the column volumes to a minimum that maintains chromatographic reproducibility from sample to sample, increase the flow rate during re-equilibration within pressure limitations, and increase the steepness of the slope to initial mobile phase composition. Chromatographic irreproducibility may not be observed until several samples later when testing lower column volumes. The steepness of the slope from final mobile phase composition to initial

mobile phase composition depends on buffer solubility. If no buffer is used then the steepness can be a vertical line, but if a buffer is used the solubility of the buffer in the organic mobile phase needs to be considered and a less steep approach to initial mobile phase compositions will prevent precipitation of salts.

Pressure

The backpressure limits of the LC pump limits the selection of columns. HPLC pumps can handle pressures less than 400 bar, and UPLC pumps can handle pressures greater than 400 bar. Long columns, high flow rates, and sub-2 µm particles cause pressures too great for an HPLC pump.[67] A method employing a sub- 2 µm column on a UPLC pump can be scaled up using a formula[85] to a column suitable for a HPLC pump without significant further optimization. This formula can also be used to scale down a HPLC method to UPLC. A larger ID of a column allows for larger loop injections. Longer length allow for further separation. Smaller particle size is for better resolution, and pore size can be altered for selectivity. Factors that can be changed to lower pressure without changing the column are higher ID of tubing and higher column temperature. Though the ID of tubing should be chosen to properly load a column of a specific ID,[67] and raising the column temperature may decrease pH stability of the column. Pressures that are under 400 bar but cause leaks can be addressed by selecting the proper ferrule. The ferrule clamps down on the PEEK tubing and prevents the line from slipping out of the ferrule resulting in a leak. A stainless steel ferrule on PEEK tubing can handle higher pressures than PEEK ferrules. The more viscous the mobile phases [86, 87] the higher pressure and increasing the column temperature will lower the viscosity though retention times may shift, and the stability of the column may decrease. During a chromatographic run that uses a gradient, the pressure will change in response to the new viscosity, and the pressure at the most viscous mixture [81, 88] will determine pump suitability. Lower pressure allows for higher flow rates, longer columns, and smaller particle size.

Carryover

An autosampler is needed for large numbers of samples as it is impractical for a person to manually inject each sample, but each new sample shares the same plumbing as the previous sample resulting in carryover. A wash solvent is used to clean the transfer tubing and wash the exterior of the needle between injections, and the sample loop is back flushed with mobile phase onto the column and remains in line during the entire chromatographic run for adequate flush. Typically 50:50 water:methanol is used as a flush but an alternative may be needed to remove certain compounds keepimg pH in mind if the sample is not neutral. However, a 6 port injection valve is used to switch from load sample loop with sample to inject sample in loop onto the column. During the switch the sample is deposited onto the stator face which can only be cleaned by turning the rotor seal, requiring blanks to be used in between samples to prevent false positives.[89] The amount of blanks in between samples depends on compound and concentration, and a sufficient amount of test blanks is used after a sample to determine when carryover is insignificant.

Tuning and Calibration

The choice of tuning and calibration solution should include calibrants whose *m/z* are above and below all compounds in an analysis and not have a large range between calibrants.

Direct infusion of Polytyrosine-1,3,6 tuning and calibration solution can be used to achieve a stable ion beam.[90] The tuning procedure changes the voltages of the tube lens, ion guides, lenses, and quadrupole offset voltages on Q1 and Q3 to achieve optimum ion transmission. The tube lens voltage is *m/z* dependent and guides ions into the opening of the skimmer while solvent, neutrals, photons, and other *m/z* ions collide with the skimmer and are pumped out by vacuum being skimmed from the ion beam. The tube lens offset voltage, also known as skimmer offset voltage, additionally accelerates ions into atmospheric gas aiding in desolvation and increasing sensitivity. The higher the voltage the more acceleration, and the collisions with atmospheric gas can result in fragmentation also known as ion source fragmentation. The

optimum voltage balances desolvation and fragmentation, but is typically set to 0V, and only one value can be used for all compounds in an analysis. The calibrating procedure corrects for any mass drift from true values setting mass accuracy and controlling mass resolution. This low flow tune file can be used for direct infusion of compounds with little or no change, and for a LC experiment after optimizing ion source parameters for LC flow and modifying the file. This same procedure should be applied for negative ion mode.

Optimization of Ion Source Parameters at LC Flow

ESI typically performs best with small droplets created by the small ID of fused silica tubing. However, high flow rates may require larger ID of fused silica tubing to slow the linear velocity of the spray. In addition, replacing fused silica with a metal needle is known to result in higher ion efficiency of negative ions.

Direct infusion into LC flow[90] is used to mimic conditions during a chromatographic run. This technique consists of filling a syringe with the most thermally labile analyte in the chromatographic method at approximately 10 µM, dissolved in LC flow solvent, to yield a signal of 10^5-10^7 intensity and changing ion source parameters for an optimum stable signal. A tee union is used to connect the LC pump and syringe with LC flow passing through the tee union with no change in direction and syringe flow entering the LC flow at a 90˚ angle at 5 µL/min, which can be adjusted higher if needed while lowering the LC flow rate so that the addition of syringe flow and LC flow equals the method flow rate. The highest percentage of water or the most nonvolatile solvent in the chromatographic method is used for LC flow to ensure evaporation at any point during a chromatographic run. The ion source position, amount of drying gases, and ion transfer tube temperature is flow dependent. The higher the flow, the further the ESI nozzle will be positioned from the orifice, more drying gases will be needed, and the higher the ion transfer tube temperature will be to get strong and stable signal. Spray voltage at an initial setting of 4000V for positive mode and 3000V for negative mode is used until position of the ion source is set as well as all other parameters. Negative mode typically uses less spray voltage than positive mode as arcing is more prone.[91] Adjusting before setting ion

source position may cause arcing in the ion source as the ESI nozzle is too close to the MS orifice. A setting of 90V for tube lens voltage is sufficient. Set the ion source position to middle settings of all parameters and an initial ion transfer tube temperature of 290˚C. Adjust the drying gases in the order of sheath, auxillary, and sweep. Sheath gas is the nebulizing gas, auxillary is the drying gas, and sweep gas attempts to maintain a stable clean environment in the ion transfer tube to prevent loss of signal. Sweep should be turned up as high as possible without losing signal intensity or stability. Adjust the ion source position, then adjust the ion transfer tube temperature. A thermally labile analyte was previously chosen to ensure that thermal decomposition of other analytes in the chromatographic method will not occur. Adjust spray voltage up or down in increments of 500V then in increments of 100V to get optimum.

SRM Optimization

Identifying Parent and Product Ions

Direct infusion of a standard at low flow rate of 5 to 20 µL/min is used to identify ions. If no standard is available then the sample to be measured can be placed in a syringe and direct infused. The solvent system should include the mobile phases used in chromatographic separations as the parent ion may be an adduct ion.[92, 93, 94] Set Q1 at FWHM 0.7 to full scan a range that includes the parent ion and possible adduct ions at a scan time of 1s and set Q3 as an ion transmission device (ITD). Then set Q1 as a mass analyzer on the identified parent ion, and set Q3 to full scan a range below the parent ion. This is also known as product scan mode. Start at a CE of 0 and increase in increments of 10 reaching 80 to observe all products at a collision gas pressure of 1.5 mtorr. Perform the same procedure in negative ion mode.

Tube Lens

Tube lens voltage is not flow dependent, and direct infusion at low flow is appropriate. Direct infuse compound and perform SRM optimization. Tube lens voltage only depends on the parent ion and the same optimum can be applied to other SRM transitions of different product ions. An optimized tube lens voltage will be found, and a percent increase to tuned tube lens voltage will

be stated. If ran a second time, another optimum tube lens voltage will result as the maximum is not smooth and the percent increase may not be as much. The tuning solution uses the monomer at 182 m/z, trimer at 508 m/z, and hexamer at 997 m/z of polytyrosine to determine the optimum tube lens voltage of all compounds by interpolating and extrapolating from the optimum value of the 3 compounds. The *m/z* values are 2 units less in negative mode. The improvement is typically no more that 10% and fluctuates, requires typing in instrument method and finding new value every 3 months as part of routine tuning; and for these reasons tuned tube lens voltage is preferred for high throughput analysis. However, if necessary, a specific tuning and calibration solution containing the compounds in the analysis can be made, and sourcing this tune file in the instrument method would remove the requirement of manual typing with the added benefit of mass calibration. If solubility issues are a problem, then make a solution that only contains compounds in a given MS acquisition segment as each requires their own tune file.

Collision Energy

Collision energy (CE) is the offset voltage on Q2 that results in acceleration of ions into the collision gas. Offset voltages are DC voltages that control acceleration and deceleration of ions, quadrupole rod pairs are equal in amplitude and sign, whereas, ramping DC controls ion stability and quadrupole rod pairs are equal but opposite in sign. The name offset is from a compared difference of applied voltage, in this case, the difference in voltage from the Q2 and the ion source resulting in acceleration of the parent ion as setting the translational kinetic energy (TKE). Increasing offset voltage increases the TKE of a parent ion which increases the energy of a parent ion and molecule of argon gas collision, or collision energy. An optimum exists at a balance as a low CE is only enough energy to cause the desired fragment in a higher energy population or from multiple collisions; and a high CE results in further fragmentation or scattering.

Collision energy is not flow dependent. Direct infuse compound and perform SRM optimization on observed product ions at 1.5 mtorr collision gas pressure. A breakdown curve will show the optimum CE. A breakdown curve is produced by monitoring the signal intensity as different CE

are applied and the parent ion is broken down into fragments producing signal.[95] The CE that resulted in the highest signal is the optimum CE. If the maximum is not smooth, a different optimum CE will result when the SRM optimization is ran a second time. Choose the SRM transitions that have the most stable breakdown down curve at the maximum as any fluctuation left or right in CE will not result in a significant change in signal. The maximum does not just result in the highest y value but also is the smallest slope so small movement in the x direction is essentially the same y value.

Although other SRM transitions are not used for further optimization, the breakdown curves should be saved as a screenshot if these SRM transitions will be needed later to resolve interference with the chosen SRM transitions. The approach now is to find the SRM transition that is most suitable for quantitation based on the sensitivity but later a less sensitive SRM transition but more selective may be used. It is common for classes of compounds with varying R groups to have the same CE when losing the same product ion, and optimizing on one compound of this class may be sufficient.[62] However, if in doubt optimize on the lowest *m/z* and the highest *m/z* to see if CE is conserved as CE controls the translational kinetic energy at which collisions take place, and heavier *m/z* would be predicted to accelerate slower at the same CE and result in less fragmentation shifting their optimum CE higher. This is analogous to the principle that allows for *m/z* separations using a time of flight mass analyzer as ions are lined up and receive the same acceleration voltage but higher TKE results for low *m/z* and lower TKE results for higher *m/z* achieving separation.

CE is compound dependent and does not depend on ion transmission. Therefore parameters of scan time, scan width, and FWHM does not change optimum CE, but would only result in a higher intensity value at optimum CE on the breakdown curve as these affect ion transmission. The quadrupole offset voltages for Q1 and Q3 are adjusted during tuning, but CE stay the same within 1 unit before and after a tune suggesting that optimum CE does not need to be reacquired.

Collision Gas Pressure

The collision gas is ultra high purity argon chosen for its inertness and mass. It is important for the collision gas to not form ions or react with the parent ion, and for the mass to be heavy enough to transfer energy into the parent ion upon collision resulting in fragmentation and not to have energy transferred to it from the parent ion moving it out of the path. The manifold which contains the mass analyzers Q1 and Q3 are under high vacuum of 10^{-6} torr as a long mean free path, preferably from ion source to detector, is desired to prevent collisions of ions resulting in scattering and lowering sensitivity. However, Q2 is also in the manifold and an aluminum chamber called the collision cell covers Q2 and traps argon gas at mtorr pressure. Lenses are used on both sides of Q2 to focus the ion beam, shield RF voltage applied to Q1 and Q3 from Q2 and vice versa, and to act as a baffles to prevent argon from entering the high vacuum region of mass analyzers Q1 and Q3. The lenses are named because they focus the ion beam that passes through them and must have a hole to allow the passage of the ion beam to the detector resulting in escape of argon gas into the high vacuum region. The previous breakdown curves were obtained at a collision gas pressure of 1.5 mtorr as this pressure typically results in enough fragmentation to achieve a breakdown curve. However, an optimum setting of collision gas does exist.

Collection-induced dissociation (CID) is the process of a parent ion colliding with an argon molecule and converting its TKE into internal energy which fragments the parent ion. The efficiency of the CID process is a balance between fragmentation and collection efficiency which both depend on the collision gas pressure. The collection efficiency is the ratio of ions that exit the collision cell to those that enter the collision cell and assumes one fragment per parent ion. The collection efficiency is nearly 100% when the collision gas pressure is 0 as no ions were scattered upon collision with argon but with other gases present in high vacuum resulting in a collision of Q2. Collection efficiency depends on the mass of the parent ion, as heavier masses are less prone to scatter than lighter masses when colliding with argon and the collision gas pressure as more pressure introduces more opportunities for collisions. Fragmentation efficiency is the fraction of desired fragment ion to the remaining parent ion at the exit of the collision cell. The fragmentation efficiency approaches 100% as the pressure of the collision gas is increased as

multiple collisions occur, but the collection efficiency is decreased due to scattering. Fragmentation efficiency is inversely proportional to the stability of a parent ion as the more stable an ion is the less likely a fragment will result from a collision and to the mass as the vibrational energy from a collision is better distributed across many bonds of a larger and heavier ion. The optimum CID efficiency will be at a collision gas pressure that is low enough to prevent scattering, but high enough for collisions to take place for fragmentation to occur.

To find the optimum setting of collision gas, perform SRM optimization at collision gas pressures of 1, 2, 3, and 4 mtorr and obtain breakdown curves. The optimum CE is typically within ± 2 units at any collision gas pressure, but it is the intensity that changes at different collision gas pressures. Identify the collision gas pressure that has the highest signal and test 0.5 mtorr higher and lower as the optimum could have been reached and decreased but still better than lower setting or the optimum was yet to be reached but decreased lower than previous setting. The optimum collision gas pressure will result in the highest intensity of fragment ions. The pressure of collision gas is not able to change fast enough for an optimal gas pressure for individual SRM transitions like voltages such as CE. In a multi component analysis the same collision gas pressure must be used for all compounds so it is best to choose a pressure that is best for all. Higher collision gas pressure is needed for massive ions to fragment, and the scattering is negated because massive ions are less prone to scatter. Therefore in a multi component analysis, it is best to have parent ions within a mass range and be careful not to confuse the *m/z* of a parent ion with its mass as a massive parent ion can exist as a low *m/z* when carrying multiple charges. Small differences in tank to tank purity of argon may exist, and the tank should not be changed mid analysis as CID efficiency may change.

FWHM

Full width at half max (FWHM) is a measure of mass resolution that is the full width of a *m/z* peak at half its maximum in a profile view spectrum. The peaks are of Gaussian shape because some population of the ion beam becomes stable as the scan enters a Mathieus stability triangle,[60] then as the scan proceeds the highest population of ions are stable at the true *m/z*, and

as the scan leaves the stability triangle a lesser population is stable. This is how a peak with width results from ions with no distribution in mass.[65] The scan is a constant DC/AC ratio that increases in magnitude resulting in a linear line of positive slope for positive ions and negative slope for negative ions. Alternating current (AC) is often referred to as radio frequency (RF) as the AC used in mass spectrometry is similar to the frequency of radio waves. If one extends a vertical line upwards at any point of the slope, it will pass through the tip of a *m/z* stability triangle that is most stable at this DC/AC ratio and can pass through the mass filter. This *m/z* stability triangle is the *m/z* on a profile spectrum when extending a vertical line downwards to intersect the *m/z* x-axis on a spectrum. When the DC/AC ratio is at a point when the target *m/z* is most stable a vertical line extending upwards will pass through the tip of the target *m/z* stability triangle. Then on the profile spectrum extending a line downwards will intersect the *m/z* x-axis of the target mass. This extension downward is also known as a centroid and is viewed in centroid view.

The idea for selectivity is for the DC/AC ratio slope to pass only through the tips of the stability triangles approaching a FWHM of 0 because at the tips only one *m/z* is stable. The FWHM increases as the DC/AC ratio slope decreases becoming farther from the stability triangle tips. Selectivity is less at any point on the slope because ions will exist in more than one *m/z* stability triangle allowing other ions to pass through the mass filter in addition to the target *m/z*. A SRM interference may be removed by decreasing the FWHM resulting in a smaller bandpass filter.[59] This increase in selectivity results in a loss of sensitivity. A 0.1 FWHM is about 30% and a 0.2 FWHM is about 60% of the signal of a 0.7 FWHM.[65] Though sensitivity is lost, LOD (Limits of Detection) may increase as the S/N ratio might increase as noise is filtered out. If sensitivity is too high, the linear range on a calibration curve will decrease as saturation of the electron multiplier occurs before a large linear range can be established and increasing selectivity by lowering FWHM will reduce ion transmission, prevent saturation of the electron multiplier, and increase linear range allowing samples to have greater range differences in concentrations. High ion efficiency, high CID efficiency, and high mass will saturate the electron multiplier at lower concentrations than other compounds decreasing linear range.

Quadrupole mass resolution is not linear, meaning a setting of 0.7 FWHM does not result in all *m/z* having a 0.7 FWHM peak. The mass resolution is less with higher *m/z*. For example, at 0.7 FWHM the 182 *m/z* of the tuning solution has baseline resolution of the +1 isotope peak but this baseline resolution is less with the 508 *m/z* isotope clusters and the 997 *m/z* has a valley height with the +1 isotope. This valley can be resolved using 0.5 FWHM meaning lower FWHM is needed at higher *m/z* for the same resolution. Each SRM transition may have its own FWHM values for Q1 and Q3 though typically the same setting for FWHM is placed on both Q1 and Q3 in an SRM experiment; a compromise that balances sensitivity, selectivity, and mass resolution for all compounds in the analysis.

Microscans

Microscans are smaller scans within allotted scan time. These microscans are averaged when determining accurate *m/z*, and in an SRM experiment where quantitation is the goal, smoothing points is used to average the chromatogram.

Scan Time

Scan time, otherwise known as dwell time, is the allotted time for transmission of ions for a given SRM transition. The higher the scan time the more sensitive the SRM transition as the electron multiplier has longer time to collect ions. The stability of an SRM transition signal also increases with scan time as small changes in scan time are less relative. A scan time of 0.050 s is commonly used, but scan times as short as 0.010 s are used when duty cycle is an issue. A balance between scan time and duty cycle is made for quantitative reproducibility. The scan time can be optimized for each SRM transition, but typically the same scan time is used for all SRM transitions in an analysis.

Scan Width

Scan width is the range of *m/z* that is scanned left and right of the specified *m/z* value in the SRM transition. For example, a scan width of 1 *m/z* means the quadrupole will start the scan 0.5 *m/z* units to the left of the specified *m/z* value, increase in *m/z*, and finish 0.5 *m/z* units to the right of the specified *m/z* value summing the entire signal as one centroid reported as the specified *m/z* value. A true SRM transition has no scan width as 100% of the scan time is spent on the apex of a peak allowing for maximum ion transmission. The selectivity and sensitivity improves with narrower scan width but the instrument must remain in mass calibration or can miss the peak entirely especially when the FWHM is low.[59] A small drift in mass calibration will not affect the sensitivity gain from a narrow scan width over a large scan width as the time spent near the apex results in a greater signal than the brief time a large scan width spent on the apex.

The scan width should be narrower when FWHM is lower, as a scan width of 0.7 *m/z* will cover most of a peak at 0.7 FWHM but the same scan width at 0.1 FWHM will spend time where no peak exists as the peak is much thinner. The easiest way to avoid this is to set the scan width equal to or less than the FWHM. A scan width of 1 *m/z* at 0.7 FWHM is common and is used with nominal mass values to ensure enough ion transmission though this practice is not the best for selectivity and sensitivity. The scan width can be optimized for each SRM transition but typically the same scan width is used for all compounds in an analysis.

Duty Cycle

Duty cycle is the time that elapses until a SRM transition can be scanned again, in other words, the time between data points on a SRM transition chromatogram.[96] Each SRM transition consists of a scan time where ion transmission occurs and a pause time to purge the quadrupoles and allow ions in the detector region to finish flight to the electron multiplier to avoid crosstalk. The scan time and pause time for each SRM transition in a scan cycle added together is the duty cycle, but in practice an approximate duty cycle is calculated by neglecting the pause times as these are small relative to the scan time and often times not reported by the manufacturer. A

scan cycle starts on the lowest parent mass and increases to the highest parent mass then the next scan cycle returns on the lowest parent mass never scanning in decreasing parent masses. SRM scans are not a continuous signal, instead the signal collected during the scan time is plotted as discrete points on a chromatogram and connected by straight lines to approximate where other discrete points would be if the duty cycle was lower.

The duty cycle should be low enough to for a chromatographic peak to have at least 10 points. The broadness of a peak in a chromatogram determines the maximum duty cycle allowed while maintaining 10 points per peak. Broad peaks can have higher duty cycles whereas narrow peaks will require a lower duty cycle. The peak is effectively traced with 10-15 points with an error in area of less than 10%.[96] More than 15 points per peak does not significantly improve area reproducibility, and at less than 10 points results in poor area reproducibility, retention time shifts, broaden peaks, lower peak heights, and loss of sensitivity.[97]

Time segments for MS acquisition are used to lower the duty cycle while maintaining a high throughput analysis. It is unnecessary to scan for a SRM transition before and after its retention time. A complete list of SRM transitions for an experiment can be divided into smaller lists of similar retention times and placed in a time segment. The duty cycle for the time segment is only of the SRM transitions in the smaller list and not of the complete SRM transitions list. If the retention time of a compound or the broadness of its peak is in between time segments, then it is placed in both time segments resulting in two duty cycles for one peak if the time segments do not have equivalent duty cycles. This will not affect area reproducibility if each duty cycle when considered separately would result in at least 10 points per peak. It is also unnecessary to scan for SRM transitions during column re-equilibration, and MS acquisition is typically stopped allowing eluent to drain out of the ion source an alternative to a divert valve which adds more dead volume resulting in peak broadening. This conserves ion sources drying gases, extends the life of the electron multiplier, and prevents contamination resulting in charging. Charging is when ions form a capacitor and repel incoming ions of the same charge resulting in steady loss of signal that can be diagnosed by switching polarities, discharging the capacitor, and observing the signal return when returning the original polarity. The solution is to sonicate or clean the contaminated part with Alcanox.

Profile and Centroid Data Type

Profile data type is typically used for tune and calibration as the ion intensities at sampling intervals are connected to form a continuous line displaying the broadness of a peak that results from a set FWHM resolution.

Centroid data type is typically used for data acquisition as scan speeds are faster and the disk space requirement to store the data is smaller than profile data type allowing faster data processing. The sum of the sampling intervals across a peak are displayed as a single centroid causing peak height to be greater than profile data type.

SRM m/z Values

The theoretical *m/z* values for SRM transitions may be rounded to the tenths place for 0.7 FWHM as the resolution of a quadrupole fluctuates beyond a tenth of a *m/z* value. Each SRM transition should have a unique parent mass as Xcalibur will combine SRM transitions of the same parent mass but different product masses into one chromatogram. To prevent this add a thousandth *m/z* value to SRM transitions that share the same parent masses. The rounding may cause the apex of the mass spectrum peak or the entire peak to be missed within a defined scan width at a lower FWHM such as 0.1 FWHM.

Conclusion

The three merits of optimizing SRM parameters are selectivity, sensitivity, and reproducibility. Parameters that affect selectivity are FWHM and scan width that sometimes result in an increase in sensitivity as noise or an interference is removed increasing S/N ratio. Parameters that affect sensitivity are those that increase ion transmission including tube lens voltage, collision energy, collision gas pressure, FWHM, scan time, scan width, and duty cycle. Parameters that affect reproducibility are tube lens voltage, collision energy, scan time, and duty cycle.

Selectivity parameters may be adjusted to remove an interference in an SRM transition. To mass filter an interference, first lower the scan width as this will increase selectivity and sensitivity then try in combination or separately lowering FWHM as this will increase selectivity but decrease sensitivity. If this fails to remove the interference, then try a different SRM transition sufficient for quantitation utilizing the same parent mass but different product mass for the compound including SRM transitions in the other polarity mode. Negative mode is typically less sensitive as a given concentration of a compound makes less negative ions than positive ions but more selective as the background also forms less negative ions than positive ions. If mass filtering does not resolve the interference, then seek another developed LC method. Understanding the instrument allows one to know its limitations and not design an experiment outside of the limits or attempt to transfer an incompatible LC/MS method.

Alternative Methods to Remove SRM Interference without Mass Filtering

As discussed before, interferences can be mass filtered by increasing selectivity, adjusting FWHM, scan width, or using a different SRM transition. Alternatives to mass filtering an interference are adjusting sample preparation, chromatography and ion source. Suitable changes to sample preparation to remove interferences include solid phase extraction (SPE), liquid-liquid extraction, and derivatization. Derivatization changes the parent mass which leads to a different SRM transition though many times the ionization efficiency, chromatographic peak shape, and RT changes.[98] Chromatography modifications can be made which result in baseline resolution of interference and target compound in the chromatogram, and these include alteration in flow rate, gradient, geometrics of column, add modifier to mobile phase to form adduct ions,[92, 93, 94] changing mobile phases, couple columns, or a column of different stationary phase. Several ion sources are available such as ESI (Electrospray Ionization), APCI (Atmospheric Pressure Chemical Ionization), and APPI (Atmospheric Pressure Photoionization). Each have their own ionization mechanism, and the interference may not ionize efficiently with another ion source. These techniques rely more on method development than method optimization and will not be discussed in detail. Method development is labor intensive whereas method optimization results from small changes to an existing method that require little time investment.

Absolute Quantitation using External Calibration Curves

Absolute quantification is achieved by a calibration curve used to determine the unknown concentration of a compound in a sample by applying a fit and interpolating. It is common for quality controls to be prepared to test the % accuracy of the fit to predict a known concentration and not rely solely on the R^2 value to determine suitability of a fit. Alternatively, the calibration levels can be used to determine % accuracy at each calibration level as the fit is better or worse at prediction of concentrations in discrete ranges due to an imperfect fit.

Calibration curves using an external standard or an external standard plus internal standard is common. The internal standard corrects for ion suppression and ion transmission losses due to the clogging of the ITT by the following formula:

$$\frac{Area\ of\ External\ Standard}{Area\ of\ Internal\ Standard} = Area\ Ratio$$

This area ratio is used as the y-value on the calibration curve. The percent loss due to ion suppression or transmission is equal for both external standard and internal standard. The percent loss of ion suppression and transmission is applied to both the external standard and internal standard which cancels the percent loss factor. For example, a 10% loss due to ion suppression and transmission at an area ratio of 200/100 becomes 180/90 which remains an area ratio of 2. The benefit of a calibration curve using external standard with internal standard is significant when a target compound free matrix is unavailable. The sample solvent is used in the absence of a target compound free matrix to mimic sample conditions,[48] but ion suppression in the sample matrix results in a lower area of target compound compared to the area of external standard at the same concentration.

ESI calibration curves are sigmoidal, and when a target compound free matrix is unavailable standard addition is not an option. Standard addition determines the amount of target compound in the matrix from the x-intercept assuming linearity to the origin and a y-intercept of 0 in a target compound free matrix which is not the case for ESI calibration curves. Calibration curves are collected from low to high concentrations to prevent erroneous carryover, and sample vials are not overfilled that would result in a vacuum. A rough calibration curve before sample analysis is useful to determine if sample levels fall in the linear range, then later a calibration curve is used for quantitation. It is important to concentrate or dilute samples to fall in the linear range of a calibration curve for ease of data processing and to maintain sensitivity. Furthermore, in high throughput analysis, it may not be possible for all compounds in a sample to be in the linear portion due to varying concentrations thus a decision must be made on which compound or compounds have priority and must be in the linear portion.

Data Processing

Xcalibur Quan Browser was used with the ICIS algorithm and 5 smoothing points. The formula for smoothing was the moving average. The effect of smoothing is more profound with more smoothing points or with chromatographic peaks of fewer data points. A smoothing point setting of 15 may smooth a chromatographic peak of a few data points to baseline noise or distort data introducing false peaks. Smoothing changes the area and RT, but no more than instrumental variation that would result if same sample was injected again.

This means if two unsmoothed chromatograms were obtained by duplicate injections of the same sample then smoothed chromatograms could have areas and RT in between the unsmoothed chromatograms. Smoothing is applied before the algorithm sweeps the chromatogram helping the algorithm identify a peak and perform automatic integration which eases data processing and increases reproducibility as manual integration is not needed.

Isotope Labeled SRM Transitions of ^{13}C Flux Experiment

To determine all of the possibilities for isotopologues and isotopomers for parent and product ion a systematic approach was used. The system of numbers separated by a hyphen is used to represent the number of ^{13}C in the parent ion and the number of ^{13}C in the product ion (Tables 12-15). To find all SRM transitions first set the parent ion to a number and determine all product ions possible. Starting with a fully labeled parent ion ask yourself if all the ^{13}C from the parent ion show up in the fragment what will the number be for the product ion. Then do the same thing but change the question to if all the ^{12}C from the parent ion show up in the fragment what will the number be for the product ion. Finally, do all integer values between the maximum and minimum numbers for the product ion. Repeat this process with the next lower labeled parent ion and continuing to a fully unlabeled parent ion.

Table 1: Isotope Labeled SRM Transitions of Aspartate in ^{13}C Flux Experiment

	Ion Formula		
Comment	**Parent $C_4H_8NO_4+$ # of ^{13}C in parent**	**Product $C_2H_4NO_2+$ # of ^{13}C in product**	**Comment**
Fully labeled	4	2	Fully labeled
Next lower labeled parent ion	3	2	Maximum # for product ion, all ^{13}C from parent in fragment
	3	1	Minimum # for product ion, all ^{12}C from parent in fragment
Next lower labeled parent ion	2	2	Maximum # for product ion, all ^{13}C from parent in fragment
	2	1	All integer values in between maximum and minimum
	2	0	Minimum # for product ion, all ^{12}C from parent in fragment
Next lower labeled parent ion	1	1	Maximum # for product ion, all ^{13}C from parent in fragment
	1	0	Minimum # for product ion, all ^{12}C from parent in fragment
Fully unlabeled	0	0	Fully unlabeled

Material for Results and Discussion taken from:

Ankrah, N. Y. D., May, A. L., Middleton J. L., Jones D. R., Hadden, M., Gooding, J. R., LeCleir, G. R. , Wilhelm S. W., Campagna, S. R. and Buchan, A. Metabolic response of a Roseobacter to phage infection: insights into the influence of viral lysis on ocean biogeochemistry, *submitted.*

Results and Discussion

Composition of Extracellular Small Molecule Components following Phage-Induced Cell Lysis

As the water-soluble metabolite component of DOM is likely to be comprised of molecules that are more readily utilized than macromolecules, which often require processing prior to uptake and utilization,[19] we set out to determine how viral infection affects these labile nutrients. Cell-free filtrates of phage-amended and control cultures were collected 480 min post infection, 240 min after initiation of phage-induced cell lysis, and were analyzed for the presence of a suite of 317 metabolites, most of which are core components of carbon and nitrogen utilization as well as the biosynthesis of macromolecular inputs. Fifty-six of these metabolites were detected in filtrates from either or both treatments (Table 2). Interestingly, the metabolite concentrations in the infected cultures were not uniformly higher for every molecule. Instead, an increase in 29%, no change in 45%, and a decrease in 27% of the concentrations for the detected metabolites were noted (Table 2), indicating that phage infection altered the labile small molecule composition of the DOM.

In general, the metabolites with decreased concentrations in the filtrates of the infected cultures were primarily those that are expected to be readily recycled, such as components of central C and N metabolism and small co-factors. This suggests intact cells in the virus-amended cultures remained active and were able to rapidly consume material from lysed cells to support their own metabolism, consequently depleting selected metabolites in the extracellular milieu (Table 2). Metabolites with increased relative concentrations in the filtrates were those typically related to cellular stress or those that are potentially too large or rarely encountered to be effectively transported into the cell. For example, the phosphatidylcholine, phosphatidylethanolamine, and ethanolamine pools in the filtrates of the phage treated culture were dramatically elevated. The

increase of these bacterial cell wall constituents in the phage-amended culture filtrates is consistent with a need for the cells to adapt to the physical stresses imposed from viral load as well as degradation of cell wall material from lysed cells. Of the amino acids that were detected in filtrates, several of those with the largest increases in concentration for the phage amended cultures are also part of the normal cellular stress response (*e.g.* asparagine, cysteine, homoserine and methionine).[99] All detected CoA-activated carboxylic acids were elevated in the infected cultures. While these compounds serve as intermediates of the TCA cycle, amino acid metabolism and fatty acid biosynthesis,[100] they may not be efficiently transported and assimilated due to their large size and/or low availability under normal conditions. However, urea, a nitrogen-rich byproduct of *de novo* nucleotide biosynthesis,[100] was also elevated in infected cultures, despite being neither large nor rarely encountered.

Table 2: Metabolite Content of Filtrates of Infected *Sulfitobacter* sp. 2047 Relative to Control Cultures at 480 min Post Infection

Amino acids	**Fold change**[a]
Asparagine	2.37↑
Methionine[b]	1.83↑
Cysteine	1.74↑
Tryptophan	0.73
Threonine	0.10↓
Homoserine	1.87↑
GABA[b]	1.45
Betaine	1.29
1-Methylhistidine	1.28
O-Acetylserine	0.91
S-Adenosylmethionine	0.88
N-Acetylornithine[b]	0.56↓
TCA cycle	
Malonyl CoA	1000.00↑
Succinyl CoA	1.34
Succinate	0.78
[Fumarate, Maleate, & Isoketovalerate][c]	0.72
Citrate	0.70
2-Oxoglutarate[b]	0.62↓
3-Phosphoglycerate	1.89↑
[1,3 & 2,3 Bisphosphoglycerate]	1.10
Phosphoenolpyruvate	0.61↓
Lipids	
[Phosphatidylcholine, Phosphatidylethanolamine & Ethanolamine][c]	1000.00↑
Propionyl CoA	79.81↑
Ethanolamine	2.93↑
Palmitate	0.97
Farnesylpyrophosphate	0.57↓
Nucleic acids, Nucleosides and Nucleotides	
Deoxyadenosine[b]	4.69↑
Thymine[b]	2.62↑
Cytosine[b]	1.55↑
dCDP	1.24
UDP	1.12
5'-Methylthioadenosine	1.05
Orotate	0.95
Dihydrooroate	0.87
N-Acetylglucosamine-1-Phosphate	0.80
TDP	0.57↓
GMP	0.42↓
5-Methyldeoxycytidine[b]	0.35↓
Pentose phosphate	
Fructose-1,6-Bisphosphate	0.99
Sedoheptulose-7-Phosphate	0.71
Erythrose-4-Phosphate	0.00↓
Cofactors, vitamins and electron carriers	
Acetyl CoA	35.17↑
Pyridoxine	4.63↑
Nicotinate	0.95
5-Methyltetrahydrofolate	0.85
Methylmalonic Acid	0.79
Thiamine	0.29↓
FAD	0.00↓
NAD	0.00↓
Other	
Urea	2.78↑
DL-Pipecolic Acid	1.89↑
Acetyl Phosphate	0.92
4-Hydroxybenzoate	0.81
Citraconate[d]	0.42↓
Phenylpyruvate[b]	0.37↓
Lactate	0.32↓

[a]Metabolite levels in infected cultures are expressed relative to levels in control cultures at 480 min. Elevated metabolites, ↑,(fold change ≥1.5) and depressed metabolites , ↓,(fold change ≤ 0.67).
[b]Not detected in intact cells, detected in filtrates only
[c]Metabolites are indistinguishable with the applied method

Alterations in Intracellular Metabolite Pools and Flux during Phage Infection

The set of molecules released upon phage-induced cell lysis is in part a reflection of the intracellular metabolite pools of the host. Yet, surprisingly little is known of the influence of phage infection on host metabolism at a global level. Much of the knowledge concerning bacteriophage-host interactions comes from studies of T-even (T2, T4, and T6) viruses that infect *Escherichia coli*. Both lysogenic and lytic coliphages initially promote similar alterations in host metabolism[101] that halt host cell DNA synthesis,[102] degrade host DNA,[103, 104, 105] and assemble the machinery for viral production.[102, 105] These activities drastically alter aspects of host metabolism.[102, 105, 106, 107] As the breakdown of host DNA may not fully supply precursors needed for phage DNA synthesis,[108] an initial lag in *E. coli* metabolism is followed by rapid uptake of nutrients to be used for *de novo* synthesis of macromolecular building blocks.[109] In fact, many viruses contain auxiliary metabolic genes (AMGs) that are present to overcome rate limiting steps in host biosynthesis[18] and genes encoding enzymes involved in pyrimidine biosynthesis are encoded in the Φ2047B phage genome (Figure 1). It is possible that these enzymes are present to support the increasing need for viral DNA during infection. A dramatic example of host manipulation has been demonstrated in marine cyanophage which encode and express photosynthesis proteins homologous to those found in their hosts.[110] The extent to which pathway specific alterations of host metabolism is a universal strategy among phages remains unknown.

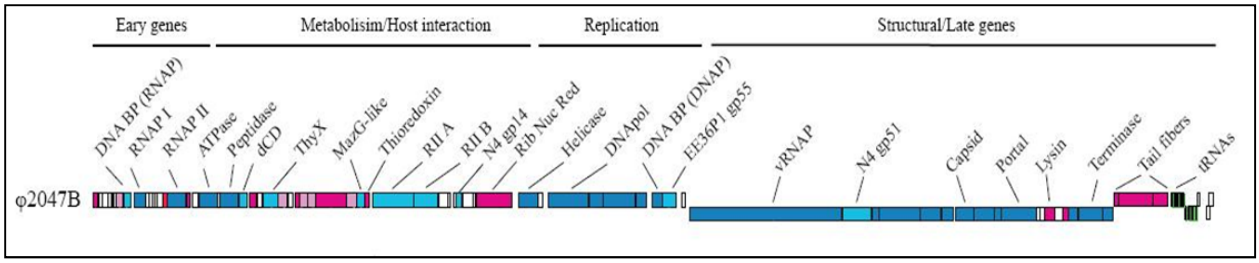


Figure 1: Phage 2047 genome. φ2047B is a N4-like lytic phage of the Podoviridae family.

As non-enveloped phage are comprised primarily of nucleic acid and protein, typically in an equal mass ratio,[111] we reasoned that if major pathway specific influences of viral infection on host metabolism were present that they would be manifested in pathways that contribute to the synthesis of the biochemical building blocks for these macromolecules. To assess the effect of phage production on the metabolism of the host population, we measured the relative concentrations of 83 central pathway metabolites (the "core metabolome") in infected and control populations at discrete time points throughout the infection cycle (Figure 2a). These data can be viewed in two ways: with the relative concentrations of intracellular metabolites either being evaluated with or without normalization to cell density. Without cell normalization, these data give a relative measure of the total pool of intracellular metabolites within an equal volume of the culture (Figure 3). Interestingly, the concentrations were nearly identical for the majority (~70%) of metabolites in phage-amended and control populations at all time points. However, significant temporal differences were evident in UDP-activated sugars (*e.g.* UDP-glucose, UDP-glucuronate/galacturonate, and UDP-*N*-acetylglucosamine) that may reflect alterations in cell wall integrity in phage-infected bacteria. The relative concentration of UDP-glucose in the infected population was elevated 15 min after phage addition and remained fairly constant throughout the experiment. UDP-glucuronate/galacturonate became elevated over time with a 13-fold spike in concentration at 120 min post infection, the time point preceding wide spread cell lysis. Conversely, the relative concentration of UDP-*N*-acetylglucosamine decreased by half at the 120 min time point. UDP-sugars are precursors of cell envelope components, including peptidoglycan and surface polysaccharides.[112] Changes in the relative concentrations of these compounds in infected populations might be expected given that alterations in host cell envelope composition and architecture typically occurs following phage infection,[3, 104] and this is consistent with an increase in cell wall components detected in the corresponding filtrates from the final time point (Table 2). As peptidoglycan is produced by actively growing and dividing cells,[112] these data may also indicate alterations in the specific growth rate of infected bacteria which is not readily evident in the net cell densities.

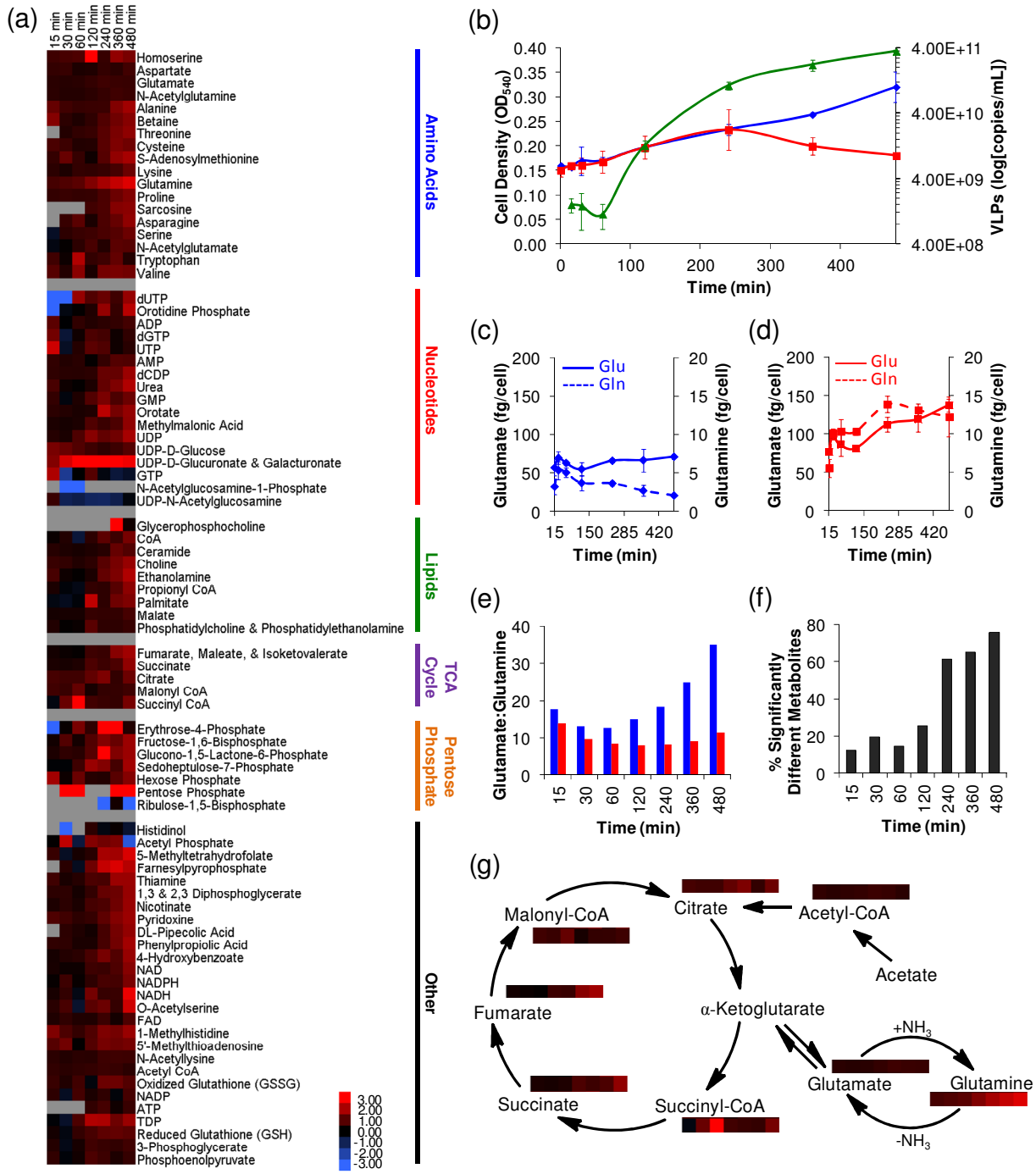


Figure 2: a) Heatmap of intracellular metabolites of phage amended and control *Sulfitobacter* sp. 2047 populations. Metabolite concentrations are normalized to bacterial cell number, determined by microscope count, and expressed relative to levels measured in the uninfected host cells at the corresponding time point. Columns correspond to minutes post infection. Values are average areas of duplicate biological and technical replicates. Ratios are log 2 transformed and plotted on a color scale. **b)** *Sulfitobacter* sp. 2047 cell density (at OD_{540}), control, phage amended, and phage concentration at each metabolite sampling time point reported in panel a. Turbidity declines are indicative of phage-induced lysis. Phage numbers were derived from qPCR assays. Averages and ranges of biological duplicates are reported. Absolute concentrations of glutamine and glutamate in **c)** control and **d)** phage amended *Sulfitobacter* sp. 2047 populations. Values represent duplicate biological and duplicate technical replicates. Error bars show the standard error of the mean. **e)** glutamate to glutamine ratios for control and phage amended populations throughout the experimental time course. **f)** Variation in intracellular metabolite concentrations between phage amended and control populations shown in panel a. Fold changes > 1.499 and p-values < 0.1 were considered significant. **g)** Biosynthesis of glutamate and glutamine is linked to TCA cycle intermediates and these two metabolites are interconverted. Data for selected metabolites are shown in heatmap format.

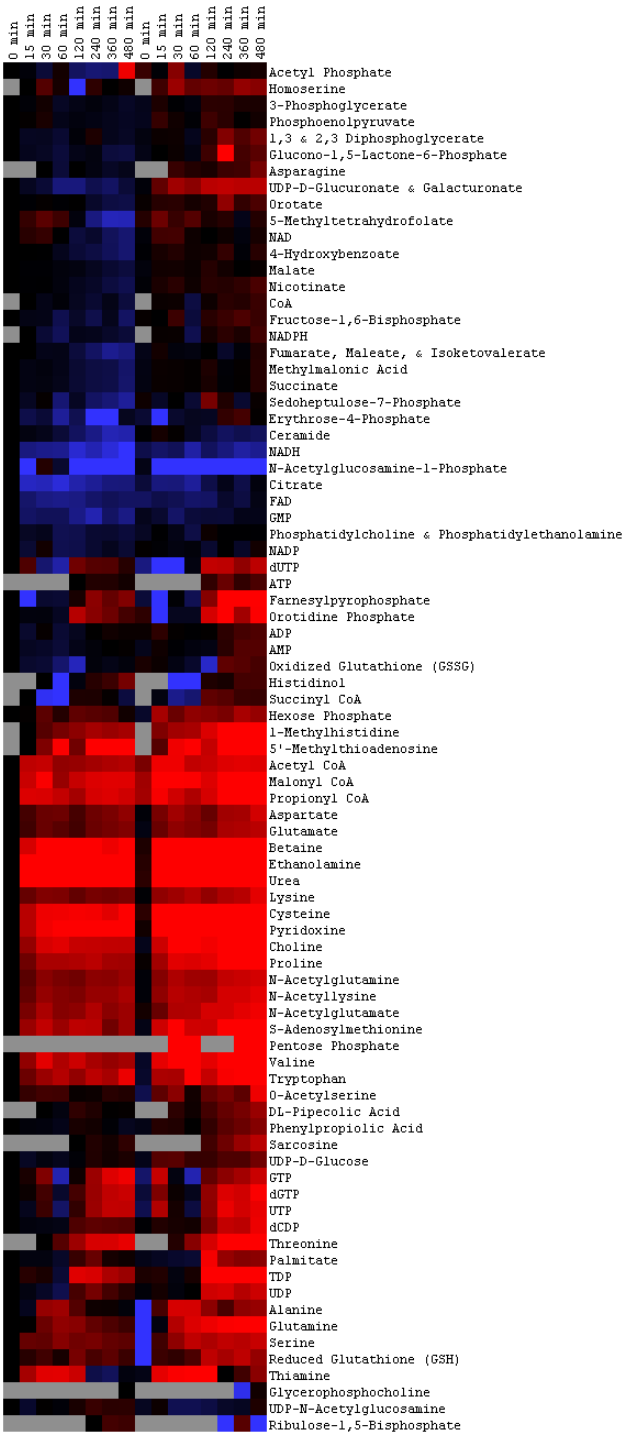
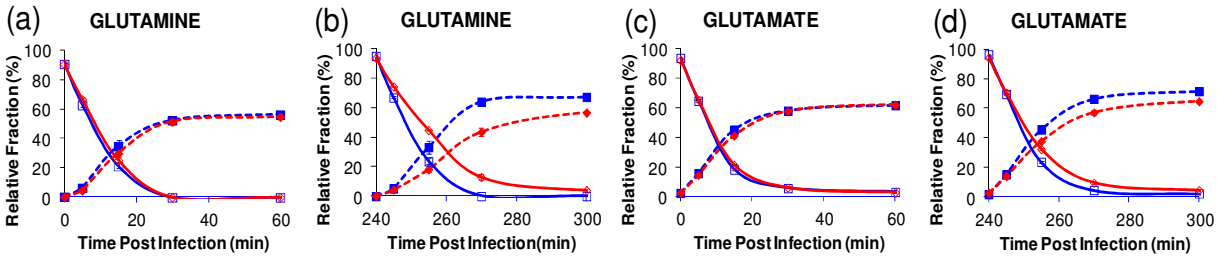


Figure 3: Metabolome dynamics during phage infection. Heatmap of intracellular metabolite levels during the course of phage infection expressed relative to levels measured in the uninfected host cells at time t=0. Ratios are $\log_2$ transformed and plotted on a color scale. Rows correspond to metabolites measured by LC-triple quadrupole MS/MS. Columns correspond to minutes post infection. The host cells and phage strains used in each time are indicated. Values are averages of duplicate independent biological and technical replicates.

To further assess the effect of phage amendment on cellular metabolism, the cell density normalized data were analyzed to evaluate the relative concentrations of metabolites within individual cells. These data highlight the distinct metabolic trajectories for, and provide insight into, the intracellular metabolic concentrations of infected versus control populations (Figure 2a). Dynamics in phage production and host population survival can be used to divide the time course of the experiment into two phases: an early infection period (0-120 min) and a later infection period (240-480 min). The per cell metabolite concentrations also support this division. During the early infection period, <25% of the measured metabolites were significantly different ($p \leq 0.05$ and at least 1.5 fold change) between the infected and control populations. During the second phase of the time-course, phage production had leveled off, the host population had dropped to half of its peak cell density (Figure 2b) and the variation in the core metabolome became more dramatic relative to the uninfected controls. In fact, 80% of metabolite concentrations were significantly elevated (p-value < 0.1) in phage-amended populations at 480 min (Figure 2f). No significant decreases were noted for any metabolite. Thus, phage-amended populations transition to having an increased intracellular metabolite concentration during the infection cycle. The statistical differences within these 66 metabolites clearly point to the population of infected cells being physiologically distinct from those that are uninfected.

Since the relative increase in pool size can result from either an increase or decrease in metabolic activity as concentrations can build up from enhanced biosynthesis or from depressed utilization of a molecule,[113] we performed experiments with ^{13}C-acetate and monitored the incorporation of the label into 14 select TCA cycle components, N assimilation intermediates, and amino acids to assess their turnover rates as well as general cellular metabolic activity. This was done for populations in both the early (immediately following virus addition) and late (240 min post infection) phases of the time course described above. While the generation time of the bacterium was ~6 hr, the incorporation of labeled substrates into metabolites occurred within minutes (Appendix, Tables 12-15). With the exception of glutamate and glutamine, the cell normalized turnover rates for all measured metabolites were indistinguishable between the control and viral treated cultures for both phases of the infection cycle. As metabolite concentrations were largely similar between infected and control populations during these early time points (Figure 2f), the rate data further supports the observation that host metabolism was not dramatically altered

within the first hour following phage addition. However, the elevated metabolite concentrations in infected populations during the later phase of infection signifies an increase in metabolic activity as a greater concentration of metabolites were being utilized and replenished by these populations in the same period of time. Although the glutamate and glutamine pools were being turned over more slowly in the infected cultures (relative rates of 0.80 and 0.72 respectively (Figure 4), glutamine displayed a dramatic increase in concentration over the time course of the experiment culminating in a 6-fold excess in the phage amended cultures by the final time point. The magnitude of the glutamate pool increase was not as dramatic (1.37-1.67 fold) and concentrations of this metabolite remained fairly consistent throughout the experiment (Figure 2d). As flux is proportional to both pool size and turnover rate, the higher cellular concentrations of these metabolites in the infected cultures results in a flux that is essentially equivalent to those of the control populations for glutamate and higher for glutamine during the late phase of the experiment, even though the entire pool is consumed more slowly (Figure 4). Collectively, these data indicate that in comparison to controls, cell metabolism is measurably higher during the later stages of the infection cycle.



Time (min)		GLUTAMINE			GLUTAMATE		
Post Infection	Post ^{13}C Addition	Fold Change[a]	Rate Constant[a], k	Flux[a]	Fold Change[a]	Rate Constant[a], k	Flux[a]
0	**0**	1.16	0.91	1.06	1.18	0.91	1.08
30	**30**	1.46	0.91	1.34	1.07	0.91	0.98
240	**0**	2.09	0.72	1.50	1.06	0.80	0.84
270	**30**	3.64	0.72	2.62	1.24	0.80	0.99

[a]calculated as a ratio of Infected/Control per time point

Figure 4: Incorporation of acetate derived ^{13}C into glutamine and glutamate in phage amended and control populations during two distinct phases of infection (0 and 240 minutes post infection). The rate constant is determined by SigmaPlot (Methods and Materials). Flux is calculated by multiplying the fold change by the rate. Fold changes between 0.67 and 1.5 were considered as no change in concentration. Values are averages of duplicate biological and duplicate technical replicates.

The small molecule composition of the filtrates as well as the intracellular metabolite concentrations and turnover rates suggest that the phage-amended cultures were incorporating nutrients from recycled sources, *i.e.* from small molecules that were liberated during viral lysis or from breakdown of unlabeled macromolecules. The strongest evidence for this phenomenon comes from the data for glutamate and glutamine, which despite showing high intracellular concentrations in infected populations were undetectable in the culture filtrates (Table 2). Given that these compounds are typically preferred sources of N (over ammonium) in bacteria [3, 4, 5, 114, 115] it is likely that these compounds were released from lysed cells and then rapidly assimilated by metabolically active cells. Furthermore, these compounds typically have high intracellular concentrations in bacteria, with glutamate identified as the single most abundant metabolite in actively growing, acetate-fed *E. coli.*[116] To verify *Sulfitobacter* sp. 2047 also contained high concentrations of both of these metabolites, their absolute concentrations were determined for the "core metabolome" experiment and found to be similar to what has been reported for *E. coli* (1.5×10^{-14} g/cell and 1.1×10^{-15} g/cell for glutamate and glutamine, respectively;[116] (Figure 2c-d), further supporting the argument that these two compounds could serve as important sources of regenerated N in phage-amended cultures. Finally, the intracellular glutamate to glutamine ratio has been used as an indicator of nitrogen availability in many bacterial species, with an increased ratio indicating growth under nitrogen limiting conditions.[117] An analysis of this ratio for both phage-amended and control cultures indicates that the control population, which shows a progressive increase in this ratio through the growth cycle, is likely experiencing decreased N availability as the cultures reach the end of exponential phase (Figure 2e). The shift in the ratio is primarily driven by a decrease in the glutamine concentration. In contrast, the phage-amended cultures maintained a constant C:N ratio throughout the experiment, and these cells were able to increase both their glutamate and glutamine concentrations. As sufficient ammonium was provided to supply the N required in both cultures, these data indicate that the liberation of higher quality C and N containing nutrient sources are likely beneficial to the remaining members of the infected population.

External Calibration Curves of Glutamine and Glutamate

The calibration curves for glutamine and glutamate have a less than optimum R^2 value due to the presence of salt. When the calibration curves were collected with standards dissolved in extraction solvent without the added ratio of minimal media the reproducibility of duplicate injections and R^2 value was improved along with an increase in slope affirming an online desalting column would improve quantitation. [68] The salt causes ion suppression, clogs the ITT, and affects shape of chromatographic peaks. The reproducibility of duplicate biological and duplicate technical replicates in Figure 4 suggest the use of an internal standard would correct for irreproducibility of duplicate injections but the slope of the calibration curves would still be less than if salt was not present meaning a loss in sensitivity would remain.

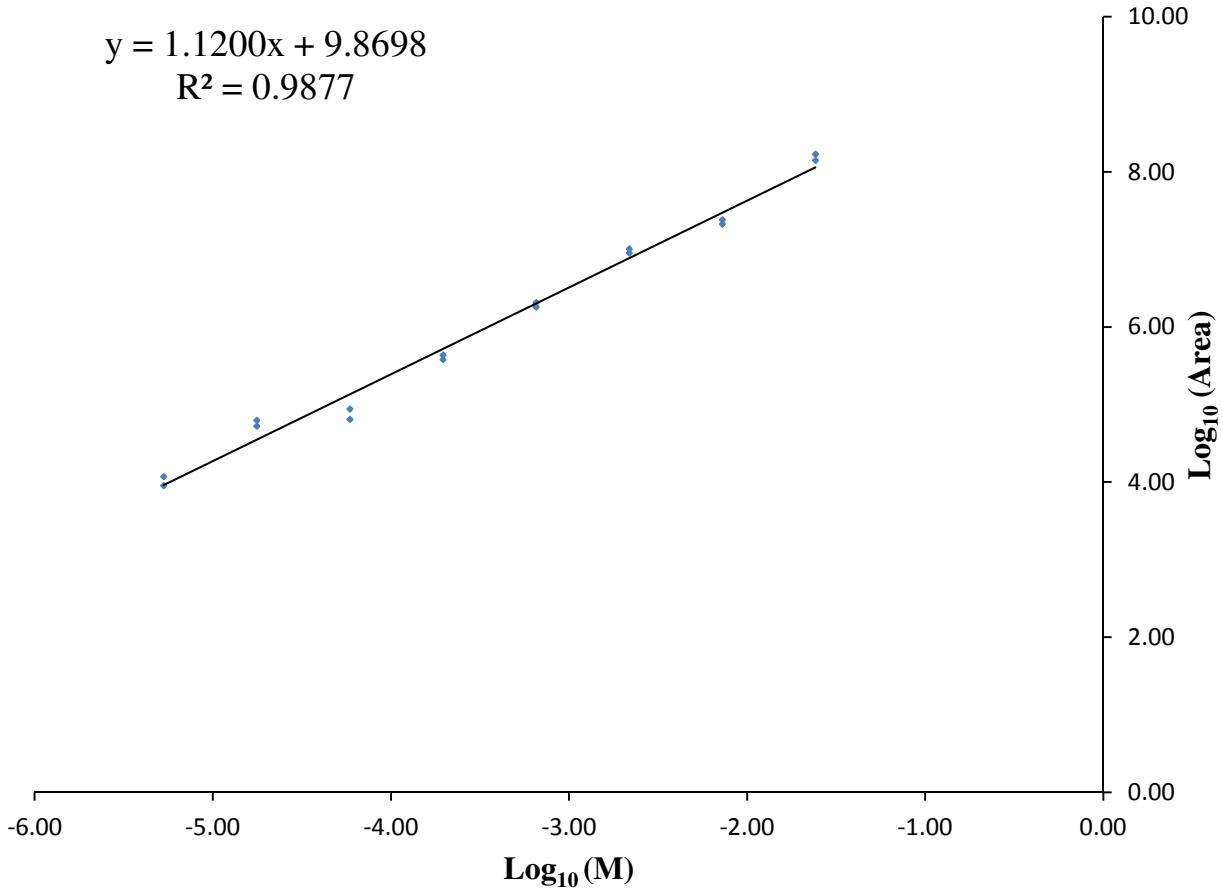


Figure 5: External standard calibration curve of glutamine. One dilution series and duplicate injections per calibration level.

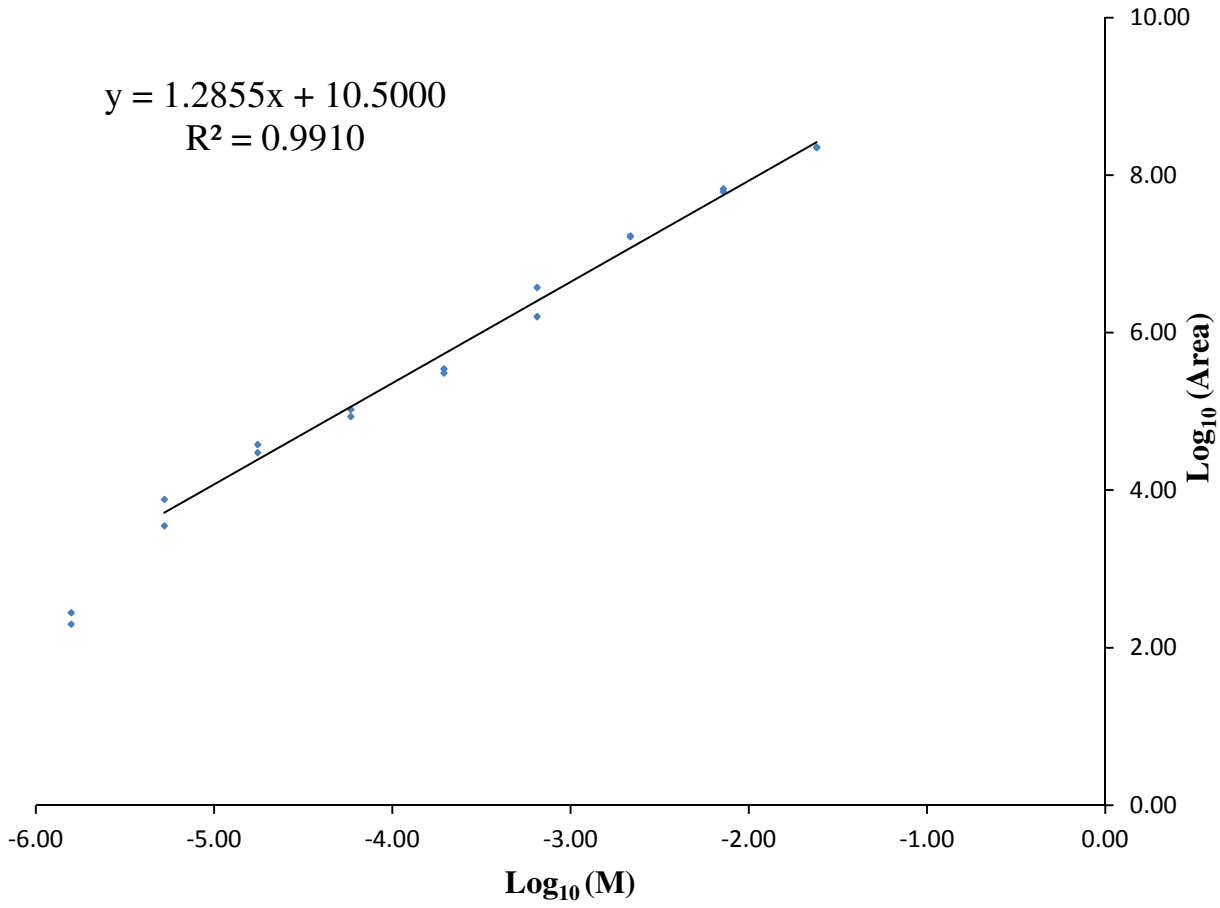


Figure 6: External standard calibration curve of glutamate. One dilution series and duplicate injections per calibration level.

Calculations

Figure 2a and Figure 3

Average areas of duplicate biological and duplicate technical replicates:

$$\frac{\left[\frac{Area\ of\ Bio.Rep.1 + Area\ of\ Tech.Rep.}{Cell\ \#\ of\ Bio.Rep.1} + \frac{Area\ of\ Bio.Rep.2 + Area\ of\ Tech.Rep.}{Cell\ \#\ of\ Bio.Rep.2}\right]}{\#\ of\ Areas}$$

The following Microsoft Excel formula was applied to calculate fold change:

=IF(AND (Avg. Infected Area = "#DIV/0!", Avg. Uninfected Area = "#DIV/0!"), "N/A", IF(Avg. Uninfected Area = "#DIV/0!", 1000, IF(Avg. Infected Area = "#DIV/0!", 0.001, Avg. Infected Area / Avg. Uninfected Area)))

Where Avg. Uninfected Area is at the relevant time point.

Figure 2c and Figure 2d

After interpolation of concentration using the linear equation of the external standard calibration curves the following formula was applied:

$$\frac{\left[[M]x\ Volume\ of\ Extraction\ Solvent\ used\ to\ Extract\ (L)x\ Molecular\ Weight\ (\frac{g}{mol})\right]}{Cell\ Count\ \left(\frac{cell}{mL}\right)x\ Aliquot\ of\ Culture\ Filtered\ (mL)}$$

Material for Isolation of Bacterial and Phage Strains, Culture Conditions and Sample Collection, and Flux Analysis taken from:

Ankrah, N. Y. D., May, A. L., Middleton J. L., Jones D. R., Hadden, M., Gooding, J. R., LeCleir, G. R. , Wilhelm S. W., Campagna, S. R. and Buchan, A. Metabolic response of a Roseobacter to phage infection: insights into the influence of viral lysis on ocean biogeochemistry, *PNAS*, *in press*.

Methods and Materials

General Methods

The HPLC-ESI-MS-MS system used for detection of metabolites included a Finnigan Surveyor MS Pump Plus, a Finnigan Surveyor Autosampler upgraded to Autosampler Plus Specifications, an Ion Max API Source operating in ESI mode, and a Finnigan TSQ Quantum Discovery MAX. A Dell Precision 390 was used as the data system. Xcalibur 2.0.7 was used for data acquisition and data processing. HPLC separations were performed using two separate columns for detection of positive ions and negative ions. Positive polarity separates in a Phenomenex Luna 250 x 2.00 mm NP column packed with 5 µm pore size, 100 Å diameter aminopropyl particles at 10.0°C column temperature and 150.0 µL/min flow rate. Negative polarity separates in a Phenomenex Synergi 150 x 2.00 mm RP column packed with 4 µm pore size, 80 Å diameter C18 particles at 25.0°C column temperature and 200.0 µL/min flow rate.

Isolation of Bacterial and Phage Strains

Sulfitobacter sp. 2047 was isolated from a mesocosm study in Raunefjorden, Norway in 2008 by enrichment with dimethylsufoniopropionate (DMSP). Agar plates (0.8% w/v Noble Agar [Difco, Sparks, MD, USA]) were made using 0.22 µm filtered fjord water and 10mM DMSP (kindly provided by J. Henrikson and W. Whitman UGA). The strain was subsequently maintained on Artificial Seawater (ASW) 1.0% agar plates [230 mMNaCl, 5.3 mMKCl, 3.9 mM $CaCl_2$, 0.1 mM H_3BO_3, 11.8 mM $MgSO_4$, 11.2 mM $MgCl_2$, 0.8 mM $NaHCO_3$, 5 mM NH_4Cl, 75 µM K_2HPO_4, and 10 mMTris-HCl (pH 7.5)] supplemented with 0.25% yeast extract (Fisher, Fair Lawn, NJ, USA) and 0.4% tryptone (Fisher, Fair Lawn, NJ, USA) at 20°C. Filter-sterilized

(0.22µm) stock solutions were added to the autoclaved basal salt solution along with vitamins, iron, and trace metals. Unless otherwise stated, all further growth experiments with the bacterium, including infections, were performed in ASW at 20°C.

Phage were isolated from viral concentrates of Raunefjorden seawater using standard bacteriophage enrichment. Viral (ca. 10X) concentrates were produced using a Labscale (tangential flow filtration) TFF System (Millipore, Billerica, MA) equipped with a Pellicon XL 50 Ultrafiltration Cassette (Millipore). Phage specific for *Sulfitobacter* sp. 2047 were enriched by adding early exponential phase host cells grown in ASW to an OD_{540} of 0.15-0.17 with the viral concentrate at a ratio of 2:1:2 of cell culture: ASW: Raunefjorden viral concentrate. Following incubation at 20°C for 48h, enrichments were centrifuged at 5000 x *g* for 10 min and then filtered through a 0.22 µm cellulosic filter (BD, Franklin Lakes, NJ, USA). Clarified phage enrichments were then subject to plaque assay using the same host. Plaques purification and preparation of phage stocks were based on standard methods.[118] Isolated phage were plaque-purified six times. Once purified, concentrated lysates were made by gently washing soft agar from 10 completely lysed plates of each phage strain using 7 mL of MSB buffer [230 mMNaCl, 5.3 mMKCl, 3.9 mM $CaCl_2$, 0.1 mM H_3BO_3, 11.8 mM $MgSO_4$, 11.2 mM $MgCl_2$, 0.8 mM $NaHCO_3$, 5 mM NH_4Cl, 75 µM K_2HPO_4, and 10 mMTris-HCl (pH 7.5)]. The final purified phage concentrate was 0.22 µm filtered and stored at 4°C in the dark.

Culture Conditions and Sample Collection

To carry out an analysis of the metabolite repertoire in phage infected *Sulfitobacter* sp. CB2047, the bacterium was grown in ASW supplemented with 10 mM sodium acetate (Fisher, Fair Lawn, NJ, USA) at 25°C in the dark with 200 rpm agitation. Once cultures reached an OD_{540} of ca. 0.17, phage were added at a multiplicity of infection of 4 (4 phage $cell^{-1}$). No phage added controls were run in parallel. Samples were collected for intracellular metabolites analysis prior to phage addition (t=0) and then 15, 30, 60, 120, 240, 360 and 480 min post phage addition. Sampling of the extracellular metabolites in the first cultures was performed after 480 min of growth in both the viral infected and control cultures.

Flux Analysis

For flux analyses, 20mM ^{13}C-acetate was added to a set of control and viral infected cultures at either 0 min or 240 min post infection. Samples were then taken from each culture to which ^{13}C-acetate had been added at 0, 5, 15, 30 60, 120 post-addition. To investigate any changes in central metabolic fluxes during infection we monitored the incorporation of ^{13}C-labeled acetate into intracellular metabolites over the course of infection cycle. ^{13}C-labeled sodium acetate (1,2-13C2, 99%, Cambridge Isotope Laboratories, Andover, MA, USA) was added to both infected and uninfected cultures at two distinct time points: immediately prior to phage addition (t=0) and at the onset of phage proliferation (t=240 min). Samples were collected 2 min following addition of ^{13}C acetate at 5, 15, 30, 60, 120, and 240, 245, 255, 270, 300, 360, 480 min.

Extraction Procedure

Extraction of metabolites followed methods developed by Rabinowitz and coworkers[119] with some modifications. Filter culture aliquots of 10 mL using Magna Nylon Filters (Millipore, Billerica MA) and house the filters cell side down in 60 x 15 mm polystyrene petri dishes holding 1.3 mL of 0.1 M formic acid 2:2:1 acetonitrile:methanol:water extraction solvent for 15 min at -20°C. Relocate the petri dishes to a 4°C controlled climate, flip filters cell side up, and pipette extraction solvent over the surface for 60 s. Transfer the solution into a 1.5 mL Eppendorf tube and centrifuge using an Eppendorf Centrifuge 5415 D set at parameters of 5 min, 13.2 rpm or 16.1 rcf, and 4°C. Aspire the supernatant and deliver 300 µL to each two autosampler vials. Data acquisition of one autosampler vial begins immediately and the other is reserved at -80°C for the opposite polarity following completion of the first chosen polarity.

Filtrates

Freeze filtrate aliquots of 5 mL at -80°C then place in a VirTis 3.5 L DBTES Lyophilizer and relocate to a 4°C controlled climate. Resuspend lyophilized filtrates in 300 µL of extraction solvent and centrifuge using an Eppendorf Centrifuge 5415 D set at parameters of 5 min, 13.2 rpm or 16.1 rcf, and 4°C. Aspire the supernatant and deliver to a 1.5 mL Eppendorf tube. Centrifuge again under same parameters. Aspire the supernatant and deliver 100 µL to each two autosampler vials.

Chromatographic Details

Partitioning of metabolites followed methods developed by Rabinowitz and coworkers with some modifications.[20, 120] A Finnigan Surveyor MS Pump Plus coupled to a Finnigan Surveyor Autosampler, upgraded to Autosampler Plus specifications, is used to perform high-performance liquid chromatography (HPLC) plumbed with 1/16 OD 0.003 in. ID polyether ether ketone (PEEK) tubing. A 4°C autosampler tray temperature and partial loop injections of 10 µL utilizing a 25 µL sample loop is used for both polarities.

Positive Mode

Positive polarity separates in a Phenomenex Luna 250 x 2.00 mm NP column packed with 5 µm pore size, 100 Å diameter aminopropyl particles at 10.0°C column temperature and 150.0 µL/min flow rate. Mobile phases are 95% 20 mM ammonium acetate, 20mM ammonium hydroxide in water, 5% ACN at pH 9.4 (solvent C) and ACN (solvent D). A 40 min gradient follows: t) 0 min, 15% solvent C : 85% solvent D; t) 15 min, 100% solvent C : 0% solvent D; t) 28 min, 100% solvent C : 0% solvent D; t) 30 min, 15% solvent C : 85% solvent D; t) 40 min, 15% solvent C : 85% solvent D.

Negative Mode

Negative polarity separates in a Phenomenex Synergi 150 x 2.00 mm RP column packed with 4 µm pore size, 80 Å diameter C18 particles at 25.0°C column temperature and 200.0 µL/min flow rate. Mobile phases are 97% 15mM acetic acid, 11mM tributylamine (TBA) in water, 3% methanol (solvent A) and methanol (solvent B). A 50 min gradient follows: t) 0 min, 100% solvent A : 0% solvent B; t) 5 min, 100% solvent A : 0% solvent B; t) 10 min, 80% solvent A : 20% solvent B; t) 15 min, 80% solvent A : 20% solvent B; t) 30 min, 35% solvent A : 65% solvent B; t) 33 min, 5% solvent A : 95% solvent B; t) 37 min, 5% solvent A : 95% solvent B; t) 38 min, 100% solvent A : 0% solvent B; t) 50 min, 100% solvent A : 0% solvent B.

Mass Spectrometric Detection Parameters

Eluent traversed through a 0.10 ID x 0.19 mm OD fused silica tube and into an Ion Max ion source housing of a Finnigan TSQ Quantum Discovery MAX operating in electrospray ionization (ESI) mode. The polyimide sheath coating of fused silica tubing was removed from the spray tip to prevent elongation due to ACN. The ion source was set at depth C, front-to-back 1.30, and side-to-side 0. Positive polarity uses a 4500 ESI spray voltage and negative polarity uses a 3000 ESI spray voltage. The ion transfer capillary temperature was 290°C. Nitrogen was used as sheath, auxillary, and sweep gas set at 40, 5, and 1 arbitrary units, respectively, and 100 psi from the source. Ultra high purity argon was used as collision gas and was set to 1.5 mTorr in the collision cell and 20 psi from the source. The scan type SRM was used with parameters 0.05 s scan time, 1 *m/z* scan width, and Q1 & Q3 peak width (FWHM) 0.7 Da, 0V skimmer offset voltage, centroid data type, and 1 microscan. Tuned tube lens voltage was used. Complete SRM parameters for the majority of metabolites have been reported by Rabinowitz and coworkers.[20] All Finnigan instruments were operated using Xcalibur 2.0.7 from a Dell Precision 390. All power systems were connected to a Toshiba 1600 EP Series UPS (Uninterruptible Power Supply).

Data Processing

Electronically stored chromatograms were viewed using Xcalibur 2.0.7 Quan Browser and peak area values were downloaded to Microsoft Excel 2007 where fold changes and p-values were calculated. Heat maps were generated using Gene Cluster 3.0 and viewed using Java TreeView 1.1.5.[121] SigmaPlot was used for calculation of rate constants. An exponential decay formula was obtained:

$$y = Ae^{-kx} + c$$

SigmaPlot solves for the rate constant *k*.

Sample Matrix for External Standards

A 10 mL volumetric flask was zeroed and filled to its mark with minimal media thermally equilibrated at 22˚C. The weight of 10 mL of minimal media was then used to calculate density:

$$\frac{Minimal\ media\ (g)}{Volume\ of\ flask\ (mL)} = Density\ of\ minimal\ media\ (g/mL)$$

Then the weight of a dry Magna Nylon Filter was recorded and the weight of a wet Magna Nylon Filter after filtering 10 mL of minimal media. The difference was taken in the wet and dry weights to obtain the weight of minimal media on the filter. This was repeated twice more and the average weight of minimal media on the filter was used for further calculations:

$$Average\ weight\ of\ minimal\ media\ on\ filter\ (g)\ \chi\ Density\ of\ minimal\ media\ (mL/g) = Average\ volume\ of\ minimal\ media\ on\ filter\ (mL)$$

The average volume of minimal media on the filter was used to determine a ratio of extraction solvent to minimal media to reproduce the sample matrix. The volume of extraction solvent is already determined by experimental design to be 1.3 mL and the average volume of minimal media on a filter was determined to be 0.1472 mL. Due to limitations of pipettes the ratio was rounded to 1.3:0.147. This ratio of extraction solvent to minimal media was used as the solvent in preparing glutamine and glutamate standards.

Data Processing for the Calculation of Absolute Quantitation using External Standard

A calibration curve was used to calculate the absolute quantitation of glutamine and glutamate as all other metabolites are relative quantitation through fold changes of area ion counts.

Glutamine

Serial dilutions were made of glutamine standard. 35 mg of glutamine (+99% Sigma-Aldrich) was weighed on weighing paper then a 10 mL volumetric flask with a glass funnel was zeroed. The glutamine was then transferred into the glass funnel and the actual weight glutamine transferred was recorded. 1.3:0.147 extraction solvent:minimal media was used to wash the glutamine down and for dilution. Then half log dilutions were performed using a pipette to transfer 750 µL x 2 from the stock to a 5 mL volumetric flask then to another 5 mL volumetric flask and so on until enough dilutions were obtained to produce a calibration curve where all measured values could be interpolated. The sampling order for chromatographic injections was from lowest concentration to highest to prevent erroneous carryover. One dilution series but each sample was injected in duplicate.

The calibration curve was graphed on a $\log_{10}$ scale to display calibration levels equidistance apart. A linear equation was obtained using least-squares linear regression of the data:

$$Area\ of\ Glutamine = m\,[Glutamine] + b$$

The [*Glutamine*] in samples can be determined as *Area of Glutamine* is known.

Glutamate

Serial dilutions were made of glutamate standard. 35 mg of glutamate (+99% Sigma Aldrich) was weighed on weighing paper then a 10 mL volumetric flask with a glass funnel was zeroed. The glutamate was then transferred into the glass funnel and the actual weight glutamate transferred was recorded. 1.3:0.147 extraction solvent:minimal media was used to wash the glutamate down and for dilution. Then half log dilutions were performed using a pipette to transfer 750 µL x 2 from the stock to a 5 mL volumetric flask then to another 5 mL volumetric flask and so on until enough dilutions were obtained to produce a calibration curve where all

measured values could be interpolated. The sampling order for chromatographic injections was from lowest concentration to highest to prevent erroneous carryover. One dilution series but each sample was injected in duplicate.

The calibration curve was graphed on a $\log_{10}$ scale to display calibration levels equidistance apart. A linear equation was obtained using least-squares linear regression of the data:

$$Area\ of\ Glutamate = m\,[Glutamate] + b$$

The [*Glutamate*] in samples can be determined as *Area of Glutamate* is known.

Chapter 2

Changes in Choline Esters in Blood and Milk during Early, Mid, and Late Lactation in Dairy Cows

Material for Abstract and Introduction taken from:

Artegoitia, V. M., Middleton, J. L., Harte, F., Campagna, S. R., and de Veth, M. J. Changes in Choline Esters in Blood and Milk during Early, Mid, and Late Lactation in Dairy Cows, *in preparation.*

Abstract

Choline is an essential nutrient for humans and production animals. The ruminant is a unique animal model as almost all dietary choline is degraded in the rumen and the requirement for choline is not established for dairy cows. Therefore, understanding what choline forms are secreted by the mammary gland may provide an understanding of the lactation requirement for choline in the dairy cow. The objective of this study was to characterize the changes in choline and choline esters in blood and milk occurring in early, mid, and late lactation. Twelve Holstein cows were selected at calving and managed under the same diet, without choline supplementation. Throughout the study milk and blood samples were collected three times during early (wk 1, 2 and 3), mid (wk 4, 5 and 6), and late lactation (wk 7, 8, and 9). Free choline (Cho) and choline esters, glycerophosphocholine (GPC), lysophosphatidylcholine (LPC), phosphatidylcholine (PtCho), phosphocholine (PCho) and sphingomyelin (SM), were analyzed using liquid chromatography-tandem mass spectrometry and quantified by using stable isotope-labeled internal standards. Fold changes reported are all $P \leq 0.01$ for these compounds. The major choline forms in plasma were PtCho (79%) and SM (14%). The main choline forms in milk were PCho (46%), PtCho (27%) and Cho (11%). The concentration of all esters decreased in milk, except PtCho and Cho (increased), and SM (remained the same). In summary, the 2-fold higher level of choline output by the mammary gland, combined with lower plasma levels, during early lactation suggest that there is a greater requirement for choline during this period.

Background and Significance

Introduction

Production animals require the essential nutrient of choline for optimal growth and performance. The primary importance of choline is the biosynthesis of phosphatidylcholine (PtCho), which has important roles in the body. The phospholipid membrane in all cells is comprised of PtCho, making dietary choline especially important in growing animals. Lipid metabolism in the liver requires choline since PtCho hastens the removal of fat and reduces fat accumulation. Triglycerides (TG) will accumulate in the liver of a lactating dairy cow during the periparturient period, which may lead to metabolic disorder known as ketosis and lower milk yield. Supplementing the diet with rumen protected (RP) choline during this period will reduce TG accumulation and improve milk yield.[122] Until now the supplementation of RP choline has been during the periparturient period.

Choline also participates in methylgenesis, in which the methyl groups on choline are used for the formation of methionine (Met) from homocysteine. Oxidation of choline occurs when methyl groups are transferred to form up to three molecules of Met. The first methyl group is transferred when the oxidative intermediate of choline, betaine, is metabolized to dimethyl glycine. Then choline is further oxidized to release the remaining two methyl groups which may enter the folate pathway.

The potential for choline to act as a surrogate for and provide Met has biological and economic importance, but choline has not been used as an alternative for Met as a supplementation for dairy cows. The reasons for this may come from reliance of information from previous studies. The studies where RP choline was supplemented did not determine the level of choline absorbed at the small intestine after degradation in the rumen. The alternative use of RP choline to provide RP Met as a feed additive must evaluate the actual choline availability to the dairy cow post rumen. The development of RP amino acids studied the post rumen availability, and this must be done with RP choline. Also, other studies have conflicting reports of the importance of methylgenesis in the lactating dairy cow. In a classical study of methylgenesis, radio labeled

choline was injected in lactating goats, and no radio labeled methyl groups on Met were found, suggesting choline was not the source of methyl groups for Met.[123] Another report stated the enzyme betaine-homocysteine methyl transferase (BHMT), which transfers the methyl group from betaine to homocysteine to form Met, had enzymatic activity 5 times lower in the sheep (a ruminant) than in rats.[124] In contrast, a different report determined that BHMT enzymatic activity alters with changes in Met supplementation in steers.[125] A more recent study[126] concluded changes in the methylation cycle due to methylgenesis is not the reason why folate and vitamin B12 increases milk production. This experiment has another important research objective of elucidating the changes in choline metabolism and methylgenesis due to choline supplementation and evaluate if choline can provide Met as a supplement in the lactating dairy cow.

Microbes in the rumen degrade the majority of naturally occurring choline in feed and choline chloride in supplements.[127] Choline is absorbed at the small intestine of ruminants. For choline supplementation to be effective it must be RP choline, and when RP choline is supplemented to the lactating dairy cow a reduction in TG in the liver has been observed.[122] A limiting factor in such studies is that they do not allow an understanding of the choline absorption at the small intestine or bioavailability to the animal. This is problematic for the following two reasons. Firstly, there is no protocol for effectively evaluating the efficacy of various RP choline products.[128, 129] Secondly, the importance of choline supplementation is established, but a requirement of choline supplementation could not be established due to variable responses of reduced TG in the liver in the lactating dairy cows. This is possibly due to the uncertainty of bioavailable choline from different RP choline products meaning absorption at the small intestine or the various responses were due to inconsistent RP choline supplementation.[127]

An approach to estimate bioavailability of choline in the lactating dairy cow is to measure choline levels in the blood when no supplementation is given and at various known amounts of RP choline supplementation, then comparing the increase in blood choline levels with the amount of RP choline supplementation. This approach has been previously applied to evaluate the bioavailability of lysine in soybean meal and RP lysine.[130, 131] An alternative approach is to evaluate bioavailability of choline through measurements of choline levels in milk and to

compare rises in milk choline levels against various known amounts of RP choline supplementation.

The advantage of measuring choline levels in the milk over blood for evaluating bioavailability of choline is that the total amount of choline secreted in the milk is measured, whereas, blood only measures changes in choline concentration since choline is secreted in the milk. A study used the approach of measuring choline levels in the milk after abomasally infusing four different levels of unprotected choline chloride and an increase in milk choline yield was correlated with increasing choline chloride dosages.[132] However, the authors noted the response of choline infusion was curvilinear and the actual recovery of choline in the milk was ~20-fold lower than previous reports in lactating cows, in other words, <5% of the choline infused was recovered in the milk. This concludes that this approach is not appropriate for quantitative evaluation of choline bioavailability. In addition, other field evaluations of using milk choline levels to assess the bioavailability of choline from RP choline supplementation have been found to not be reproducible, but the lack of reproducibility may be in large part due to the sample preparation.[132] It seems that they did not extract the choline from milk fat, which is a major choline containing component in milk,[133] therefore, milk choline levels may still be a reliable indicator of bioavailability. An LC/MS/MS method has been developed that is suitable for quantitation of choline and choline esters in blood and milk at different physiological states of the lactating cow such as early, mid, and late lactation. Up until now there have been difficulties in quantifying the total bovine milk choline,[133] and this approach will correlate milk choline and other milk components to determine if choline levels can be predicted form these components.

Internal Standard

Internal standards (ISTD) may be an isotope of the target compound or a different compound typically used to correct for ion suppression and loss of signal due to the ITT clogging. An ISTD works best when it shares the same RT as the target and is chemically similar to the target compound, in other words, an isotope of the target compound. The forepump pressure decreases as the ID of the ITT is narrowed by accumulation of contaminants creating a better vacuum but decreasing ion transmission. The loss of signal can be monitored by plotting the area of an ISTD and forepump pressure versus injection number. Typically a two-fold decrease in ISTD area is observed before total loss of signal meaning target compounds in samples at the end of a high number sample analysis is subject to lower signal response even if they share the same concentration of target compound as the samples in the beginning of the analysis. The correction for ion suppression is an estimation because no two SRM transitions can be measured at the same time. An ISTD that has a different RT is affected by ion suppression during a chromatographic window that does not equally affect the target compound. [48, 134] The closer the RT to the target compound the more similar the ion suppression conditions are if the ion suppression zone is broad and encompasses both. The signal loss due to ITT clogging is typically a slow change, and an ISTD does not need the same RT as a target compound to correct for signal loss.

Impurities can be added to the sample when adding internal standard.[134] The ISTD stock solution should screened for target compounds and the sample for ISTD compounds to ensure both solutions are free of each other's compounds. An isotope labeled target compound should be labeled enough to not have interference from the natural isotope of the target compound in the SRM transition and be pure enough that unlabeled target compound does not interfere with the target compound SRM transition. If interferences exist, than a correction formula must be used that subtracts the contribution of area from the interference.[135]

The concentration of ISTD must be constant in all samples. The area of the target compound is divided by the area of the ISTD, and two samples with the same concentration of target

compound will yield two different area ratios if the concentration of the ISTD is different in both.[134] Any factors resulting in differences in ISTD area are assumed to equally affect the target compound but the area ratios of samples are only valid for comparison when the concentration of ISTD is the same in samples. A difference in ISTD area due to addition to samples will cause error in analysis as the differences in ISTD area do not equally affect the target compound area, and it is best to not use area ratios.[134] To prevent this, one batch of ISTD with enough volume to last the entire experiment is prepared, and the same pipets are used. ISTD is also used to correct for transfer loss, degradation, loop injections, and is best added to the sample directly after collection and prior to storage.

Classes of compounds usually elute at different RT due to the varying lengths of R groups. To have an ISTD that covers the entire peak of a target compound, one would need an isotope labeled target compound of each. This is unrealistic due to issues of solubility, introduction of contaminants, and the cost. Typically one ISTD for each class of compounds is used in experiments where the RT of the compounds are similar.

Foregoing the external standard calibration curve with internal standard and applying the formula for absolute quantitation using isotope dilution saves much lab work not preparing external standard curves but there are differences. The formula assumes that double the area of a target compound to the area of an isotope standard is double concentration, only reports concentration of monoisotopic mass when an SRM scan is set for the monoisotopic parent mass, and neglects mass discrimination of the electron multiplier.

Double the area of a target compound to the area of an isotope standard is double concentration when measurements are within the linear portion of the sigmoidal curve and the slope of the external calibration curve is 1 for both isotopomers. Since ESI external calibration curves are ~1 in slope at the set mass spectrometry parameters that affect sensitivity this is a good approximation. A doubling in scan time would increase the slope. Signal is twice as much with

direct injection on a constant concentration, but with chromatographic peaks the concentration is changing over time and twice the scan time does not result in twice the signal in each individual scan. Conversely, a loss in steepness of slope and linearity can be observed when increasing scan time as an increase in duty cycle can lead to a loss in signal due to missing the apex of the peak and irreproducible areas.

The formula only reports the concentration of monoisotopic mass when an SRM scan is set for the monoisotopic parent mass. The concentration reported using an external standard calibration curve is for all isotopic masses regardless that the SRM scan is for a monoisotopic parent mass. The actual concentration of the compound including all isotopic forms is larger and becomes more significant with much larger compounds as the largest peak in a full scan MS may not be the monoisotope. Scanning for the most abundant isotope instead of the monoisotope would lower LOD in MS analysis, but in MS/MS analysis the LOD may not be lowered because the desired fragment is a combination of isotopes due to the random positions of isotopes in the parent mass which is not the case with a monoisotopic parent mass. A theoretical correction factor[136] would need to be applied to obtain the concentration of all isotopic masses. An alternative is the empirical determination by direct infusion of the compound to obtain a full scan mass spectrum with a sufficient number of microscans then using the centroid peak heights to determine the percent abundance of the monoisotopic form compared to the total isotopic forms. The correction factor would be the multiplication of (100 / % abundance of monoisotope) to the entire formula.

Mass discrimination of the electron multiplier is neglected in the formula. The heavier isotope standard product ion will eject more electrons upon impact of the conversion dynode than the lighter target compound product ion resulting in a higher signal for the isotope standard at the same concentration. The increase in signal for the isotope standard is higher at higher concentrations, but because a constant concentration of isotope standard is used the factor of mass discrimination remains constant. The formula places the isotope standard area in the denominator and equal concentrations result in a ratio less than 1, which slightly underestimates

the concentration of target compound. The mass discrimination of the electron multiplier is typically not disclosed, and an empirical determination can be made of the mass discrimination factor by direct infusion of a standard with a known isotopic ratio then obtaining a full scan mass spectrum with a sufficient number of microscans. The centroid peak heights are used to determine the isotopic ratio of the monoisotope and the +1 isotope peak. This isotopic ratio can be compared to an accepted value, and the error is from mass discrimination. A typical value is 0 to 6% increase[64] in signal for each amu. This means the heavier the product ion of the isotope standard compared to the product ion of the target compound the more significant the correction becomes. The correction factor would be the multiplication of (1 + % increase as a decimal) to the entire formula.

The alternative of using an external calibration curve with internal standard has the same errors of neglecting thermal degradation, assuming a purity of 100% considering all weight to be from the compound, mass transfer into the first stock and analytical balance rounding error, dilution error, in addition to, neglecting changes in instrument response, ignoring SRM interferences from matrix when a target compound free matrix cannot be obtained,[59] and an imperfect fit of the curve. An error in dilution may be seen on the graph as a sharp change in pattern of calibration levels affecting the fit, but an error in mass transfer results in the entire curve to be lower and the fit is not affected. Multiple dilution series are used to account for mass transfer error into stock and analytical balance rounding error. An empirical comparison can be made by preparing an external standard calibration curve with the isotope standard as the internal standard. Then compare the concentrations predicted by the fit to the concentrations predicted using the formula to determine the difference between the two techniques. In conclusion, neither approach is 100% accurate but are within acceptable error when applied correctly.

Please Refer to Chapter 1 for a Fuller Discussion on Analytical Techniques

Results and Discussion

The concentrations were typically < 20% RSD for triplicate injections. Classes of compounds eluted at slightly different RT depending on the length of their chains. For example, a short chain PtCho has a higher RT than a long chain PtCho, and RT shifts were observed between milk samples but not plasma samples. The time segmentation of MS acquisition placed PtCho close to the end of its time segment. To ensure detection of an entire chromatographic peak, the shortest chain PtCho was monitored using Qual Browser throughout the analysis. Other compounds that bordered their time segment were also monitored. As the column aged time segmentation was shifted to the right due to an increase in RT of compounds. If a comparison of milk samples to plasma samples is made at each corresponding week of lactation, then the following general statements can be made. Milk has a higher concentration of AcCho, Cho, GPC, and Pcho than plasma. Plasma has a higher concentration of PtCho, LPC, and SM. Betaine is in near equal amounts in both milk and plasma samples.

Determination of Heatmap Log Scale

Heatmaps were tested on $\log_2$ – $\log_{10}$ scales to determine a suitable log scale. A lower base log is more sensitive than a higher base log and can show small differences more efficiently, whereas, the higher base log would be more efficient for large differences. A log scale is needed to remove fraction bias. A difference between two data sets would give two different fold changes depending on which set is selected for the numerator. For example, if the larger data set is chosen for the numerator then (2x / x) = 2 which is 200% greater, whereas, if the smaller data set is chosen for the numberator then (x / 2x) = 0.5 which is 50% lower. However, the log (2) = +Y and the log (0.5) = -Y which is the same distance from 0. A compromise must be made for all compounds and a $\log_3$ scale was determined to be the most suitable to show differences in all compounds.

Changes in Milk and Plasma at Different Weeks of Lactation Relative to Week 1

Milk samples displayed an overall decrease in AcCho, Pcho, and Bet. An increase was observed for Cho and PtCho. Sphingomyelin stayed nearly the same. Acetylcholine was not detected in early lactation (Figure 7). Plasma samples showed an overall increase in LPC, PtCho, and SM. Betaine and Cho remained nearly the same. No decreases were observed (Figure 8). Phosphocholine had a %RSD greater than 20% for triplicate injections because the concentration was at LOD. Acetylcholine was not detected but the recovery of AcCho ISTD was sporadic suggesting that AcCho may be present in significant amounts in plasma. The addition of an enzyme inhibitor upon collection of plasma sample has been discussed for future work.

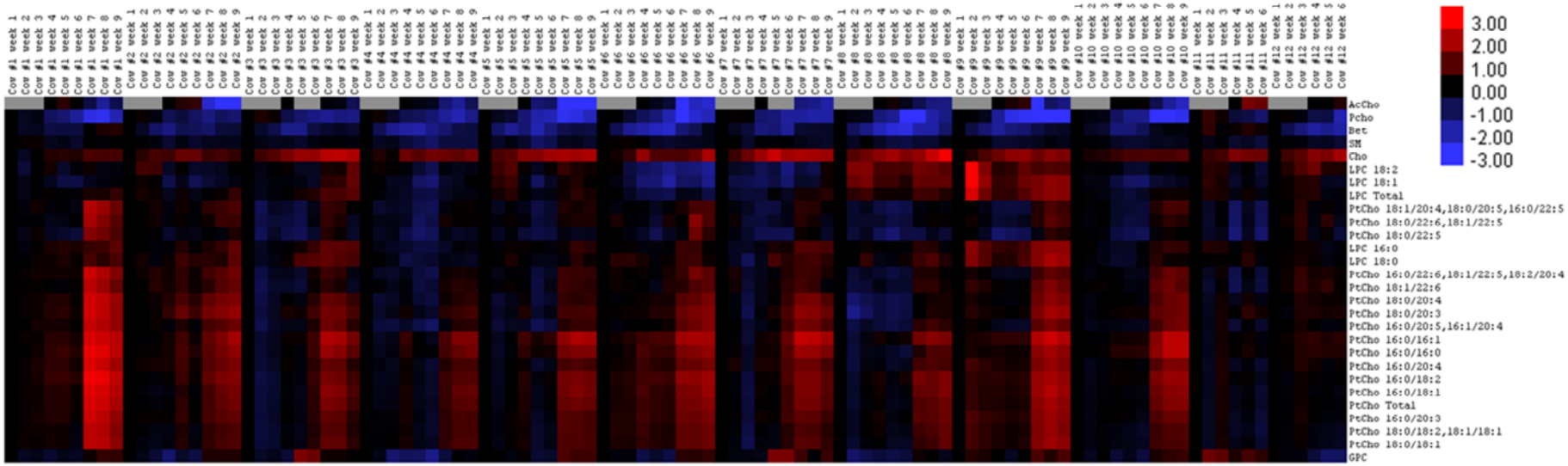


Figure 7: Heatmap of milk samples from 12 cows. Rows are lipids detected and columns are weeks of lactation with each cow in staggered formation. Each color square represents the fold change of average absolute concentration of triplicate injections. Blue is decrease and red is increase in fold change relative to week 1 or 1st week of detection for each cow displayed on a $\log_3$ scale. Black is no change and gray is undetected. Cows #11 and #12 were unavailable for late lactation sampling.

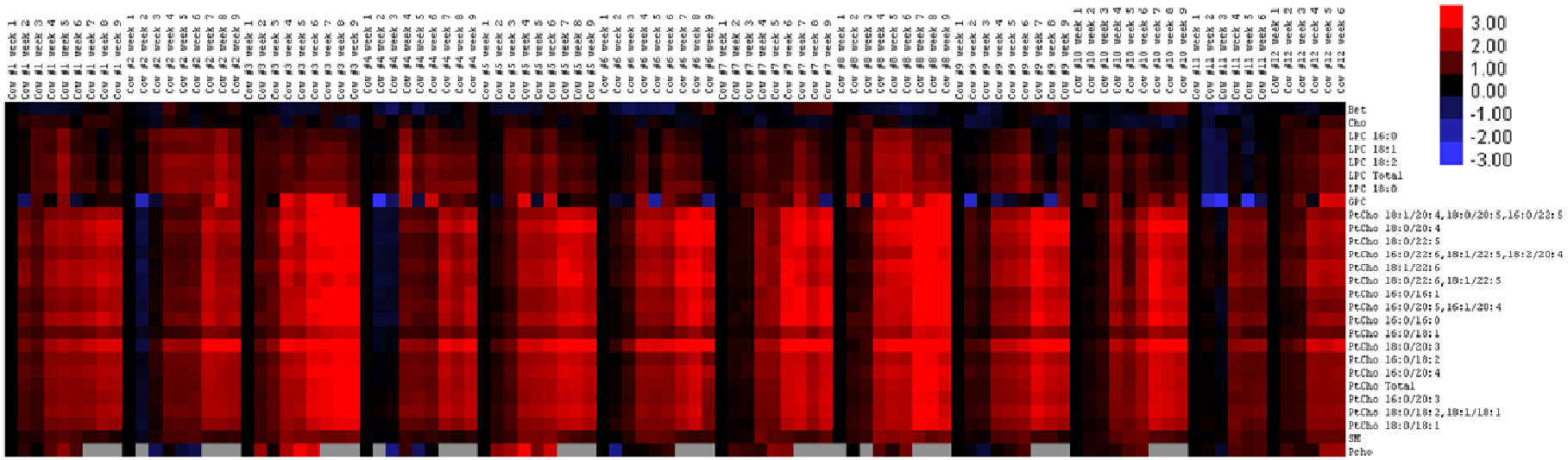


Figure 8: Heatmap of plasma samples from 12 cows. Rows are lipids detected and columns are weeks of lactation with each cow in staggered formation. Each color square represents the fold change of average absolute concentration of triplicate injections. Blue is decrease and red is increase in fold change relative to week 1 or 1st week of detection for each cow displayed on a $\log_3$ scale. Black is no change and gray is undetected. Cows #11 and #12 were unavailable for late lactation sampling.

Extraction Reproducibility of Milk and Plasma Samples

To confirm that the color changes on the heatmaps (Figures 7 and 8) were not due to differences in extractions, three samples were selected and extracted twice. The difference in extractions were no more than 30% for all compounds, and because the changes in the heatmaps (Figures 7 and 8) at different weeks of lactation (WOL) are far greater than 30% the color changes shown are not induced from extraction irreproducibility. To further prove this the extraction replicates were placed on a heatmap of the same log scale with fold changes relative to the first extraction. The heatmaps (Figures 9 and 10) appear black because the irreproducibility of extractions is not large enough to induce color changes on the chosen log scale.

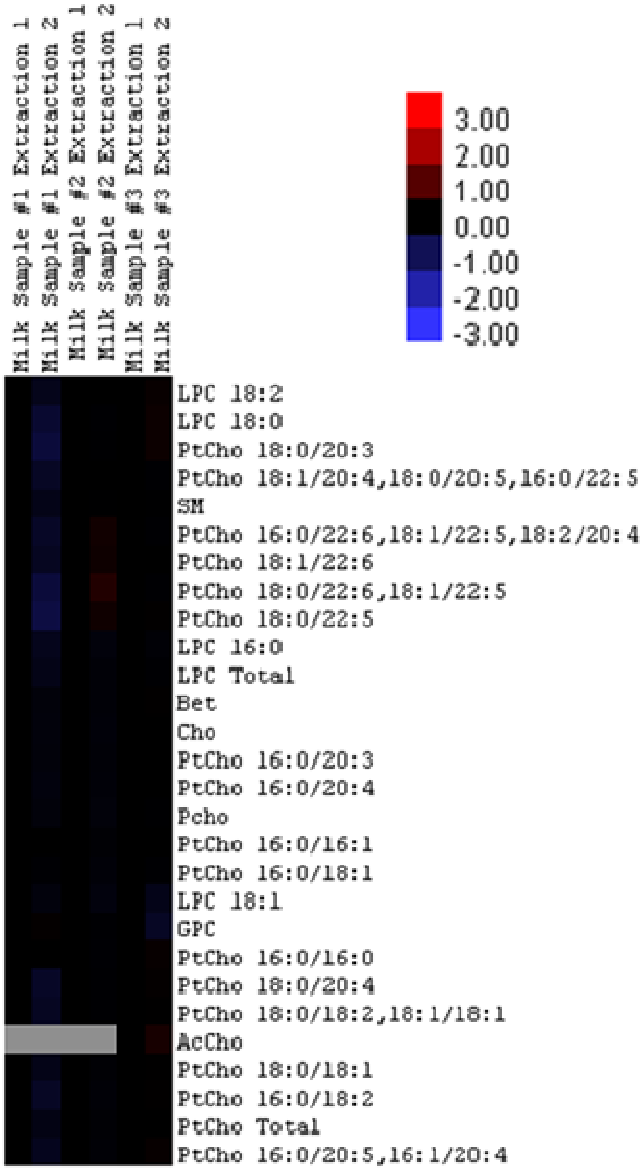


Figure 9: Heatmap of duplicate extractions of milk samples. Rows are lipids detected and columns are extractions with each milk sample in staggered formation. Each color square represents the fold change of average absolute concentration of triplicate injections. Blue is decrease and red is increase in fold change relative to extraction 1 for each milk sample displayed on a $\log_3$ scale. Black is no change and gray is undetected.

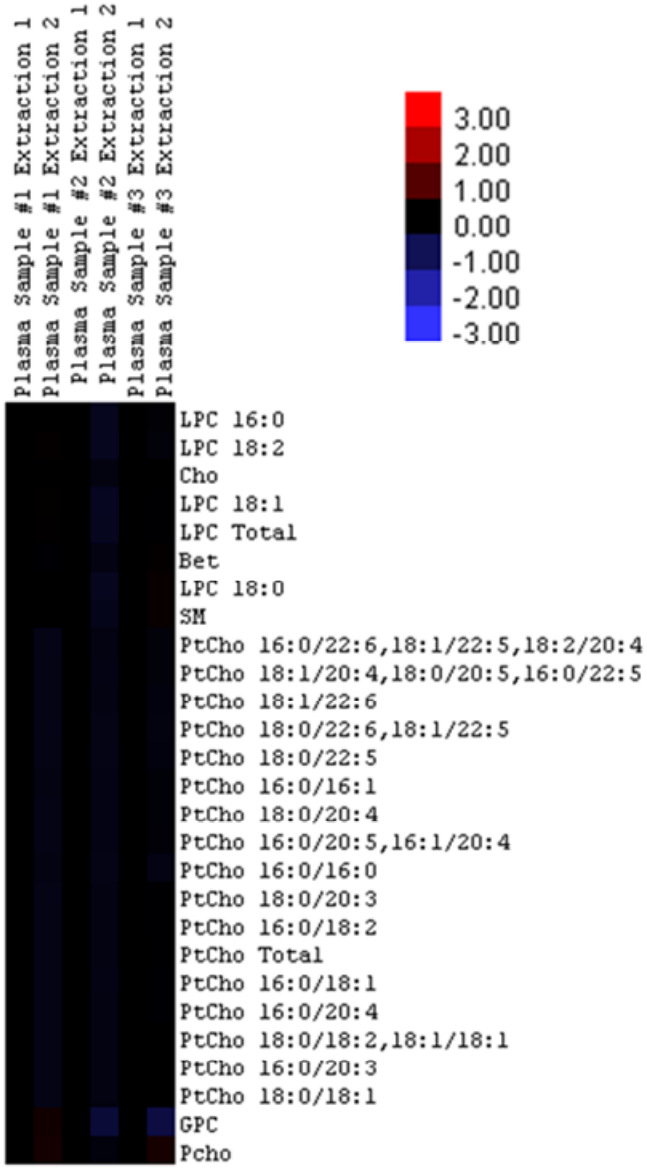


Figure 10: Heatmap of duplicate extractions of plasma samples. Rows are lipids detected and columns are extractions with each plasma sample in staggered formation. Each color square represents the fold change of average absolute concentration of triplicate injections. Blue is decrease and red is increase in fold change relative to extraction 1 for each plasma sample displayed on a $\log_3$ scale. Black is no change and gray is undetected.

External Calibration Curve of GPC

The GPC standard was dissolved in the same sample solvent used for resuspension and dilution of samples. However, a target compound free matrix is not available such as milk or plasma free of GPC. Therefore any matrix effects such as ion suppression that exist in samples do not exist when measuring the calibration curve. This results in an inability to correct for ion suppression and ion transmission losses in real time as the target compound is being measured like the ISTD does. Using the fit to predict concentrations of GPC may explain why GPC does not develop a well-defined trend on the heatmaps (Figures 7 and 8).

Internal Standard SRM Determination

To determine the SRM transitions of the ISTDs a strategic approach was used. The labeled parent ion was screened at the product ion used for the unlabeled parent ion and all +1 product ions up to a product ion that would result if all of the labels from the parent were included. Surprisingly, many other product ions were observed in addition to the product ion that results when the position of the labels are in the position stated by the distributor. However, the strongest signal was the product ion from the parent ion with position of labels as stated by the distributor and was chosen for quantitation (Table 3). This raises questions about unanticipated internal standard impurities as interferences will not just result from target compound and stated labeled compound but also from other labeled versions. Only the labeled parent ion was scanned at different product ions to find the SRM transition of the ISTD, but if a scan for other parent ions may result in signal from a less labeled parent ion and then other product ions due to random labeling of the parent ion. The ISTD stock solution was screened for target compounds and both plasma and milk samples were screened at the ISTD SRM transitions. Samples and the ISTD stock solution were determined to be free of each other's compounds, and no correction factors were applied.

Table 3: Internal Standard SRM Determination of AcCho-IS as AcCho-d13

AcCho-d13 Parent *m/z*	loss of quaternary amine Product *m/z*	# of deuteriums in product ion	Observed peak
159.4	87.2	0	N
159.4	88.2	1	N
159.4	89.2	2	Y
159.4	90.2	3	Y
159.4	91.2	4	Y
159.4	92.2	5	Y
159.4	93.2	6	N
159.4	94.2	7	N
159.4	95.2	8	N
159.4	96.2	9	N
159.4	97.2	10	Y
159.4	98.2	11	Y
159.4	99.2	12	N
159.4	100.2	13	N

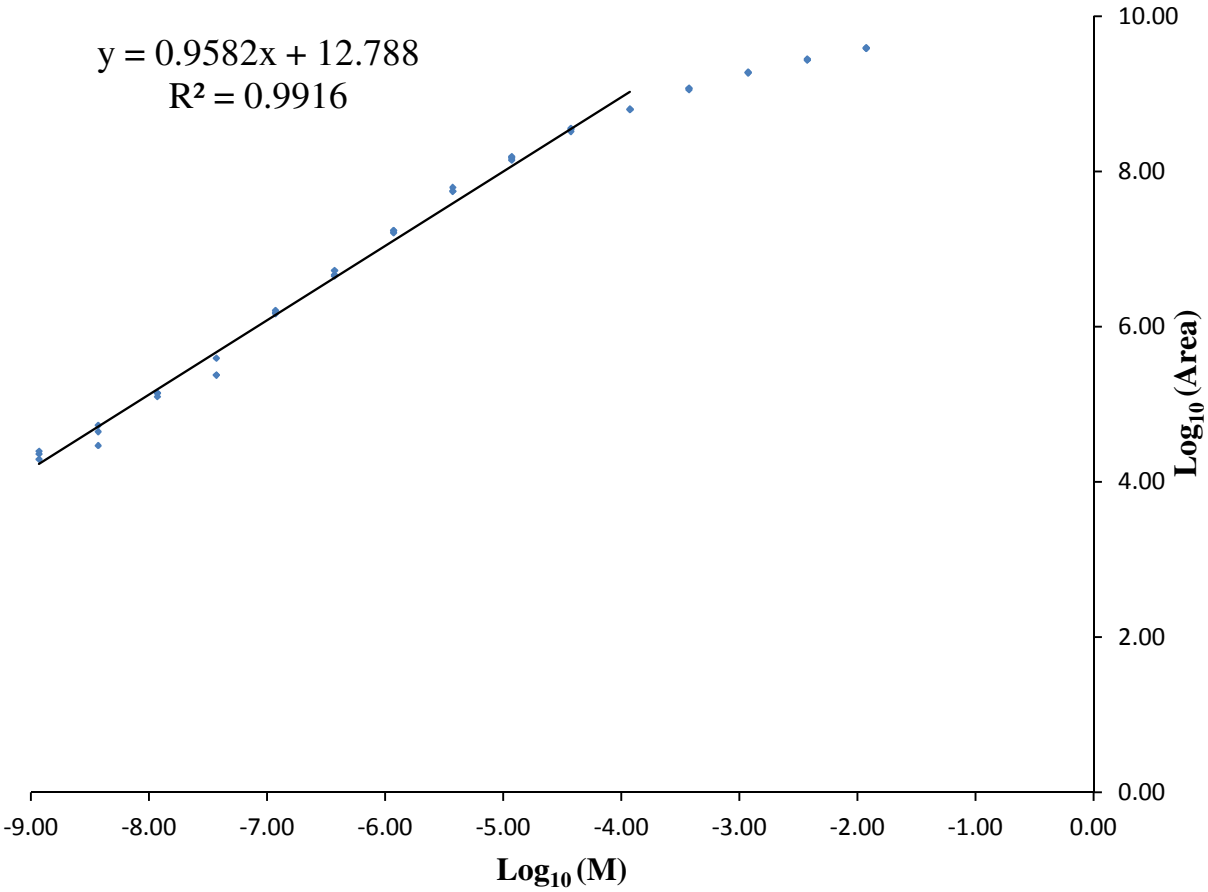


Figure 11: External standard calibration curve of GPC. One dilution series and triplicate injections per calibration level.

Calculations

Determination of 5x LOD in Autosampler Vial

To determine how much ISTD to add to the ISTD stock solution to result in a final concentration at 5x lower limit of linearity (LLOL), a retro calculation was done:

$$[5xLLOL]\, x \frac{Volume\ of\ Resuspension\ (L)}{Volume\ of\ ISTD\ Stock\ Added\ to\ Sample\ (L)} = [ISTD\ Stock]$$

Figures 7-10

After applying the formula for absolute quantitation using isotope dilution in methods and materials, the following formula was used to calculate the concentration in milk and plasma samples:

$$[Target\ Compound] x \frac{Resuspension\ Volume\ (L)}{Volume\ of\ Sample\ (L)} = [Sample]$$

Then the following was applied to calculate fold changes:

$$\frac{Avg.\ of\ Triplicate\ Injections\ of\ a\ Sample}{Avg.\ of\ Triplicate\ Injections\ of\ Relative\ Sample} = Fold\ Change$$

Methods and Materials

General Methods

The HPLC-ESI-MS-MS system used for detection of lipids included a Finnigan Surveyor MS Pump Plus, a Finnigan Surveyor Autosampler upgraded to Autosampler Plus Specifications, an Ion Max API Source operating in ESI mode, and a Finnigan TSQ Quantum Discovery MAX. A Dell Precision 390 was used as the data system. Xcalibur 2.0.7 was used for data acquisition and data processing. HPLC separations were achieved using an Ascentis Express HILIC column 150 x 2.1 mm, 2.7 µm particles at 25.0°C column temperature and a 200.0 µL/min flow rate.

Extraction Procedure

Blood was taken from the Coccygeal tail vein of each cow, and centrifugation was performed to remove the plasma from the blood sample and store at -80˚C until further analysis. Milk was also stored at -80˚C until further analysis after initial collection from the specimen. Aliquots of 0.01% relative milk production of AM and PM consecutive milkings were mixed as one sample before the start of milk extractions. Both plasma and milk samples were extracted following an extraction procedure developed by Zhao and coworkers.[62] Samples were thawed and remained on ice throughout the extraction procedure. Briefly, 1 mL of extraction solvent (chloroform, methanol, water 1:2:0.8) was added to 200 µL of sample and 40 µL ISTD stock solution in a 1.5 mL eppendorf tube. The samples were centrifuged at 16,000 rpm for 5 min at 4˚C. The supernatant was transferred to a glass vial capable of holding 5 mL, and the extraction procedure was repeated twice transferring the supernatant to the same glass vial. The combined extracts were dried under nitrogen while being kept on ice and resuspended with 5 mL of methanol kept on ice. Then 300 µL of sample was transferred to an autosampler vial for analysis.

Internal Standard Stock Solution

Internal standards in the methanol stock solution were prepared at concentrations that would result in a final concentration in the autosampler vial of approximately 5 times greater than lower limit of linearity[62] of the target compound to ensure signal greater than 10:1 but not to suppress the target compound signal.[137] Internal standards are as follows: 1,2 Distearoyl-sn-glycero-3-phosphocholine-N, N, N-trimethyl-d9 (PtCho-IS, Avanti Polar Lipids, 860362), Sphingomyelin-d13-c13 (SM-IS, Ricerca-custom made), Acetylcholine bromide-d13 (AcCho-IS, C/D/N Isotopes INC, D-1780), L-a-Lysophosphatidylcholine-palmitoyl-d3 (LPC-IS, Larodan, 71-2826), Choline chloride-trimethyl-d9 (Cho-IS, Cambridge Isotopes, DLM 549-1), Betaine-d11 (Bet-IS, Cambridge Isotopes, DLM 407), Phosphorylcholine chloride-d9 (PCho-IS, Cambridge Isotopes, DLM-298). Solids were weighed on weighing paper, transferred to the stock solutions, and the weighing paper was weighed again. The difference in weights was recorded as the mass transferred to the stock solution. Liquids were delivered with a calibrated pipet. The recorded weight on the vial was used when the entire contents were needed and the vial was rinsed 6 times with methanol. The addition of volumes to the initial volume of stock was accounted for in calculating stock concentration.

Chromatographic Details

Chromatography followed methods developed by Zhao and coworkers with some modifications.[62] A Finnigan Surveyor MS Pump Plus coupled to a Finnigan Surveyor Autosampler, upgraded to Autosampler Plus specifications, is used to perform high-performance liquid chromatography (HPLC) plumbed with 1/16 OD 0.003 in. ID polyether ether ketone (PEEK) tubing. The autosampler tray temperature is 4°C and full loop injections of 10 µL. Separation is with an Ascentis Express HILIC column 150 x 2.1 mm, 2.7 µm particles at 25.0°C column temperature and 200.0 µL/min flow rate. Mobile phases are ACN (solvent A) and 10mM ammonium formate in water buffered to pH 3.0 with formic acid (solvent B). A 30 min gradient follows: t) 0 min, 8% solvent B; t) 0.1 min, 8% solvent B; t) 10 min, 30% solvent B; t) 15 min, 70% solvent B; t) 18 min, 70% solvent B; t) 18.01 min, 8% solvent B; t) 30 min, 8%

solvent B. At the end of analysis the column was flushed with 100% ACN for 30 min prior to storage to prevent an increase in retention times.

Mass Spectrometric Detection Parameters

An Ion Max ion source equipped with an electrospray ionization (ESI) probe and plumbed with fused silica tubing 0.10 ID x 0.19 mm OD interfaced a Finnigan TSQ Quantum Discovery MAX. The polyimide sheath coating of fused silica tubing was removed from the spray tip to prevent elongation due to ACN. The ion source was set at depth C, front-to-back 1.30, and side-to-side 0. Ion source parameters are 4500 ESI spray voltage and 290°C ion transfer capillary temperature. Nitrogen was used as sheath, auxillary, and sweep gas set at 40, 5, and 1 arbitrary units, respectively, and 100 psi from the source. Ultra high purity argon was used as collision gas and was set to 1.5 mTorr in the collision cell and 20 psi from the source. The scan type SRM was used with parameters 0.05 s scan time, 1 *m/z* scan width, and Q1 & Q3 peak width (FWHM) 0.7 Da, 0V skimmer offset voltage, positive polarity, centroid data type, and 1 microscan. Tuned tube lens voltage was used. Parent and product masses were rounded to a tenth *m/z* unit, collision energies (CE) to unit, and complete masses and CE are reported by Zhao and coworkers.[62] SRM transitions of internal standards are: PtCho-IS 799.7 to 193, SM-IS 735.6 to 188, AcCho-IS 159.4 to 91.2, LPC-IS 499.3 to 184, Cho-IS 113.2 to 69.1, Bet-IS 129.1 to 66.2, and PCho-IS 193 to 125.1. Two segments were used to lower duty cycle. Segment 1: 0-10.8 min; PtCho and SM. Segment 2: 10.8-30 min; SM, AcCho, LPC, Cho, Bet, GPC, and PCho. The TSQ was tuned and calibrated every 3 months by direct infusion at low flow of Polytyrosine-1,3,6 tuning and calibration solution provided by Fisher Scientific. The ion source optimum sheath, auxillary, and sweep gases and optimum X, Y, Z positions were manually determined at method flow rate by infusing a 1:10 dilution of ISTD stock into LC flow at highest percentage of water in method. All Finnigan instruments were operated using Xcalibur 2.0.7 from a Dell Precision 390. All power systems were connected to a Toshiba 1600 EP Series UPS (Uninterruptible Power Supply).

Data Processing

Electronically stored chromatograms were integrated using Xcalibur 2.0.7 Quan Browser and peak area values were downloaded to Microsoft Excel 2007 to calculate absolute concentrations and fold changes. Heat maps were generated using Gene Cluster 3.0 and viewed using Java TreeView 1.1.5.[121]

Data Processing for the Calculation of Absolute Quantitation using Isotope Dilution

The following equation was used:

$$\frac{Area\ of\ Target\ Compound}{Area\ of\ ISTD}\ \chi\ [ISTD] = [Target\ Compound]$$

The ratio of *Area of Target Compound* and *Area of ISTD* and the known [*ISTD*] was used to calculate the [*Target Compound*]. The purity of ISTDs are greater than 99% therefore no corrections for target compound introduced by ISTD addition are applied.

Data Processing for the Calculation of Absolute Quantitation using External Standard

A calibration curve was used to calculate the absolute quantitation of GPC (+99% Bachem) due to the lack of commercial availability of an isotopic labeled ISTD.

Serial dilutions were made of GPC standard. 30 mg of GPC was weighed on weighing paper then a 10 mL volumetric flask with a glass funnel was zeroed. The GPC was then transferred into the glass funnel and the actual weight GPC transferred was recorded. Methanol was used to wash the GPC down and for dilution. Then half log dilutions were performed using a pipette to transfer 790 µL x 2 from the stock to a 5 mL volumetric flask then to another 5 mL volumetric

flask and so on until enough dilutions were obtained to produce a calibration curve where all measured values could be interpolated. The sampling order for chromatographic injections was from lowest concentration to highest to prevent erroneous carryover. One dilution series but each sample was injected in triplicate.

The calibration curve was graphed on a $\log_{10}$ scale to display calibration levels equidistance apart. A linear equation was obtained using least-squares linear regression of the data:

$$Area\ of\ GPC = m\ [GPC] + b$$

The [*GPC*] in samples can be determined as *Area of GPC* is known.

Calibration of Pipets

Pipets used in analysis should be calibrated with the solvent that they will be dispensing. A calibration solvent that is similar in viscosity and volatility can be used. Viscosity accounts for solvent remaining in the tip and volatility accounts for evaporation. Water is a suitable substitute here as milk and plasma is mostly water and any viscosity differences are negated by prewetting the tip.

The 5 mL pipet was calibrated using thermally equilibrated water. Density was determined using a 10 mL volumetric flask. Mass loss to evaporation was neglected because water was used as a solvent. Then ten 5 mL aliquots were dispensed in a beaker, and the weight was measured. The average weight of water delivered in one aliquot was used with density to get the average volume delivered:

$$\frac{Average\ Weight\ of\ Water\ (g)}{Aliquot}\ \chi\ Density\ (mL/g)\ = \frac{Average\ Volume}{Aliquot}$$

Rather than try to perfectly calibrate the pipet to 5 mL, 5.106 mL was used in the calculations to determine the final diluted concentration of internal standards. The other pipets used to aliquot sample and add internal standard in this study were new out of the box and certified to deliver nominal values within error.

Conclusion

The choice of analytical technique for quantitation and the experimental design are both strategic approaches that determine the success or failure or an experiment. The capability of the TSQ to perform SRM scan mode in individual time segments to lower duty cycle allows for reliable and reproducible quantitation. Achieving separation using liquid chromatography before the introduction of compounds into the ESI source prevents ion suppression and provides retention times for compounds. The retention time is used to confirm the identity of the compound but also allows for time segmentation of MS acquisition. The experimental design of both studies was based mostly on previous findings which are critical for informed decisions to the success of the experiment and some guesswork which is needed to explore new frontiers in science. In addition, both can have individual compounds placed in respective pathways, and data interpretation can be made on how the imposed condition changed the biological system with respect to the control.

Chapter 1 studied metabolomics of *Rosebacter*, and the time points are on the minute scale. The ability to quickly quench the metabolism is most important when the next time point is a small addition in time and changes in the system are rapid. The early time points of 0 and 5 minutes were the most difficult. Each time point collection was clocked at ~2 minutes to finish with the division of labor amongst myself and my collaborator. This means the sample collected first is ~2 minutes different in time than the sample collected last even though both are represented as the same time in analysis. The upside of working with bacteria and time points on the minute scale is that the time point of zero is controlled and all samples are collected in one day. The absolute concentration of glutamate and glutamine was not previously known for *Roseobacter*.

Chapter 2 studied lipidomics of Holstein cows, and the time points are on the week scale. Quenching the metabolism is within the hour because milk and plasma samples need to be taken from twelve Holstein cows. Since the time scale is on the order of weeks a relaxed collection during one hour does not introduce significant error. However, the samples are collected over months and the time point of zero is determined by Mother Nature. This results in multiple individual LC/MS/MS analysis to be performed and the risk of losing a subject to death or a technicality is present. Change in absolute lipid concentrations in milk and plasma over weeks of lactation is now known for Holstein cows.

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Appendix

Table 4A: Fold Change of Intracellular Metabolites of Phage Amended and Control *Sulfitobacter* sp. 2047 Populations with Cell Normalization Used in Figure 2a

	Fold Change Relative to Control at Corresponding Time Point						
	Time Point in Minutes Post Infection						
Metabolite	**15**	**30**	**60**	**120**	**240**	**360**	**480**
1,3 & 2,3 Diphosphoglycerate	1.443	1.451	1.190	1.506	2.286	2.412	3.417
1-Methylhistidine	2.844	1.865	1.520	1.912	2.808	2.588	3.480
3-Phosphoglycerate	1.348	0.835	1.212	1.701	1.578	1.502	1.696
4-Hydroxybenzoate	1.202	1.339	1.418	1.805	2.651	1.946	3.419
5-Methyltetrahydrofolate	1.579	0.746	1.206	1.380	3.898	3.738	5.944
5'-Methylthioadenosine	2.012	2.530	1.026	1.985	2.637	2.426	2.613
Acetyl CoA	1.502	1.407	1.474	1.520	1.625	1.563	1.417
Acetyl Phosphate	1.027	4.742	0.606	2.928	2.520	2.765	0.168
ADP	2.109	0.983	1.490	1.321	1.422	1.517	1.740
Alanine	2.288	1.699	1.568	1.598	1.615	2.886	3.728
AMP	1.498	1.330	1.403	1.042	1.527	1.982	1.921
Asparagine	0.000	1.572	2.093	1.241	1.855	2.303	3.236
Aspartate	1.530	1.573	1.264	1.291	1.530	1.420	1.710
ATP	0.000	0.000	0.000	1.500	1.765	1.141	1.585
Betaine	2.958	1.435	1.284	1.475	1.931	2.500	3.003
Ceramide	1.366	1.272	1.212	1.282	1.377	2.304	1.956
Choline	1.582	1.501	1.316	1.445	2.044	2.664	3.422
Citrate	1.777	1.683	1.688	2.220	2.795	1.514	2.413
CoA	1.093	1.326	0.625	1.441	2.134	1.625	2.651
Cysteine	1.834	1.636	1.329	1.352	2.063	2.788	2.500
dCDP	1.473	1.391	1.384	1.428	2.313	2.280	3.545
dGTP	2.543	0.639	1.145	1.873	1.736	1.139	1.492
DL-Pipecolic Acid	0.000	1.435	1.289	1.172	1.761	2.596	3.316
dUTP	‡0.001	‡0.001	3.415	1.951	2.349	1.663	3.399
Erythrose-4-Phosphate	‡0.001	1.075	2.706	1.461	†1000	†1000	1.329
Ethanolamine	1.800	1.125	1.039	1.253	2.236	2.775	4.218
FAD	1.298	1.484	1.310	1.294	1.633	1.186	1.889
Farnesylpyrophosphate	0.000	1.408	0.774	2.459	4.983	6.030	4.424
Fructose-1,6-Bisphosphate	1.165	1.958	1.185	1.941	2.965	2.342	3.559
Fumarate, Maleate, & Isoketovalerate	1.180	1.102	1.046	1.649	1.588	3.030	3.729
Glucono-1,5-Lactone-6-Phosphate	1.264	1.473	1.397	2.001	11.238	2.873	3.549
Glutamate	1.419	1.371	1.403	1.519	1.672	1.597	1.575
Glutamine	1.772	1.916	2.082	2.871	3.918	5.012	6.171
Glycerophosphocholine	0.000	0.000	0.000	0.000	0.000	†1000	1.155
GMP	1.526	0.891	1.283	1.962	2.727	1.849	2.406
GTP	4.150	0.305	0.993	1.998	0.878	0.617	0.722
Hexose Phosphate	3.782	1.121	2.398	1.370	1.330	2.062	2.953
Histidinol	0.000	‡0.001	0.000	1.443	0.875	1.070	0.670
Homoserine	1.663	1.800	1.882	†1000	1.568	3.298	2.672
Lysine	1.429	1.260	1.529	1.490	1.564	1.772	2.404
Malate	1.192	1.212	1.214	1.552	1.506	1.510	1.779
Malonyl CoA	1.699	1.593	2.279	1.211	1.520	1.537	1.669
Methylmalonic Acid	1.261	1.204	1.258	1.931	1.615	1.864	3.243
N-Acetylglucosamine-1-Phosphate	0.000	‡0.001	‡0.001	0.000	0.000	0.000	0.000
N-Acetylglutamate	0.869	1.045	1.436	1.807	1.341	2.091	2.014
N-Acetylglutamine	1.426	1.350	1.352	1.432	1.546	1.688	1.599
N-Acetyllysine	1.427	1.330	1.419	1.394	1.653	1.664	1.638
NAD	1.227	1.125	1.126	1.654	1.605	1.880	2.998

Table 4A: Continued

	Fold Change Relative to Control at Corresponding Time Point						
	Time Point in Minutes Post Infection						
Metabolite	**15**	**30**	**60**	**120**	**240**	**360**	**480**
NADH	1.226	1.508	0.844	2.585	1.557	1.584	6.889
NADP	1.448	0.754	1.757	1.248	1.471	0.919	1.769
NADPH	1.067	1.654	1.074	1.452	1.789	1.757	2.727
Nicotinate	1.277	1.428	1.257	1.760	1.874	2.480	3.477
O-Acetylserine	1.409	1.806	0.690	2.081	2.409	1.537	5.401
Orotate	1.425	1.340	1.242	1.289	4.641	2.615	3.338
Orotidine Phosphate	‡0.001	0.866	1.034	1.293	3.362	1.548	4.491
Oxidized Glutathione (GSSG)	1.593	1.308	1.693	0.901	2.418	2.418	1.960
Palmitate	0.865	0.786	0.922	4.763	1.455	2.461	2.806
Pentose Phosphate	0.000	†1000	†1000	0.000	0.000	†1000	†1000
Phenylpropiolic Acid	1.482	1.462	1.238	1.238	1.787	2.535	3.785
Phosphatidylcholine & Phosphatidylethanolamine	1.311	1.039	1.093	2.080	1.466	1.449	1.575
Phosphoenolpyruvate	1.496	0.926	1.166	1.874	1.644	1.273	1.546
Proline	1.523	1.594	1.528	1.555	1.854	2.403	2.892
Propionyl CoA	1.253	0.926	0.840	1.509	1.452	2.029	3.162
Pyridoxine	1.898	1.690	1.436	1.555	2.147	2.931	3.562
Reduced Glutathione (GSH)	1.185	0.872	1.528	1.800	2.155	2.231	2.087
Ribulose-1,5-Bisphosphate	0.000	0.000	0.000	0.000	‡0.001	1.178	‡0.001
S-Adenosylmethionine	1.531	2.326	1.552	1.136	2.012	3.280	3.923
Sarcosine	0.000	0.000	0.000	1.706	1.868	2.958	3.108
Sedoheptulose-7-Phosphate	0.956	0.956	1.664	3.502	2.901	1.752	3.756
Serine	0.736	1.452	1.586	1.649	1.820	1.996	2.407
Succinate	1.251	1.210	1.279	1.873	1.640	1.862	3.256
Succinyl CoA	0.874	2.349	7.946	1.712	1.583	1.527	2.643
TDP	0.951	0.711	1.864	4.242	4.068	2.441	3.508
Thiamine	1.681	1.228	1.360	1.860	1.791	3.548	3.509
Threonine	0.000	1.342	1.505	1.579	2.050	2.701	3.203
Tryptophan	1.681	1.114	4.272	1.520	1.667	2.238	1.339
UDP	1.356	1.241	1.898	3.058	2.242	2.521	3.922
UDP-D-Glucose	2.681	2.337	1.947	1.655	1.461	1.637	1.997
UDP-D-Glucuronate & Galacturonate	2.905	5.771	8.627	12.732	9.192	10.151	7.182
UDP-N-Acetylglucosamine	1.601	0.502	0.550	0.466	0.452	0.536	0.902
Urea	1.806	1.125	1.040	1.260	2.253	2.819	4.227
UTP	5.382	0.740	1.352	2.121	1.913	1.135	1.396
Valine	2.014	1.327	3.079	1.229	2.591	2.690	2.531

†A value of 1000 is used when only measured in infected

‡A value of 0.001 is used when only measured in control

Table 4B: P-value of Intracellular Metabolites of Phage Amended and Control *Sulfitobacter* sp. 2047 Populations with Cell Normalization Used in Figure 2a

	p-values						
	Time Point in Minutes Post Infection						
Metabolite	**15**	**30**	**60**	**120**	**240**	**360**	**480**
1,3 & 2,3 Diphosphoglycerate	5.81E-03	1.57E-02	1.47E-01	1.06E-01	1.32E-01	3.90E-03	1.75E-03
1-Methylhistidine	N/A	2.90E-01	3.35E-01	5.35E-04	1.80E-02	6.02E-02	3.36E-02
3-Phosphoglycerate	1.16E-01	4.18E-01	3.40E-01	1.34E-01	3.95E-02	4.91E-02	5.74E-03
4-Hydroxybenzoate	2.76E-01	1.90E-02	3.91E-02	4.79E-02	2.44E-01	9.04E-02	1.16E-04
5-Methyltetrahydrofolate	3.72E-01	3.48E-01	7.07E-01	3.06E-01	1.28E-01	1.06E-01	7.06E-02
5'-Methylthioadenosine	3.55E-01	1.45E-03	9.27E-01	1.26E-01	3.46E-02	4.80E-03	2.49E-02
Acetyl CoA	2.50E-01	1.42E-02	2.73E-01	3.26E-02	2.54E-03	1.30E-02	3.47E-02
Acetyl Phosphate	9.64E-01	1.34E-01	1.23E-01	3.08E-01	3.67E-02	4.33E-01	N/A
ADP	2.80E-01	9.44E-01	2.46E-01	2.17E-02	1.02E-01	2.46E-02	8.34E-03
Alanine	1.85E-02	1.12E-03	8.98E-03	6.43E-03	4.93E-02	2.45E-02	2.09E-04
AMP	1.18E-01	1.13E-01	3.29E-02	7.56E-01	7.71E-02	3.25E-02	1.26E-03
Asparagine	N/A	N/A	1.48E-01	6.18E-02	4.09E-03	4.90E-03	N/A
Aspartate	2.07E-02	6.86E-03	2.66E-01	1.09E-01	2.16E-03	5.60E-02	4.09E-03
ATP	N/A	N/A	N/A	1.79E-02	1.67E-01	5.81E-01	1.51E-01
Betaine	2.49E-01	6.58E-04	1.97E-02	4.05E-04	4.67E-05	3.52E-03	1.48E-04
Ceramide	2.09E-01	1.30E-02	4.34E-02	6.29E-02	1.70E-01	1.85E-02	1.96E-02
Choline	1.69E-01	1.38E-02	5.98E-03	9.19E-03	5.49E-04	1.56E-03	1.16E-03
Citrate	1.08E-01	1.64E-02	2.02E-01	3.67E-01	3.25E-02	2.72E-01	2.79E-02
CoA	7.54E-01	4.10E-01	1.91E-01	8.88E-02	7.27E-04	7.30E-02	5.13E-03
Cysteine	1.55E-01	1.24E-01	2.20E-01	4.58E-02	4.70E-04	2.69E-04	1.19E-02
dCDP	1.14E-01	6.06E-02	1.99E-02	4.27E-01	5.80E-02	5.18E-02	3.75E-06
dGTP	2.21E-01	3.86E-01	6.55E-01	1.66E-01	1.13E-02	5.40E-01	1.26E-01
DL-Pipecolic Acid	N/A	3.21E-01	3.43E-01	4.65E-01	6.13E-02	6.75E-03	4.98E-04
dUTP	N/A	N/A	N/A	1.35E-01	1.10E-01	3.22E-01	3.46E-02
Erythrose-4-Phosphate	N/A	N/A	N/A	5.70E-01	N/A	N/A	N/A
Ethanolamine	1.51E-01	6.71E-01	8.95E-01	1.60E-01	1.93E-02	6.87E-03	1.09E-02
FAD	2.05E-01	1.07E-01	3.12E-01	1.10E-01	8.04E-03	4.32E-01	4.13E-03
Farnesylpyrophosphate	N/A	N/A	N/A	3.02E-01	2.85E-01	9.73E-02	1.64E-01
Fructose-1,6-Bisphosphate	6.65E-01	7.22E-02	6.40E-01	1.81E-01	1.70E-02	1.56E-01	9.53E-03
Fumarate, Maleate, & Isoketovalerate	5.32E-02	6.78E-01	8.29E-01	3.34E-02	1.98E-02	1.37E-01	5.42E-02
Glucono-1,5-Lactone-6-Phosphate	8.99E-02	8.13E-03	4.84E-02	1.18E-01	3.56E-01	3.13E-03	1.66E-03
Glutamate	5.51E-02	9.60E-03	7.60E-02	1.16E-02	1.17E-03	8.05E-03	3.07E-04
Glutamine	2.40E-02	1.07E-03	3.52E-03	1.24E-04	9.99E-05	1.04E-06	4.22E-03
Glycerophosphocholine	N/A	N/A	N/A	N/A	N/A	N/A	N/A
GMP	2.52E-01	7.40E-01	4.49E-01	1.23E-02	2.83E-02	2.19E-02	4.35E-03
GTP	3.48E-01	2.87E-01	9.89E-01	2.56E-01	7.25E-01	4.19E-01	5.64E-01
Hexose Phosphate	7.95E-02	8.38E-01	7.94E-02	5.52E-01	5.81E-01	1.50E-01	7.90E-02
Histidinol	N/A	N/A	N/A	5.03E-02	6.81E-01	8.86E-01	2.72E-02
Homoserine	3.33E-01	2.46E-02	8.31E-02	N/A	N/A	1.39E-01	N/A
Lysine	1.74E-01	1.69E-01	1.41E-01	1.18E-02	2.39E-04	1.40E-02	2.01E-04
Malate	1.17E-01	4.26E-02	7.02E-03	1.44E-01	1.34E-02	3.08E-02	6.98E-03
Malonyl CoA	9.10E-02	1.72E-02	8.10E-02	5.06E-01	1.01E-02	2.23E-01	4.79E-02
Methylmalonic Acid	3.10E-02	1.81E-03	4.47E-02	1.48E-01	1.25E-03	3.83E-03	6.58E-03
N-Acetylglucosamine-1-Phosphate	N/A	N/A	N/A	N/A	N/A	N/A	N/A
N-Acetylglutamate	N/A	4.17E-01	9.39E-02	2.10E-01	1.19E-02	1.57E-03	3.06E-03
N-Acetylglutamine	6.33E-02	1.32E-02	8.98E-02	2.53E-02	2.12E-02	8.13E-03	2.48E-02
N-Acetyllysine	4.86E-02	4.05E-02	9.74E-02	1.97E-02	3.72E-03	3.17E-03	2.16E-03
NAD	3.51E-01	4.40E-01	4.60E-01	3.08E-02	1.56E-01	2.22E-01	2.63E-04

Table 4B: Continued

	p-values						
	Time Point in Minutes Post Infection						
Metabolite	**15**	**30**	**60**	**120**	**240**	**360**	**480**
NADH	5.60E-01	2.60E-01	5.79E-01	6.71E-02	5.98E-02	2.62E-01	4.07E-02
NADP	4.64E-01	4.21E-01	1.85E-01	5.08E-01	2.57E-01	7.55E-01	5.66E-02
NADPH	5.60E-01	1.20E-01	9.01E-01	2.83E-02	6.48E-03	8.08E-02	2.04E-04
Nicotinate	1.51E-02	2.41E-03	2.11E-02	5.67E-02	2.48E-03	5.12E-03	7.08E-04
O-Acetylserine	6.59E-01	1.29E-01	4.89E-01	N/A	2.54E-01	4.57E-01	1.65E-02
Orotate	1.04E-02	7.70E-02	1.21E-01	1.57E-02	2.75E-01	5.73E-02	2.28E-03
Orotidine Phosphate	N/A	7.46E-01	9.27E-01	5.22E-01	2.59E-01	7.17E-02	N/A
Oxidized Glutathione (GSSG)	4.57E-01	4.87E-01	2.48E-01	8.42E-01	9.51E-03	4.16E-03	1.23E-01
Palmitate	7.85E-01	5.46E-01	8.44E-01	3.27E-01	4.15E-01	6.03E-02	2.75E-02
Pentose Phosphate	N/A	N/A	N/A	N/A	N/A	N/A	N/A
Phenylpropiolic Acid	2.74E-03	1.60E-02	1.19E-01	6.37E-01	2.45E-02	5.91E-03	1.29E-04
Phosphatidylcholine & Phosphatidylethanolamine	3.09E-01	8.68E-01	4.71E-01	2.89E-01	8.80E-04	1.33E-03	3.57E-03
Phosphoenolpyruvate	1.04E-01	6.98E-01	4.95E-01	1.68E-01	1.52E-02	1.16E-01	1.08E-03
Proline	1.33E-01	1.83E-02	6.43E-02	9.24E-03	4.50E-05	1.10E-03	1.09E-03
Propionyl CoA	5.58E-01	7.72E-01	5.87E-01	2.14E-02	6.66E-02	3.31E-03	1.84E-03
Pyridoxine	9.90E-02	4.14E-02	8.76E-02	6.67E-03	9.80E-03	1.59E-03	2.53E-04
Reduced Glutathione (GSH)	8.02E-01	7.00E-01	4.17E-01	3.84E-03	9.53E-03	7.44E-02	1.22E-01
Ribulose-1,5-Bisphosphate	N/A	N/A	N/A	N/A	N/A	N/A	N/A
S-Adenosylmethionine	3.80E-01	5.36E-02	5.78E-02	7.70E-01	2.95E-02	2.34E-02	2.82E-03
Sarcosine	N/A	N/A	N/A	2.19E-02	4.81E-03	2.21E-02	4.73E-04
Sedoheptulose-7-Phosphate	9.26E-01	9.06E-01	3.74E-02	3.85E-01	9.97E-02	3.66E-01	1.40E-01
Serine	N/A	3.24E-01	N/A	1.08E-01	9.19E-02	1.88E-02	4.48E-03
Succinate	4.84E-02	9.99E-04	2.35E-02	1.33E-01	5.94E-04	3.98E-03	6.84E-03
Succinyl CoA	8.37E-01	1.78E-01	1.27E-01	2.23E-02	7.20E-03	2.24E-02	3.16E-02
TDP	8.72E-01	3.72E-01	2.41E-01	3.34E-01	3.54E-01	2.57E-03	1.70E-02
Thiamine	6.58E-02	2.15E-02	3.32E-02	2.84E-03	1.30E-01	1.49E-02	5.10E-02
Threonine	N/A	N/A	2.13E-01	3.81E-02	7.40E-03	1.13E-03	3.28E-08
Tryptophan	4.99E-01	7.55E-01	2.85E-01	2.08E-01	1.87E-01	6.75E-02	2.43E-01
UDP	2.53E-01	4.61E-01	5.60E-02	1.75E-01	1.04E-01	1.04E-02	3.06E-03
UDP-D-Glucose	2.83E-03	1.29E-03	8.71E-05	2.18E-01	1.51E-02	4.85E-03	1.47E-03
UDP-D-Glucuronate & Galacturonate	6.70E-03	6.54E-03	4.19E-05	4.43E-02	2.85E-04	2.34E-03	1.02E-03
UDP-N-Acetylglucosamine	9.75E-02	1.06E-02	9.66E-03	4.64E-02	5.03E-04	2.37E-02	4.15E-01
Urea	1.42E-01	6.72E-01	8.91E-01	1.41E-01	2.27E-02	5.49E-03	1.07E-02
UTP	1.46E-01	5.89E-01	6.07E-01	2.96E-01	3.77E-02	6.73E-01	1.90E-01
Valine	1.50E-01	1.68E-02	1.20E-01	3.09E-01	6.01E-03	2.18E-04	2.64E-03

N/A is result of an insufficient number of measurements

Table 5A: Virus (Φ2047) Counts Used in Figure 2b

	Virus Counts			
Time Post Infection in Minutes	**Replicate 1**	**Replicate 2**	**Average**	**Range**
15	1.40E+09	1.79E+09	1.60E+09	3.90E+08
30	1.09E+09	1.95E+09	1.52E+09	8.60E+08
60	9.07E+08	1.38E+09	1.15E+09	4.80E+08
120	1.13E+10	1.41E+10	1.27E+10	2.40E+09
240	9.91E+10	1.13E+11	1.06E+11	1.40E+10
360	1.99E+11	2.41E+11	2.20E+11	4.10E+10
480	3.62E+11	3.47E+11	3.55E+11	1.50E+10

Table 5B: *Sulfitobacter* sp. CB2047 Grown on 10mM Acetate Infected With Virus Used in Figure 2b

	Cell Density at OD_{540}			
Time Post Infection in Minutes	**Replicate 1**	**Replicate 2**	**Average**	**Range**
0	0.144	0.157	0.151	0.013
15	0.158	0.162	0.160	0.004
30	0.153	0.168	0.161	0.015
60	0.179	0.156	0.168	0.023
120	0.185	0.208	0.197	0.023
240	0.212	0.254	0.233	0.042
360	0.208	0.191	0.200	0.017
480	0.181	0.180	0.181	0.001

Table 5C: *Sulfitobacter* sp. CB2047 Controls Grown on 10mM Acetate Used in Figure 2b

	Cell Density at OD_{540}			
Time Post Infection in Minutes	**Replicate 1**	**Replicate 2**	**Average**	**Range**
0	0.157	0.163	0.160	0.003
15	0.155	0.158	0.157	0.003
30	0.184	0.155	0.170	0.029
60	0.173	0.166	0.170	0.007
120	0.196	0.197	0.197	0.001
240	0.228	0.239	0.234	0.011
360	0.262	0.266	0.264	0.004
480	0.305	0.336	0.321	0.031

Table 6: Glutamate and Glutamine Concentrations in Control (fg/cell) Used in Figure 2c

	Glutamate		Glutamine	
Time Post Infection in Minutes	**Control**	**STDev**	**Control**	**STDev**
15	56.66	10.85	3.19	1.01
30	69.50	8.38	5.32	1.16
60	62.70	2.79	5.02	0.63
120	54.88	8.89	3.67	0.94
240	65.72	2.54	3.61	0.33
360	66.45	14.93	2.67	0.72
480	70.84	3.42	2.03	0.19

Table 7: Glutamate and Glutamine Concentrations in Infected (fg/cell) Used in Figure 2d

	Glutamate		Glutamine	
Time Post Infection in Minutes	**Infected**	**STDev**	**Infected**	**STDev**
15	77.14	3.61	5.58	1.18
30	96.68	2.20	10.12	0.64
60	86.13	14.68	10.35	1.58
120	81.67	1.05	10.36	0.45
240	112.54	10.00	13.90	1.05
360	119.39	16.61	13.15	0.78
480	137.99	8.01	12.27	2.58

Table 8: Glutamate to Glutamine Ratios for Control and Infected Used in Figure 2e

Time Post Infection in Minutes	Control	Infected
15	17.76	13.82
30	13.07	9.56
60	12.49	8.32
120	14.94	7.88
240	18.21	8.10
360	24.89	9.08
480	34.97	11.25

Table 9: % Significantly Different Metabolites Used in Figure 2f

Time Post Infection in Minutes	% Significantly Different
15	12.05
30	19.28
60	14.46
120	25.30
240	61.45
360	65.06
480	75.90

Table 10: Fold Change of Intracellular Metabolites of Phage Amended and Control *Sulfitobacter* sp. 2047 Populations without Cell Normalization Used in Figure 3

	Fold Change Relative to Control at Time Point 0															
	Time Point in Minutes Post Infection															
Metabolite	**0**	**15**	**30**	**60**	**120**	**240**	**360**	**480**	**0**	**15**	**30**	**60**	**120**	**240**	**360**	**480**
1,3 & 2,3 Diphosphoglycerate	1.000	0.742	0.763	0.699	1.031	1.581	1.245	1.227	0.929	0.835	0.775	0.628	1.082	1.962	1.199	1.211
1-Methylhistidine	0.000	1.000	1.876	2.412	3.103	4.282	4.742	5.729	0.000	1.921	2.332	2.800	4.112	6.521	4.897	5.777
3-Phosphoglycerate	1.000	0.924	1.166	0.773	0.912	1.115	1.304	1.207	0.851	0.965	0.671	0.712	1.097	0.968	0.775	0.595
4-Hydroxybenzoate	1.000	0.972	0.987	0.834	0.710	0.714	0.745	0.663	1.025	0.921	0.921	0.892	0.888	1.014	0.586	0.656
5-Methyltetrahydrofolate	1.000	1.543	2.100	1.743	0.990	0.436	0.347	0.363	1.438	1.854	1.117	1.607	0.952	0.950	0.504	0.631
5'-Methylthioadenosine	0.000	1.000	2.613	7.541	2.582	11.057	15.220	29.534	0.000	1.415	4.601	5.937	3.543	16.266	14.445	22.044
Acetyl CoA	1.000	4.542	4.921	3.392	3.692	4.650	6.403	7.141	2.813	5.362	4.863	3.783	3.961	4.171	3.993	2.909
Acetyl Phosphate	1.000	0.908	0.670	1.232	0.510	0.490	0.683	11.034	1.691	0.727	2.053	0.588	1.033	0.674	0.618	0.607
ADP	1.000	0.670	1.052	0.678	0.855	1.239	1.789	1.795	1.142	1.099	0.717	0.771	0.787	0.961	1.085	0.893
Alanine	1.000	2.965	4.007	2.376	1.434	1.576	1.526	1.831	1.397	3.936	4.575	2.675	1.324	1.828	1.669	1.596
AMP	1.000	0.739	0.713	0.665	0.912	1.134	1.575	1.728	0.856	0.867	0.662	0.712	0.663	0.950	1.234	0.943
Asparagine	0.000	0.000	1.000	0.655	1.084	1.086	1.105	1.333	0.000	0.000	1.150	1.029	0.949	1.108	1.054	1.181
Aspartate	1.000	1.745	2.371	2.574	1.970	3.041	4.228	5.432	0.991	2.041	2.624	2.442	1.782	2.577	2.423	2.663
ATP	0.000	0.000	0.000	0.000	1.000	1.487	1.807	1.724	0.000	0.000	0.000	0.000	1.039	1.407	0.870	0.789
Betaine	1.000	5.751	9.519	10.653	9.901	10.041	10.981	13.354	1.351	12.932	9.522	10.363	10.143	10.714	10.908	11.703
Ceramide	1.000	0.851	0.812	0.667	0.515	0.417	0.363	0.404	1.026	0.911	0.723	0.613	0.456	0.322	0.333	0.226
Choline	1.000	3.363	5.769	6.629	5.581	6.161	7.497	8.026	0.854	4.049	6.055	6.610	5.629	6.969	8.084	7.859
Citrate	1.000	0.191	0.219	0.176	0.276	0.364	0.578	0.616	0.659	0.274	0.257	0.233	0.422	0.553	0.342	0.425
CoA	0.000	1.000	0.866	1.017	0.885	0.865	1.382	1.048	0.000	0.872	0.806	0.476	0.898	1.013	0.875	0.798
Cysteine	1.000	4.443	7.033	7.547	8.372	9.118	9.732	14.429	1.525	6.300	7.966	7.542	7.857	10.389	11.167	10.556
dCDP	1.000	0.848	0.869	0.884	2.146	2.668	3.175	3.261	1.020	1.007	0.849	0.940	2.105	3.354	2.872	3.358
dGTP	1.000	1.056	1.759	0.719	1.902	4.333	7.569	8.536	0.675	2.129	0.758	0.632	2.528	4.129	3.459	3.699
DL-Pipecolic Acid	0.000	0.000	1.000	0.938	1.665	1.637	1.568	1.760	0.000	0.000	1.024	0.914	1.358	1.584	1.680	1.688
dUTP	1.000	1.636	0.441	0.276	2.728	2.510	2.902	2.313	0.380	‡0.001	‡0.001	0.754	3.799	3.247	1.961	2.256
Erythrose-4-Phosphate	1.000	0.461	0.684	0.305	0.537	‡0.001	‡0.001	1.373	0.673	‡0.001	0.495	0.566	0.575	0.937	0.950	0.459
Ethanolamine	1.000	15.356	50.442	48.352	43.922	47.158	68.012	56.188	1.453	20.916	40.467	38.939	38.279	58.687	76.596	68.785

Table 10: Continued

	Fold Change Relative to Control at Time Point 0															
	Time Point in Minutes Post Infection															
Metabolite	**0**	**15**	**30**	**60**	**120**	**240**	**360**	**480**	**0**	**15**	**30**	**60**	**120**	**240**	**360**	**480**
FAD	1.000	0.411	0.344	0.340	0.377	0.528	0.755	0.750	0.480	0.416	0.352	0.337	0.342	0.474	0.357	0.402
Farnesylpyrophosphate	1.000	‡0.001	0.624	0.670	1.507	3.879	3.527	4.732	0.671	‡0.001	0.699	0.376	2.313	10.072	8.467	6.049
Fructose-1,6-Bisphosphate	1.000	0.819	0.843	0.545	0.802	0.668	1.286	0.751	1.119	0.752	1.122	0.497	1.078	1.125	1.131	0.763
Fumarate, Maleate, & Isoketovalerate	1.000	0.988	0.825	0.869	0.671	0.552	0.467	0.592	0.874	0.892	0.640	0.695	0.775	0.486	0.550	0.627
Glucono-1,5-Lactone-6-Phosphate	1.000	0.777	0.746	0.652	0.905	1.093	0.954	0.973	0.889	0.768	0.766	0.687	1.276	6.342	1.098	0.987
Glutamate	1.000	1.657	2.345	2.171	1.905	2.967	4.084	4.799	0.908	1.815	2.254	2.293	2.024	2.736	2.619	2.172
Glutamine	1.000	2.129	3.585	3.568	2.717	3.015	2.795	2.238	0.693	2.861	4.821	5.615	5.452	6.527	5.690	3.950
Glycerophosphocholine	0.000	0.000	0.000	0.000	0.000	0.000	0.000	1.000	0.000	0.000	0.000	0.000	0.000	0.000	0.068	0.337
GMP	1.000	0.438	0.474	0.507	0.369	0.290	0.670	0.559	0.845	0.514	0.297	0.508	0.499	0.434	0.512	0.392
GTP	1.000	1.100	2.908	0.247	1.210	4.391	9.699	11.537	0.446	3.875	0.592	0.196	1.725	2.164	2.456	2.436
Hexose Phosphate	1.000	0.966	2.175	1.427	2.396	2.537	2.906	1.881	0.748	2.977	1.611	2.559	2.303	1.782	2.395	1.642
Histidinol	0.000	0.000	1.000	‡0.001	1.061	1.863	2.620	4.759	0.000	0.000	‡0.001	‡0.001	1.075	0.883	1.102	0.936
Homoserine	0.000	1.000	1.989	1.245	‡0.001	1.822	1.573	1.948	0.000	1.278	2.519	1.847	1.904	1.447	2.169	1.544
Lysine	1.000	2.263	2.862	2.955	2.460	3.301	3.988	4.540	1.015	2.498	2.560	3.419	2.573	2.853	2.830	3.112
Malate	1.000	0.947	0.959	0.950	0.975	0.958	1.038	1.002	0.961	0.881	0.810	0.875	1.049	0.793	0.630	0.511
Malonyl CoA	1.000	5.065	7.568	3.575	5.445	7.410	9.601	10.223	3.846	6.637	8.441	6.079	4.656	6.250	5.958	4.968
Methylmalonic Acid	1.000	0.849	0.896	0.869	0.735	0.723	0.865	0.698	0.913	0.830	0.750	0.828	0.984	0.645	0.650	0.651
N-Acetylglucosamine-1-Phosphate	1.000	‡0.001	1.451	0.702	‡0.001	‡0.001	‡0.001	‡0.001	0.905	‡0.001	‡0.001	‡0.001	‡0.001	‡0.001	‡0.001	‡0.001
N-Acetylglutamate	1.000	2.677	4.079	3.150	3.451	5.305	4.973	5.927	1.283	1.791	3.006	3.388	4.456	3.950	4.126	3.461
N-Acetylglutamine	1.000	2.046	3.087	2.835	2.754	3.921	4.758	5.842	0.987	2.247	2.910	2.889	2.764	3.325	3.250	2.671
N-Acetyllysine	1.000	2.242	3.362	3.095	3.074	4.261	5.352	6.639	0.987	2.454	3.142	3.304	3.000	3.885	3.591	3.128
NAD	1.000	1.341	1.518	1.031	0.682	0.861	0.745	0.662	0.976	1.303	1.202	0.886	0.771	0.765	0.539	0.572
NADH	1.000	0.335	0.318	0.322	0.196	0.286	0.255	0.074	0.573	0.331	0.334	0.205	0.355	0.247	0.163	0.142
NADP	1.000	0.644	1.143	0.545	0.582	0.828	1.224	1.063	1.087	0.727	0.610	0.722	0.519	0.671	0.446	0.561
NADPH	0.000	1.000	0.656	0.520	0.934	1.017	1.123	1.059	0.000	0.833	0.754	0.427	0.939	1.001	0.781	0.836
Nicotinate	1.000	0.957	0.979	0.940	0.873	0.897	0.922	0.872	1.005	0.950	0.977	0.897	1.071	0.925	0.920	0.871
O-Acetylserine	1.000	1.363	1.753	1.718	1.061	1.358	2.261	2.121	0.544	1.623	2.158	0.882	1.695	1.777	1.211	3.259

Table 10: Continued

	Fold Change Relative to Control at Time Point 0															
	Time Point in Minutes Post Infection															
Metabolite	**0**	**15**	**30**	**60**	**120**	**240**	**360**	**480**	**0**	**15**	**30**	**60**	**120**	**240**	**360**	**480**
Orotate	1.000	0.952	1.039	1.012	1.122	0.876	0.923	0.914	1.134	1.051	0.983	0.951	1.004	2.137	0.930	0.889
Orotidine Phosphate	1.000	0.769	0.914	0.813	4.940	3.780	3.543	3.137	1.533	‡0.001	0.522	0.634	4.420	6.680	2.409	3.993
Oxidized Glutathione (GSSG)	1.000	0.711	0.681	0.433	0.233	1.132	1.287	1.483	1.309	0.841	0.598	0.580	0.150	1.505	1.257	0.850
Palmitate	1.000	0.844	0.897	0.747	1.781	2.901	1.810	1.820	0.874	0.578	0.487	0.518	5.796	2.278	1.817	1.488
Pentose Phosphate	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	†1000	†1000	0.000	0.000	†1000	†1000
Phenylpropiolic Acid	1.000	0.903	0.888	0.860	1.354	1.445	1.449	1.297	0.890	1.040	0.911	0.803	1.171	1.411	1.492	1.413
Phosphatidylcholine & Phosphatidylethanolamine	1.000	0.717	0.794	0.537	0.590	0.831	1.078	1.023	0.792	0.749	0.574	0.447	0.856	0.675	0.621	0.462
Phosphoenolpyruvate	1.000	1.036	1.231	0.854	0.969	1.056	1.259	1.250	0.842	1.182	0.788	0.751	1.286	0.952	0.634	0.556
Proline	1.000	2.476	3.972	4.146	4.621	5.668	5.890	6.113	1.123	2.846	4.410	4.870	5.008	5.812	5.681	5.079
Propionyl CoA	1.000	5.687	5.705	5.114	4.166	7.057	9.247	9.019	3.932	5.711	3.704	3.241	4.384	5.688	7.698	8.140
Pyridoxine	1.000	4.484	7.257	8.325	11.577	12.499	12.520	14.524	1.220	6.544	8.514	9.112	12.477	14.700	14.642	14.799
Reduced Glutathione (GSH)	1.000	1.219	1.624	1.264	2.891	2.209	3.260	2.694	0.083	1.224	1.007	1.399	3.619	2.608	2.889	1.585
Ribulose-1,5-Bisphosphate	0.000	0.000	0.000	0.000	0.000	1.000	2.354	2.114	0.000	0.000	0.000	0.000	0.000	‡0.001	0.920	‡0.001
S-Adenosylmethionine	1.000	3.541	4.984	3.721	4.983	5.659	4.162	5.945	0.891	4.124	7.890	4.373	4.118	6.332	5.220	6.733
Sarcosine	0.000	0.000	0.000	0.000	1.000	1.467	1.663	2.196	0.000	0.000	0.000	0.000	1.184	1.508	1.942	1.961
Sedoheptulose-7-Phosphate	1.000	0.715	1.040	0.412	0.816	0.550	0.630	0.477	1.192	0.555	0.690	0.514	2.038	0.885	0.419	0.498
Serine	1.000	1.937	2.076	3.124	3.045	3.298	3.547	3.498	‡0.001	1.264	2.222	3.483	3.133	3.292	2.927	2.449
Succinate	1.000	0.849	0.887	0.853	0.729	0.723	0.856	0.691	0.918	0.823	0.746	0.827	0.947	0.655	0.642	0.647
Succinyl CoA	0.000	1.000	0.136	0.055	1.438	1.618	1.683	1.005	0.000	0.687	0.225	0.336	1.735	1.420	1.060	0.769
TDP	1.000	1.353	1.277	0.680	6.777	7.335	6.186	5.877	1.329	1.004	0.612	0.960	20.703	15.680	6.222	5.918
Thiamine	1.000	3.821	6.323	6.679	5.809	0.681	0.779	1.383	0.888	4.952	5.427	6.900	7.477	0.658	1.033	1.470
Threonine	0.000	0.000	1.000	1.927	3.704	6.660	8.489	10.229	0.000	0.000	0.820	2.212	4.067	7.493	9.148	9.443
Tryptophan	1.000	2.271	3.449	4.546	3.629	5.722	6.194	11.444	0.738	3.079	2.791	15.541	3.855	5.190	5.343	4.457
UDP	1.000	0.830	0.745	0.565	1.986	3.258	2.715	2.243	0.991	0.907	0.635	0.813	4.235	3.945	2.742	2.538
UDP-D-Glucose	1.000	0.737	0.882	0.838	1.051	1.621	1.813	2.085	0.865	1.533	1.438	1.241	1.205	1.302	1.186	1.202
UDP-D-Glucuronate & Galacturonate	1.000	0.735	0.642	0.381	0.398	0.650	0.699	1.051	0.982	1.701	2.594	2.508	3.550	3.313	2.841	2.178
UDP-N-Acetylglucosamine	1.000	0.701	1.017	0.975	1.347	1.967	2.229	2.355	0.815	0.846	0.358	0.414	0.439	0.489	0.485	0.615

Table 10: Continued

	Fold Change Relative to Control at Time Point 0															
	Time Point in Minutes Post Infection															
Metabolite	**0**	**15**	**30**	**60**	**120**	**240**	**360**	**480**	**0**	**15**	**30**	**60**	**120**	**240**	**360**	**480**
Urea	1.000	16.511	55.658	52.759	47.722	51.333	73.039	60.296	1.512	22.529	44.657	42.526	41.784	64.429	83.592	73.935
UTP	1.000	0.737	1.647	0.450	1.604	4.411	7.665	7.838	0.562	3.241	0.810	0.456	2.430	4.623	3.511	3.198
Valine	1.000	3.313	6.455	4.403	5.900	4.869	5.372	6.343	0.772	5.019	6.001	10.463	5.027	6.912	5.792	4.601

†A value of 1000 is used when only measured in infected

‡A value of 0.001 is used when only measured in control

Table 11: Expanded Table Used in Figure 4

Time in Minutes		Glutamine			Glutamate		
Post Infection	Post ^{13}C Addition	Fold Change[a]	Rate Constant[a], k	Flux[a]	Fold Change[a]	Rate Constant[a], k	Flux[a]
0	0	1.16	0.91	1.06	1.19	0.91	1.08
5	5	1.08	0.91	0.99	1.09	0.91	1.00
15	15	1.34	0.91	1.22	1.14	0.91	1.04
30	30	1.46	0.91	1.34	1.07	0.91	0.98
60	60	1.90	0.91	1.74	1.19	0.91	1.09
120	120	2.17	0.91	1.98	1.09	0.91	0.99
240	0	2.09	0.72	1.50	1.06	0.80	0.84
245	5	2.13	0.72	1.53	0.97	0.80	0.77
255	15	2.78	0.72	2.00	0.99	0.80	0.78
270	30	3.64	0.72	2.62	1.24	0.80	0.99
300	60	3.20	0.72	2.30	1.23	0.80	0.98
360	120	3.20	0.72	2.30	1.24	0.80	0.98

[a]Calculated as a ratio of infected / uninfected per time point

Table 12A: Average Percent of Glutamine Flux Early Post Infection Used in Figure 4a

		Average Percent at Time Point									
Time in Minutes		Control					Infected				
Post Infection	Post ^{13}C Addition	0-0	2-2	3-2	3-3	5-4	0-0	2-2	3-2	3-3	5-4
0	0	91.06	8.55	0.00	0.00	0.39	90.93	8.73	0.00	0.00	0.34
5	5	62.82	26.61	0.00	4.02	6.55	66.95	23.76	0.00	4.64	4.66
15	15	21.24	25.68	0.00	18.24	34.84	25.39	27.80	0.00	16.84	29.97
30	30	0.00	14.59	18.04	14.93	52.44	0.00	15.11	18.23	15.03	51.62
60	60	0.00	8.85	21.88	12.85	56.42	0.00	11.74	19.61	13.81	54.83
120	120	0.00	7.22	19.46	13.60	59.72	0.00	9.46	15.85	13.69	61.00

X-Y : Where X is the number of ^{13}C in the parent ion and Y is the number of ^{13}C in the product ion

Table 12B: Range of Glutamine Flux Early Post Infection Used in Figure 4a

		Range									
Time in Minutes		Control					Infected				
Post Infection	Post ^{13}C Addition	0-0	2-2	3-2	3-3	5-4	0-0	2-2	3-2	3-3	5-4
0	0	0.27	0.66	0.00	0.00	0.39	0.34	0.00	0.00	0.00	0.34
5	5	1.39	0.30	0.00	1.01	0.08	1.33	1.53	0.00	0.25	0.05
15	15	1.39	1.33	0.00	1.26	3.98	1.12	0.01	0.00	0.52	1.65
30	30	0.00	2.44	1.45	2.63	1.26	0.00	1.75	3.19	2.40	2.54
60	60	0.00	1.80	2.64	0.26	0.58	0.00	0.08	1.24	0.53	0.63
120	120	0.00	0.40	1.02	0.26	0.36	0.00	2.55	0.49	0.18	2.86

X-Y : Where X is the number of ^{13}C in the parent ion and Y is the number of ^{13}C in the product ion

Table 13A: Average Percent of Glutamine Flux Late Post Infection Used in Figure 4b

		Average Percent at Time Point									
Time in Minutes		Control					Infected				
Post Infection	Post ^{13}C Addition	0-0	2-2	3-2	3-3	5-4	0-0	2-2	3-2	3-3	5-4
240	0	95.33	4.67	0.00	0.00	0.00	93.94	5.73	0.00	0.00	0.32
245	5	66.99	24.96	0.00	2.77	5.29	74.50	17.90	0.00	3.17	4.43
255	15	23.69	26.16	0.00	16.90	33.26	44.99	26.79	0.00	9.96	18.27
270	30	0.00	8.67	15.88	11.21	64.24	12.99	18.04	10.55	14.85	43.57
300	60	0.00	5.46	14.59	12.49	67.46	4.12	12.23	14.56	12.33	56.76
360	120	0.00	4.75	13.64	8.81	72.80	2.20	7.87	14.13	13.38	62.42

X-Y : Where X is the number of ^{13}C in the parent ion and Y is the number of ^{13}C in the product ion

Table 13B: Range of Glutamine Flux Late Post Infection Used in Figure 4b

		Range									
Time in Minutes		Control					Infected				
Post Infection	Post ^{13}C Addition	0-0	2-2	3-2	3-3	5-4	0-0	2-2	3-2	3-3	5-4
240	0	1.59	1.59	0.00	0.00	0.00	0.68	1.00	0.00	0.00	0.32
245	5	0.67	1.46	0.00	0.08	2.05	0.17	0.43	0.00	1.20	1.79
255	15	2.02	6.08	0.00	0.19	4.25	0.24	1.48	0.00	0.58	2.29
270	30	0.00	2.72	1.30	0.68	2.10	1.87	1.40	1.37	0.78	2.63
300	60	0.00	1.18	1.72	1.30	1.83	0.43	0.41	0.76	0.74	0.01
360	120	0.00	0.50	0.83	1.17	0.83	0.04	0.57	2.90	2.50	1.01

X-Y : Where X is the number of ^{13}C in the parent ion and Y is the number of ^{13}C in the product ion

Table 14A: Average Percent of Glutamate Flux Early Post Infection Used in Figure 4c

		Average Percent at Time Point							
Time in Minutes		Control				Infected			
Post Infection	Post ^{13}C Addition	0-0	3-2	3-3	5-4	0-0	3-2	3-3	5-4
0	0	93.88	1.20	2.39	2.52	92.45	1.64	3.29	2.62
5	5	64.95	6.42	12.60	16.03	65.69	6.34	13.22	14.74
15	15	18.36	15.01	21.25	45.38	21.57	15.75	21.29	41.39
30	30	5.72	18.52	17.49	58.27	6.14	18.31	17.72	57.83
60	60	3.13	18.98	16.05	61.83	2.97	18.37	16.37	62.28
120	120	2.06	20.05	15.20	62.69	2.34	19.54	15.02	63.10

X-Y : Where X is the number of ^{13}C in the parent ion and Y is the number of ^{13}C in the product ion

Table 14B: Range of Glutamate Flux Early Post Infection Used in Figure 4c

		Range							
Time in Minutes		Control				Infected			
Post Infection	Post ^{13}C Addition	0-0	3-2	3-3	5-4	0-0	3-2	3-3	5-4
0	0	0.47	0.14	0.25	0.08	1.62	0.28	0.81	0.53
5	5	1.05	0.04	0.44	0.66	0.54	0.05	0.39	0.10
15	15	0.40	0.38	0.58	0.20	0.40	0.38	0.03	0.80
30	30	0.21	0.85	0.29	0.93	0.53	0.19	0.22	0.94
60	60	0.42	0.40	0.21	0.23	0.13	0.09	0.29	0.25
120	120	0.14	0.85	0.30	0.41	0.03	0.37	0.13	0.48

X-Y : Where X is the number of ^{13}C in the parent ion and Y is the number of ^{13}C in the product ion

Table 15A: Average Percent of Glutamate Flux Late Post Infection Used in Figure 4d

		Average Percent at Time Point							
Time in Minutes		Control				Infected			
Post Infection	Post ^{13}C Addition	0-0	3-2	3-3	5-4	0-0	3-2	3-3	5-4
240	0	96.29	0.56	1.39	1.76	94.04	1.01	2.28	2.67
245	5	69.56	4.21	11.13	15.10	70.40	4.41	11.34	13.84
255	15	23.50	10.34	20.64	45.51	32.21	11.23	19.01	37.55
270	30	4.51	14.30	14.90	66.29	9.44	16.03	17.51	57.02
300	60	2.21	14.48	11.87	71.44	4.29	15.78	15.36	64.57
360	120	1.50	13.56	10.71	74.23	2.32	16.07	13.38	68.23

X-Y : Where X is the number of ^{13}C in the parent ion and Y is the number of ^{13}C in the product ion

Table 15B: Range of Glutamate Flux Late Post Infection Used in Figure 4d

		Range							
Time in Minutes		Control				Infected			
Post Infection	Post ^{13}C Addition	0-0	3-2	3-3	5-4	0-0	3-2	3-3	5-4
240	0	0.32	0.01	0.10	0.21	0.03	0.03	0.05	0.02
245	5	0.15	0.02	0.71	0.59	0.73	0.06	0.45	0.34
255	15	0.39	0.49	0.37	1.24	0.54	0.29	0.44	0.19
270	30	0.02	0.13	0.63	0.51	0.81	0.26	0.86	1.41
300	60	0.18	0.76	0.06	0.88	0.24	0.18	0.48	0.41
360	120	0.03	0.19	0.24	0.40	0.13	0.45	0.05	0.37

X-Y : Where X is the number of 13C in the parent ion and Y is the number of 13C in the product ion

Table 16: Glutamine External Calibration Curve Used in Figure 5

Calibration Level	M	Injection #	Area
1	2.42E-02	1	1.41E+08
		2	1.70E+08
2	7.26E-03	1	2.42E+07
		2	2.14E+07
3	2.18E-03	1	9.01E+06
		2	1.02E+07
4	6.53E-04	1	1.82E+06
		2	2.07E+06
5	1.96E-04	1	3.80E+05
		2	4.37E+05
6	5.88E-05	1	6.46E+04
		2	8.79E+04
7	1.76E-05	1	5.30E+04
		2	6.29E+04
8	5.29E-06	1	9.05E+03
		2	1.18E+04
9	1.59E-06	1	0.00E+00
		2	0.00E+00
10	4.76E-07	1	0.00E+00
		2	0.00E+00
11	1.43E-07	1	0.00E+00
		2	0.00E+00
12	4.29E-08	1	0.00E+00
		2	0.00E+00

Table 17: Glutamate External Calibration Curve Used in Figure 6

Calibration Level	M	Injection #	Area
1	2.40E-02	1	2.22E+08
		2	2.28E+08
2	7.20E-03	1	6.71E+07
		2	6.20E+07
3	2.16E-03	1	1.66E+07
		2	1.71E+07
4	6.48E-04	1	1.61E+06
		2	3.76E+06
5	1.94E-04	1	3.48E+05
		2	3.08E+05
6	5.83E-05	1	8.62E+04
		2	1.06E+05
7	1.75E-05	1	3.01E+04
		2	3.81E+04
8	5.25E-06	1	7.67E+03
		2	3.54E+03
9	1.57E-06	1	1.99E+02
		2	2.79E+02
10	4.72E-07	1	0.00E+00
		2	0.00E+00
11	1.42E-07	1	0.00E+00
		2	0.00E+00
12	4.25E-08	1	0.00E+00
		2	0.00E+00

Table 18A: Cow #1 Average µM in Milk Used in Figure 7

	Average µM								
	Week of Lactation								
Lipid	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**
AcCho	0.00	0.00	0.00	0.02	0.04	0.02	0.01	0.00	0.01
Bet	27.84	18.32	19.15	11.71	12.22	12.40	26.93	40.23	44.11
Cho	72.28	47.07	75.61	113.20	121.22	109.17	187.96	175.14	175.57
LPC 16:0	0.64	0.53	0.66	0.90	1.01	0.88	1.27	1.37	2.12
LPC 18:2	0.28	0.14	0.29	0.36	0.16	0.25	0.33	0.22	0.24
LPC 18:1	0.77	0.57	0.78	0.53	0.33	0.32	0.55	0.63	0.71
LPC 18:0	0.23	0.21	0.26	0.28	0.33	0.26	0.57	0.38	0.58
LPC Total	1.91	1.45	1.99	2.06	1.82	1.71	2.72	2.61	3.64
PtCho 16:0/16:1	6.43	7.23	7.14	10.64	10.87	8.30	104.92	92.97	69.70
PtCho 16:0/22:6,18:1/22:5,18:2/20:4	0.47	0.51	0.59	0.61	0.60	0.56	6.86	4.51	2.53
PtCho 18:1/20:4,18:0/20:5,16:0/22:5	2.00	1.76	2.55	1.82	1.85	1.59	20.44	14.45	7.91
PtCho 18:0/20:4	1.28	1.43	1.53	1.89	1.94	1.41	19.18	15.37	8.97
PtCho 18:0/20:3	0.67	0.74	0.79	1.05	1.14	0.79	10.48	7.77	4.49
PtCho 18:1/22:6	0.22	0.23	0.24	0.22	0.20	0.23	2.46	1.61	0.81
PtCho 18:0/22:6,18:1/22:5	0.40	0.38	0.37	0.29	0.32	0.38	3.50	2.20	1.21
PtCho 18:0/22:5	0.47	0.36	0.54	0.32	0.27	0.23	2.68	1.98	1.23
PtCho 16:0/16:0	19.10	19.41	23.48	34.20	40.26	29.57	394.24	359.20	216.93
PtCho 16:0/18:2	13.38	14.67	17.89	26.20	28.17	21.71	317.19	281.24	155.11
PtCho 16:0/18:1	41.43	46.96	50.93	63.10	66.76	46.50	749.96	669.74	422.56
PtCho 16:0/20:5,16:1/20:4	0.42	0.43	0.55	0.55	0.54	0.44	5.18	4.27	2.77
PtCho 16:0/20:4	2.93	3.28	4.18	5.07	5.10	3.74	49.93	38.95	22.97
PtCho 16:0/20:3	11.23	12.61	14.85	17.18	17.29	12.22	158.67	127.61	72.93
PtCho 18:0/18:2,18:1/18:1	25.76	29.30	30.72	32.59	30.60	21.42	290.07	253.47	140.49
PtCho 18:0/18:1	13.87	16.24	15.57	17.64	16.93	10.96	158.24	149.10	83.28
PtCho Total	140.08	155.53	171.92	213.35	222.83	160.04	2293.98	2024.46	1213.90
Pcho	490.11	331.22	393.95	147.73	115.49	68.35	25.63	21.77	50.22
SM	45.73	50.07	52.23	31.59	35.57	26.26	44.60	42.15	44.12
GPC	78.07	49.84	57.72	42.15	57.77	49.77	95.92	155.10	144.54

Average of triplicate injections

Table 18B: Cow #1 Fold Change in Milk Used in Figure 7

	Fold Change Relative to Week 1								
	Week of Lactation								
Lipid	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**
AcCho	0.00	0.00	0.00	1.00	1.71	1.04	0.40	0.14	0.36
Bet	1.00	0.66	0.69	0.42	0.44	0.45	0.97	1.45	1.58
Cho	1.00	0.65	1.05	1.57	1.68	1.51	2.60	2.42	2.43
LPC 16:0	1.00	0.83	1.04	1.41	1.58	1.38	2.00	2.15	3.32
LPC 18:2	1.00	0.48	1.01	1.29	0.57	0.90	1.19	0.79	0.84
LPC 18:1	1.00	0.74	1.01	0.69	0.42	0.42	0.71	0.83	0.92
LPC 18:0	1.00	0.94	1.15	1.22	1.44	1.13	2.51	1.69	2.55
LPC Total	1.00	0.76	1.04	1.08	0.95	0.89	1.42	1.36	1.90
PtCho 16:0/16:1	1.00	1.12	1.11	1.65	1.69	1.29	16.32	14.46	10.84
PtCho 16:0/22:6,18:1/22:5,18:2/20:4	1.00	1.08	1.23	1.28	1.27	1.18	14.45	9.51	5.33
PtCho 18:1/20:4,18:0/20:5,16:0/22:5	1.00	0.88	1.27	0.91	0.93	0.79	10.21	7.22	3.95
PtCho 18:0/20:4	1.00	1.11	1.19	1.47	1.51	1.10	14.94	11.98	6.99
PtCho 18:0/20:3	1.00	1.10	1.18	1.57	1.70	1.18	15.59	11.56	6.68
PtCho 18:1/22:6	1.00	1.05	1.10	0.99	0.91	1.05	11.29	7.39	3.72
PtCho 18:0/22:6,18:1/22:5	1.00	0.94	0.94	0.74	0.79	0.96	8.76	5.51	3.04
PtCho 18:0/22:5	1.00	0.76	1.14	0.68	0.58	0.48	5.68	4.20	2.62
PtCho 16:0/16:0	1.00	1.02	1.23	1.79	2.11	1.55	20.64	18.81	11.36
PtCho 16:0/18:2	1.00	1.10	1.34	1.96	2.11	1.62	23.70	21.01	11.59
PtCho 16:0/18:1	1.00	1.13	1.23	1.52	1.61	1.12	18.10	16.16	10.20
PtCho 16:0/20:5,16:1/20:4	1.00	1.03	1.31	1.33	1.28	1.04	12.41	10.23	6.63
PtCho 16:0/20:4	1.00	1.12	1.43	1.73	1.74	1.28	17.03	13.28	7.83
PtCho 16:0/20:3	1.00	1.12	1.32	1.53	1.54	1.09	14.12	11.36	6.49
PtCho 18:0/18:2,18:1/18:1	1.00	1.14	1.19	1.27	1.19	0.83	11.26	9.84	5.45
PtCho 18:0/18:1	1.00	1.17	1.12	1.27	1.22	0.79	11.41	10.75	6.00
PtCho Total	1.00	1.11	1.23	1.52	1.59	1.14	16.38	14.45	8.67
Pcho	1.00	0.68	0.80	0.30	0.24	0.14	0.05	0.04	0.10
SM	1.00	1.09	1.14	0.69	0.78	0.57	0.98	0.92	0.96
GPC	1.00	0.64	0.74	0.54	0.74	0.64	1.23	1.99	1.85

Table 19A: Cow #2 Average µM in Milk Used in Figure 7

	Average µM								
	Week of Lactation								
Lipid	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**
AcCho	0.00	0.00	0.00	0.03	0.05	0.06	0.00	0.00	0.00
Bet	78.38	48.15	21.99	14.95	27.62	25.23	20.42	35.60	39.50
Cho	32.09	54.22	79.72	72.51	122.30	118.21	67.30	80.41	60.54
LPC 16:0	0.75	0.77	0.67	0.71	1.14	0.79	1.08	1.44	2.57
LPC 18:2	0.37	0.88	0.81	0.24	0.39	0.22	0.24	0.26	0.36
LPC 18:1	1.03	1.22	1.09	0.39	0.75	0.35	0.53	0.67	0.85
LPC 18:0	0.26	0.33	0.22	0.30	0.56	0.42	0.44	0.39	0.83
LPC Total	2.41	3.20	2.79	1.63	2.84	1.78	2.29	2.76	4.61
PtCho 16:0/16:1	6.67	6.38	6.21	8.59	15.28	7.84	22.74	33.62	54.87
PtCho 16:0/22:6,18:1/22:5,18:2/20:4	0.45	0.53	0.72	0.57	0.84	0.54	1.04	1.33	1.89
PtCho 18:1/20:4,18:0/20:5,16:0/22:5	1.95	2.04	2.52	1.66	2.40	1.82	3.36	4.08	5.15
PtCho 18:0/20:4	1.01	1.00	1.21	1.56	2.62	1.65	3.39	4.60	5.08
PtCho 18:0/20:3	0.45	0.45	0.52	0.86	1.71	0.99	2.00	2.46	3.05
PtCho 18:1/22:6	0.16	0.17	0.26	0.21	0.29	0.19	0.27	0.46	0.59
PtCho 18:0/22:6,18:1/22:5	0.36	0.35	0.44	0.32	0.39	0.33	0.44	0.76	0.74
PtCho 18:0/22:5	0.46	0.45	0.48	0.27	0.37	0.35	0.43	0.39	0.67
PtCho 16:0/16:0	17.22	18.65	19.08	22.08	43.68	26.73	53.74	76.96	109.20
PtCho 16:0/18:2	20.68	20.72	18.85	22.75	43.85	23.76	69.23	100.73	113.62
PtCho 16:0/18:1	58.09	53.05	45.31	49.70	87.29	52.81	137.31	212.85	266.21
PtCho 16:0/20:5,16:1/20:4	0.51	0.49	0.58	0.46	0.76	0.47	1.25	1.48	2.02
PtCho 16:0/20:4	3.46	4.42	4.54	4.58	8.00	4.11	9.81	12.89	16.60
PtCho 16:0/20:3	13.75	15.19	14.94	14.67	25.73	14.35	33.34	42.07	47.92
PtCho 18:0/18:2,18:1/18:1	35.50	33.02	27.91	28.84	51.19	28.77	63.94	81.59	94.75
PtCho 18:0/18:1	18.17	15.23	13.29	15.89	28.94	15.73	33.85	44.84	49.73
PtCho Total	178.87	172.14	156.86	172.99	313.36	180.45	436.13	621.12	772.07
Pcho	490.45	560.79	403.12	166.30	222.55	142.75	94.82	147.89	119.04
SM	58.92	54.80	48.70	30.88	47.97	33.23	40.58	42.04	41.66
GPC	104.01	79.12	53.50	25.10	61.28	43.77	156.52	157.11	155.01

Average of triplicate injections

Table 19B: Cow #2 Fold Change in Milk Used in Figure 7

	Fold Change Relative to Week 1								
	Week of Lactation								
Lipid	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**
AcCho	0.00	0.00	0.00	1.00	1.63	1.94	0.07	0.03	0.04
Bet	1.00	0.61	0.28	0.19	0.35	0.32	0.26	0.45	0.50
Cho	1.00	1.69	2.48	2.26	3.81	3.68	2.10	2.51	1.89
LPC 16:0	1.00	1.03	0.89	0.95	1.52	1.05	1.44	1.92	3.43
LPC 18:2	1.00	2.37	2.19	0.63	1.04	0.60	0.63	0.70	0.95
LPC 18:1	1.00	1.18	1.06	0.38	0.73	0.34	0.52	0.65	0.82
LPC 18:0	1.00	1.30	0.85	1.15	2.17	1.65	1.71	1.50	3.24
LPC Total	1.00	1.33	1.16	0.68	1.18	0.74	0.95	1.15	1.91
PtCho 16:0/16:1	1.00	0.96	0.93	1.29	2.29	1.18	3.41	5.04	8.23
PtCho 16:0/22:6,18:1/22:5,18:2/20:4	1.00	1.19	1.59	1.26	1.87	1.19	2.32	2.96	4.22
PtCho 18:1/20:4,18:0/20:5,16:0/22:5	1.00	1.05	1.29	0.85	1.23	0.94	1.73	2.09	2.64
PtCho 18:0/20:4	1.00	1.00	1.20	1.55	2.60	1.64	3.37	4.58	5.05
PtCho 18:0/20:3	1.00	0.99	1.14	1.91	3.78	2.19	4.41	5.45	6.73
PtCho 18:1/22:6	1.00	1.06	1.63	1.29	1.81	1.21	1.67	2.87	3.66
PtCho 18:0/22:6,18:1/22:5	1.00	0.98	1.20	0.87	1.08	0.92	1.21	2.11	2.04
PtCho 18:0/22:5	1.00	0.97	1.03	0.57	0.80	0.75	0.93	0.85	1.45
PtCho 16:0/16:0	1.00	1.08	1.11	1.28	2.54	1.55	3.12	4.47	6.34
PtCho 16:0/18:2	1.00	1.00	0.91	1.10	2.12	1.15	3.35	4.87	5.49
PtCho 16:0/18:1	1.00	0.91	0.78	0.86	1.50	0.91	2.36	3.66	4.58
PtCho 16:0/20:5,16:1/20:4	1.00	0.96	1.15	0.91	1.51	0.93	2.47	2.93	3.99
PtCho 16:0/20:4	1.00	1.28	1.31	1.32	2.31	1.19	2.83	3.72	4.80
PtCho 16:0/20:3	1.00	1.10	1.09	1.07	1.87	1.04	2.42	3.06	3.48
PtCho 18:0/18:2,18:1/18:1	1.00	0.93	0.79	0.81	1.44	0.81	1.80	2.30	2.67
PtCho 18:0/18:1	1.00	0.84	0.73	0.87	1.59	0.87	1.86	2.47	2.74
PtCho Total	1.00	0.96	0.88	0.97	1.75	1.01	2.44	3.47	4.32
Pcho	1.00	1.14	0.82	0.34	0.45	0.29	0.19	0.30	0.24
SM	1.00	0.93	0.83	0.52	0.81	0.56	0.69	0.71	0.71
GPC	1.00	0.76	0.51	0.24	0.59	0.42	1.50	1.51	1.49

Table 20A: Cow #3 Average µM in Milk Used in Figure 7

	Average µM								
	Week of Lactation								
Lipid	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**
AcCho	0.00	0.00	0.00	0.01	0.00	0.00	0.01	0.01	0.00
Bet	83.89	48.68	32.34	14.47	14.45	35.27	45.53	53.81	46.54
Cho	13.02	28.45	38.12	62.02	91.37	88.82	139.17	151.54	121.20
LPC 16:0	0.69	0.47	0.61	0.65	0.87	1.36	2.39	2.20	1.98
LPC 18:2	0.26	0.20	0.25	0.29	0.18	0.17	0.35	0.33	0.91
LPC 18:1	0.51	0.37	0.46	0.45	0.41	0.32	0.87	0.94	1.85
LPC 18:0	0.28	0.18	0.22	0.32	0.75	0.84	0.98	0.80	0.68
LPC Total	1.74	1.23	1.54	1.72	2.21	2.70	4.59	4.27	5.42
PtCho 16:0/16:1	5.09	2.81	3.48	5.81	5.91	11.10	49.98	43.42	23.22
PtCho 16:0/22:6,18:1/22:5,18:2/20:4	0.71	0.29	0.49	0.51	0.41	0.89	1.54	1.28	0.55
PtCho 18:1/20:4,18:0/20:5,16:0/22:5	2.77	0.95	1.78	1.60	1.14	2.05	5.70	5.00	2.71
PtCho 18:0/20:4	1.50	0.62	0.96	1.52	1.21	2.49	5.38	4.89	2.88
PtCho 18:0/20:3	0.64	0.29	0.43	0.87	0.76	1.45	2.80	2.75	1.64
PtCho 18:1/22:6	0.30	0.11	0.18	0.18	0.15	0.33	0.52	0.41	0.19
PtCho 18:0/22:6,18:1/22:5	0.60	0.24	0.31	0.28	0.23	0.50	1.14	0.87	0.39
PtCho 18:0/22:5	0.60	0.20	0.37	0.24	0.20	0.34	0.97	0.70	0.36
PtCho 16:0/16:0	18.04	9.77	12.86	18.30	15.94	38.36	127.17	101.39	57.87
PtCho 16:0/18:2	19.20	8.10	10.24	17.92	16.37	38.49	89.69	79.82	43.15
PtCho 16:0/18:1	45.37	20.84	25.89	40.87	34.48	78.31	270.47	237.43	120.89
PtCho 16:0/20:5,16:1/20:4	0.48	0.18	0.30	0.36	0.36	0.69	2.01	1.67	0.81
PtCho 16:0/20:4	3.78	1.97	2.52	4.13	3.31	6.29	13.43	12.53	5.99
PtCho 16:0/20:3	13.82	6.80	8.55	14.16	11.12	22.91	47.03	43.15	22.42
PtCho 18:0/18:2,18:1/18:1	30.18	14.18	17.20	26.79	22.19	44.01	90.95	86.25	45.01
PtCho 18:0/18:1	15.02	7.37	8.66	13.99	11.67	24.37	52.03	49.59	26.72
PtCho Total	158.11	74.72	94.22	147.52	125.45	272.57	760.80	671.15	354.80
Pcho	435.44	629.19	496.41	164.91	105.86	81.55	67.89	86.39	77.06
SM	42.80	33.80	28.33	30.28	24.95	34.37	38.15	39.32	35.60
GPC	137.63	83.86	100.46	71.10	698.19	540.64	167.72	170.33	128.08

Average of triplicate injections

Table 20B: Cow #3 Fold Change in Milk Used in Figure 7

	Fold Change Relative to Week 1								
	Week of Lactation								
Lipid	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**
AcCho	0.00	0.00	0.00	1.00	0.00	0.00	0.88	0.78	0.34
Bet	1.00	0.58	0.39	0.17	0.17	0.42	0.54	0.64	0.55
Cho	1.00	2.18	2.93	4.76	7.02	6.82	10.69	11.64	9.31
LPC 16:0	1.00	0.68	0.88	0.94	1.26	1.97	3.46	3.18	2.86
LPC 18:2	1.00	0.78	0.97	1.12	0.67	0.66	1.35	1.27	3.49
LPC 18:1	1.00	0.73	0.90	0.89	0.81	0.63	1.70	1.85	3.63
LPC 18:0	1.00	0.65	0.80	1.16	2.68	3.02	3.51	2.86	2.43
LPC Total	1.00	0.70	0.89	0.99	1.27	1.55	2.64	2.45	3.11
PtCho 16:0/16:1	1.00	0.55	0.68	1.14	1.16	2.18	9.81	8.52	4.56
PtCho 16:0/22:6,18:1/22:5,18:2/20:4	1.00	0.41	0.69	0.72	0.57	1.25	2.18	1.80	0.78
PtCho 18:1/20:4,18:0/20:5,16:0/22:5	1.00	0.34	0.64	0.58	0.41	0.74	2.06	1.81	0.98
PtCho 18:0/20:4	1.00	0.41	0.64	1.01	0.81	1.66	3.58	3.25	1.92
PtCho 18:0/20:3	1.00	0.46	0.67	1.35	1.18	2.25	4.35	4.27	2.54
PtCho 18:1/22:6	1.00	0.36	0.60	0.60	0.50	1.08	1.73	1.37	0.64
PtCho 18:0/22:6,18:1/22:5	1.00	0.39	0.52	0.47	0.38	0.83	1.89	1.45	0.65
PtCho 18:0/22:5	1.00	0.34	0.61	0.39	0.34	0.56	1.60	1.16	0.59
PtCho 16:0/16:0	1.00	0.54	0.71	1.01	0.88	2.13	7.05	5.62	3.21
PtCho 16:0/18:2	1.00	0.42	0.53	0.93	0.85	2.01	4.67	4.16	2.25
PtCho 16:0/18:1	1.00	0.46	0.57	0.90	0.76	1.73	5.96	5.23	2.66
PtCho 16:0/20:5,16:1/20:4	1.00	0.38	0.62	0.75	0.75	1.44	4.18	3.47	1.69
PtCho 16:0/20:4	1.00	0.52	0.67	1.09	0.87	1.66	3.55	3.31	1.58
PtCho 16:0/20:3	1.00	0.49	0.62	1.02	0.80	1.66	3.40	3.12	1.62
PtCho 18:0/18:2,18:1/18:1	1.00	0.47	0.57	0.89	0.74	1.46	3.01	2.86	1.49
PtCho 18:0/18:1	1.00	0.49	0.58	0.93	0.78	1.62	3.46	3.30	1.78
PtCho Total	1.00	0.47	0.60	0.93	0.79	1.72	4.81	4.24	2.24
Pcho	1.00	1.44	1.14	0.38	0.24	0.19	0.16	0.20	0.18
SM	1.00	0.79	0.66	0.71	0.58	0.80	0.89	0.92	0.83
GPC	1.00	0.61	0.73	0.52	5.07	3.93	1.22	1.24	0.93

Table 21A: Cow #4 Average µM in Milk Used in Figure 7

	Average µM								
	Week of Lactation								
Lipid	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**
AcCho	0.00	0.00	0.00	0.02	0.02	0.01	0.00	0.00	0.01
Bet	93.41	31.75	15.23	11.55	10.23	16.44	23.30	37.11	42.10
Cho	27.84	42.42	30.91	111.00	83.91	125.24	111.53	93.51	124.21
LPC 16:0	1.48	1.16	0.94	0.87	0.67	0.67	0.90	1.32	1.19
LPC 18:2	0.35	0.42	0.37	0.32	0.19	0.12	0.31	0.25	0.28
LPC 18:1	0.77	0.74	0.53	0.53	0.22	0.18	0.45	0.54	0.74
LPC 18:0	0.56	0.56	0.46	0.24	0.28	0.32	0.44	0.39	0.36
LPC Total	3.16	2.89	2.29	1.96	1.37	1.30	2.11	2.51	2.58
PtCho 16:0/16:1	7.92	5.03	5.95	15.57	7.92	5.65	20.89	41.76	40.00
PtCho 16:0/22:6,18:1/22:5,18:2/20:4	1.22	0.78	0.72	0.91	0.55	0.46	1.42	1.65	1.56
PtCho 18:1/20:4,18:0/20:5,16:0/22:5	4.63	2.57	3.03	2.77	1.73	1.55	3.82	4.89	5.13
PtCho 18:0/20:4	3.00	1.57	1.83	2.66	1.61	1.31	3.61	4.60	5.32
PtCho 18:0/20:3	1.25	0.73	0.94	1.58	0.95	0.71	2.06	2.49	3.00
PtCho 18:1/22:6	0.37	0.34	0.26	0.34	0.16	0.20	0.49	0.63	0.57
PtCho 18:0/22:6,18:1/22:5	0.81	0.62	0.63	0.55	0.25	0.32	1.06	0.86	0.86
PtCho 18:0/22:5	1.02	0.67	0.79	0.42	0.27	0.34	0.82	0.63	0.70
PtCho 16:0/16:0	29.72	20.61	24.68	47.83	28.51	27.98	71.50	108.68	130.68
PtCho 16:0/18:2	31.02	21.95	23.39	38.17	24.57	18.84	64.80	87.93	89.26
PtCho 16:0/18:1	65.08	48.25	50.23	90.46	46.48	38.63	130.96	231.79	237.75
PtCho 16:0/20:5,16:1/20:4	1.24	0.58	0.55	0.75	0.48	0.35	1.05	2.01	2.06
PtCho 16:0/20:4	6.40	5.32	4.45	7.43	4.83	3.40	10.15	15.41	15.35
PtCho 16:0/20:3	18.36	16.89	16.63	24.53	15.14	10.93	32.03	47.32	44.53
PtCho 18:0/18:2,18:1/18:1	36.70	33.05	34.19	46.27	27.37	21.46	63.69	90.65	89.09
PtCho 18:0/18:1	18.61	17.23	18.24	25.36	14.37	12.55	34.65	49.65	49.17
PtCho Total	227.35	176.17	186.51	305.60	175.21	144.67	442.99	690.95	715.03
Pcho	413.00	498.11	254.66	143.67	127.30	94.80	56.90	39.92	71.16
SM	66.61	42.57	47.98	31.92	24.07	17.86	30.13	29.52	29.50
GPC	151.43	110.50	36.89	46.72	44.96	25.78	69.48	141.17	124.83

Average of triplicate injections

Table 21B: Cow #4 Fold Change in Milk Used in Figure 7

	Fold Change Relative to Week 1								
	Week of Lactation								
Lipid	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**
AcCho	0.00	0.00	0.00	1.00	0.78	0.66	0.23	0.14	0.33
Bet	1.00	0.34	0.16	0.12	0.11	0.18	0.25	0.40	0.45
Cho	1.00	1.52	1.11	3.99	3.01	4.50	4.01	3.36	4.46
LPC 16:0	1.00	0.79	0.64	0.59	0.45	0.46	0.61	0.89	0.81
LPC 18:2	1.00	1.18	1.04	0.91	0.55	0.35	0.87	0.72	0.80
LPC 18:1	1.00	0.96	0.68	0.68	0.29	0.23	0.58	0.70	0.96
LPC 18:0	1.00	1.00	0.82	0.43	0.51	0.58	0.79	0.71	0.65
LPC Total	1.00	0.91	0.72	0.62	0.43	0.41	0.67	0.79	0.82
PtCho 16:0/16:1	1.00	0.63	0.75	1.96	1.00	0.71	2.64	5.27	5.05
PtCho 16:0/22:6,18:1/22:5,18:2/20:4	1.00	0.64	0.59	0.75	0.45	0.37	1.17	1.35	1.28
PtCho 18:1/20:4,18:0/20:5,16:0/22:5	1.00	0.56	0.65	0.60	0.37	0.33	0.83	1.06	1.11
PtCho 18:0/20:4	1.00	0.52	0.61	0.89	0.54	0.44	1.21	1.54	1.78
PtCho 18:0/20:3	1.00	0.59	0.75	1.26	0.76	0.57	1.65	2.00	2.40
PtCho 18:1/22:6	1.00	0.91	0.71	0.91	0.44	0.53	1.30	1.68	1.53
PtCho 18:0/22:6,18:1/22:5	1.00	0.76	0.78	0.68	0.31	0.40	1.31	1.06	1.06
PtCho 18:0/22:5	1.00	0.65	0.78	0.41	0.27	0.33	0.80	0.61	0.69
PtCho 16:0/16:0	1.00	0.69	0.83	1.61	0.96	0.94	2.41	3.66	4.40
PtCho 16:0/18:2	1.00	0.71	0.75	1.23	0.79	0.61	2.09	2.83	2.88
PtCho 16:0/18:1	1.00	0.74	0.77	1.39	0.71	0.59	2.01	3.56	3.65
PtCho 16:0/20:5,16:1/20:4	1.00	0.47	0.44	0.61	0.39	0.28	0.85	1.63	1.66
PtCho 16:0/20:4	1.00	0.83	0.69	1.16	0.75	0.53	1.59	2.41	2.40
PtCho 16:0/20:3	1.00	0.92	0.91	1.34	0.82	0.60	1.74	2.58	2.43
PtCho 18:0/18:2,18:1/18:1	1.00	0.90	0.93	1.26	0.75	0.58	1.74	2.47	2.43
PtCho 18:0/18:1	1.00	0.93	0.98	1.36	0.77	0.67	1.86	2.67	2.64
PtCho Total	1.00	0.77	0.82	1.34	0.77	0.64	1.95	3.04	3.15
Pcho	1.00	1.21	0.62	0.35	0.31	0.23	0.14	0.10	0.17
SM	1.00	0.64	0.72	0.48	0.36	0.27	0.45	0.44	0.44
GPC	1.00	0.73	0.24	0.31	0.30	0.17	0.46	0.93	0.82

Table 22A: Cow #5 Average µM in Milk Used in Figure 7

	Average µM								
	Week of Lactation								
Lipid	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**
AcCho	0.00	0.00	0.00	0.16	0.04	0.04	0.00	0.00	0.00
Bet	97.75	26.97	14.82	22.16	9.90	20.59	35.43	43.53	40.23
Cho	14.68	37.63	45.36	85.40	83.73	116.89	129.14	119.46	135.44
LPC 16:0	0.69	0.63	0.77	0.76	0.69	0.60	0.78	1.37	1.10
LPC 18:2	0.18	0.38	0.53	0.20	0.12	0.20	0.35	0.26	0.23
LPC 18:1	0.46	0.78	0.99	0.38	0.14	0.29	0.55	0.68	0.49
LPC 18:0	0.30	0.29	0.43	0.28	0.23	0.23	0.27	0.51	0.34
LPC Total	1.63	2.08	2.72	1.63	1.18	1.31	1.96	2.82	2.17
PtCho 16:0/16:1	4.90	3.34	4.60	11.48	6.63	8.97	30.89	47.68	40.75
PtCho 16:0/22:6,18:1/22:5,18:2/20:4	0.78	0.59	0.73	0.71	0.38	0.50	1.68	1.61	1.48
PtCho 18:1/20:4,18:0/20:5,16:0/22:5	3.04	1.96	2.84	2.23	1.25	1.74	4.49	3.89	4.58
PtCho 18:0/20:4	2.05	1.09	1.49	2.31	0.92	1.23	4.00	3.48	3.82
PtCho 18:0/20:3	0.88	0.54	0.69	1.29	0.50	0.70	2.02	2.07	2.15
PtCho 18:1/22:6	0.26	0.24	0.28	0.25	0.11	0.20	0.56	0.49	0.44
PtCho 18:0/22:6,18:1/22:5	0.51	0.45	0.55	0.46	0.24	0.29	0.63	0.71	0.71
PtCho 18:0/22:5	0.72	0.46	0.64	0.37	0.30	0.33	0.57	0.49	0.52
PtCho 16:0/16:0	22.08	14.01	19.82	39.19	26.08	28.47	96.37	113.89	115.41
PtCho 16:0/18:2	21.45	14.68	21.14	33.91	18.21	24.13	96.52	101.24	88.02
PtCho 16:0/18:1	50.87	37.49	51.97	74.31	36.58	52.69	214.58	268.38	219.17
PtCho 16:0/20:5,16:1/20:4	0.91	0.46	0.60	0.59	0.31	0.42	1.45	1.82	1.81
PtCho 16:0/20:4	5.45	3.90	5.24	6.04	3.11	4.19	13.46	15.23	12.55
PtCho 16:0/20:3	16.48	13.61	18.52	21.41	10.33	13.98	48.96	45.97	39.64
PtCho 18:0/18:2,18:1/18:1	33.01	27.41	36.11	40.34	18.98	25.30	95.04	91.36	78.33
PtCho 18:0/18:1	17.27	13.40	18.21	22.94	10.03	13.54	47.84	46.89	43.57
PtCho Total	180.64	133.62	183.43	257.83	133.96	176.67	659.06	745.20	652.93
Pcho	725.52	480.33	390.18	161.91	81.92	69.56	31.68	38.90	63.86
SM	61.23	36.98	49.53	34.75	16.86	21.88	42.97	44.07	36.10
GPC	64.79	67.36	52.75	71.21	34.16	37.61	177.52	139.90	144.50

Average of triplicate injections

Table 22B: Cow #5 Fold Change in Milk Used in Figure 7

	Fold Change Relative to Week 1								
	Week of Lactation								
Lipid	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**
AcCho	0.00	0.00	0.00	1.00	0.23	0.28	0.01	0.02	0.02
Bet	1.00	0.28	0.15	0.23	0.10	0.21	0.36	0.45	0.41
Cho	1.00	2.56	3.09	5.82	5.70	7.96	8.80	8.14	9.23
LPC 16:0	1.00	0.92	1.12	1.11	1.00	0.87	1.13	1.99	1.60
LPC 18:2	1.00	2.09	2.97	1.13	0.68	1.10	1.97	1.45	1.27
LPC 18:1	1.00	1.70	2.15	0.83	0.30	0.62	1.21	1.49	1.07
LPC 18:0	1.00	0.98	1.45	0.94	0.77	0.76	0.91	1.70	1.16
LPC Total	1.00	1.28	1.68	1.00	0.72	0.81	1.20	1.74	1.33
PtCho 16:0/16:1	1.00	0.68	0.94	2.34	1.36	1.83	6.31	9.74	8.32
PtCho 16:0/22:6,18:1/22:5,18:2/20:4	1.00	0.75	0.94	0.90	0.48	0.64	2.14	2.06	1.89
PtCho 18:1/20:4,18:0/20:5,16:0/22:5	1.00	0.65	0.93	0.73	0.41	0.57	1.48	1.28	1.50
PtCho 18:0/20:4	1.00	0.53	0.73	1.13	0.45	0.60	1.95	1.70	1.86
PtCho 18:0/20:3	1.00	0.61	0.78	1.46	0.57	0.79	2.30	2.35	2.44
PtCho 18:1/22:6	1.00	0.92	1.09	0.99	0.44	0.77	2.18	1.91	1.71
PtCho 18:0/22:6,18:1/22:5	1.00	0.88	1.09	0.90	0.47	0.58	1.25	1.41	1.41
PtCho 18:0/22:5	1.00	0.64	0.90	0.51	0.42	0.46	0.79	0.69	0.72
PtCho 16:0/16:0	1.00	0.63	0.90	1.77	1.18	1.29	4.36	5.16	5.23
PtCho 16:0/18:2	1.00	0.68	0.99	1.58	0.85	1.13	4.50	4.72	4.10
PtCho 16:0/18:1	1.00	0.74	1.02	1.46	0.72	1.04	4.22	5.28	4.31
PtCho 16:0/20:5,16:1/20:4	1.00	0.50	0.66	0.65	0.35	0.46	1.60	2.01	1.99
PtCho 16:0/20:4	1.00	0.72	0.96	1.11	0.57	0.77	2.47	2.80	2.30
PtCho 16:0/20:3	1.00	0.83	1.12	1.30	0.63	0.85	2.97	2.79	2.41
PtCho 18:0/18:2,18:1/18:1	1.00	0.83	1.09	1.22	0.58	0.77	2.88	2.77	2.37
PtCho 18:0/18:1	1.00	0.78	1.05	1.33	0.58	0.78	2.77	2.71	2.52
PtCho Total	1.00	0.74	1.02	1.43	0.74	0.98	3.65	4.13	3.61
Pcho	1.00	0.66	0.54	0.22	0.11	0.10	0.04	0.05	0.09
SM	1.00	0.60	0.81	0.57	0.28	0.36	0.70	0.72	0.59
GPC	1.00	1.04	0.81	1.10	0.53	0.58	2.74	2.16	2.23

Table 23A: Cow #6 Average µM in Milk Used in Figure 7

	Average µM								
	Week of Lactation								
Lipid	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**
AcCho	0.00	0.00	0.00	0.10	0.07	0.05	0.00	0.01	0.01
Bet	184.34	65.43	28.29	21.36	15.81	13.10	41.29	62.71	138.70
Cho	19.55	24.73	37.25	132.13	53.18	53.25	51.29	72.74	175.80
LPC 16:0	0.86	0.88	0.93	0.71	0.75	0.79	1.82	1.68	0.89
LPC 18:2	1.12	1.20	0.51	0.35	0.30	0.19	0.26	0.23	0.16
LPC 18:1	2.01	1.63	1.01	0.47	0.45	0.24	0.58	0.59	0.33
LPC 18:0	0.23	0.39	0.42	0.31	0.21	0.27	0.48	0.53	0.28
LPC Total	4.22	4.11	2.86	1.85	1.71	1.50	3.14	3.03	1.66
PtCho 16:0/16:1	3.34	4.13	5.22	7.14	8.49	8.47	27.10	29.68	27.74
PtCho 16:0/22:6,18:1/22:5,18:2/20:4	0.67	0.82	0.68	0.75	0.61	0.90	1.32	3.06	2.10
PtCho 18:1/20:4,18:0/20:5,16:0/22:5	2.58	2.07	2.51	2.41	1.81	2.36	3.63	6.44	6.70
PtCho 18:0/20:4	1.35	1.22	1.40	1.53	1.76	1.75	4.03	4.70	6.51
PtCho 18:0/20:3	0.62	0.67	0.72	0.74	0.95	1.01	2.36	3.03	3.41
PtCho 18:1/22:6	0.27	0.32	0.30	0.26	0.20	0.40	0.44	0.76	0.85
PtCho 18:0/22:6,18:1/22:5	0.54	0.76	0.51	0.48	0.29	0.74	0.52	3.15	1.41
PtCho 18:0/22:5	0.62	0.64	0.52	0.39	0.22	0.41	0.54	2.57	1.09
PtCho 16:0/16:0	15.10	15.92	19.79	38.67	30.49	29.75	72.30	83.39	100.92
PtCho 16:0/18:2	13.00	19.30	21.07	29.50	30.98	27.90	84.83	80.79	98.60
PtCho 16:0/18:1	33.73	45.74	51.92	86.95	61.38	55.53	161.54	171.71	242.73
PtCho 16:0/20:5,16:1/20:4	0.47	0.39	0.45	0.48	0.48	0.59	1.51	2.00	2.63
PtCho 16:0/20:4	3.39	4.04	4.46	7.31	5.81	5.14	12.20	12.69	15.97
PtCho 16:0/20:3	10.56	15.50	15.77	23.77	18.91	16.90	42.84	40.35	51.18
PtCho 18:0/18:2,18:1/18:1	22.17	33.77	32.59	46.36	33.49	30.43	74.35	75.41	91.15
PtCho 18:0/18:1	12.20	17.44	16.83	22.87	18.77	17.54	40.63	41.10	50.23
PtCho Total	120.60	162.75	174.75	269.59	214.62	199.84	530.14	560.83	703.22
Pcho	502.10	488.35	270.37	158.49	109.41	48.99	15.99	33.68	46.65
SM	45.69	48.41	50.78	45.08	28.29	24.74	29.64	29.53	33.92
GPC	135.38	114.92	120.29	53.88	79.71	54.98	234.12	223.67	261.53

Average of triplicate injections

Table 23B: Cow #6 Fold Change in Milk Used in Figure 7

	Fold Change Relative to Week 1								
	Week of Lactation								
Lipid	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**
AcCho	0.00	0.00	0.00	1.00	0.71	0.49	0.01	0.09	0.07
Bet	1.00	0.35	0.15	0.12	0.09	0.07	0.22	0.34	0.75
Cho	1.00	1.26	1.90	6.76	2.72	2.72	2.62	3.72	8.99
LPC 16:0	1.00	1.03	1.09	0.83	0.87	0.93	2.12	1.96	1.04
LPC 18:2	1.00	1.07	0.45	0.31	0.27	0.17	0.23	0.21	0.15
LPC 18:1	1.00	0.81	0.50	0.23	0.22	0.12	0.29	0.29	0.16
LPC 18:0	1.00	1.68	1.77	1.33	0.89	1.15	2.06	2.26	1.19
LPC Total	1.00	0.97	0.68	0.44	0.40	0.36	0.74	0.72	0.39
PtCho 16:0/16:1	1.00	1.24	1.56	2.13	2.54	2.53	8.10	8.87	8.29
PtCho 16:0/22:6,18:1/22:5,18:2/20:4	1.00	1.23	1.02	1.13	0.92	1.36	1.98	4.59	3.15
PtCho 18:1/20:4,18:0/20:5,16:0/22:5	1.00	0.80	0.97	0.93	0.70	0.92	1.41	2.50	2.60
PtCho 18:0/20:4	1.00	0.91	1.04	1.14	1.31	1.30	2.99	3.49	4.83
PtCho 18:0/20:3	1.00	1.08	1.16	1.20	1.53	1.63	3.82	4.90	5.51
PtCho 18:1/22:6	1.00	1.17	1.11	0.97	0.73	1.49	1.64	2.82	3.15
PtCho 18:0/22:6,18:1/22:5	1.00	1.41	0.95	0.88	0.53	1.37	0.96	5.84	2.62
PtCho 18:0/22:5	1.00	1.04	0.85	0.63	0.35	0.66	0.87	4.17	1.77
PtCho 16:0/16:0	1.00	1.05	1.31	2.56	2.02	1.97	4.79	5.52	6.68
PtCho 16:0/18:2	1.00	1.49	1.62	2.27	2.38	2.15	6.53	6.22	7.59
PtCho 16:0/18:1	1.00	1.36	1.54	2.58	1.82	1.65	4.79	5.09	7.20
PtCho 16:0/20:5,16:1/20:4	1.00	0.83	0.96	1.02	1.03	1.26	3.22	4.26	5.61
PtCho 16:0/20:4	1.00	1.19	1.32	2.15	1.71	1.52	3.60	3.74	4.71
PtCho 16:0/20:3	1.00	1.47	1.49	2.25	1.79	1.60	4.06	3.82	4.85
PtCho 18:0/18:2,18:1/18:1	1.00	1.52	1.47	2.09	1.51	1.37	3.35	3.40	4.11
PtCho 18:0/18:1	1.00	1.43	1.38	1.87	1.54	1.44	3.33	3.37	4.12
PtCho Total	1.00	1.35	1.45	2.24	1.78	1.66	4.40	4.65	5.83
Pcho	1.00	0.97	0.54	0.32	0.22	0.10	0.03	0.07	0.09
SM	1.00	1.06	1.11	0.99	0.62	0.54	0.65	0.65	0.74
GPC	1.00	0.85	0.89	0.40	0.59	0.41	1.73	1.65	1.93

Table 24A: Cow #7 Average µM in Milk Used in Figure 7

	Average µM								
	Week of Lactation								
Lipid	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**
AcCho	0.00	0.00	0.00	0.06	0.00	0.00	0.01	0.01	0.01
Bet	88.68	57.17	43.43	17.74	20.64	12.41	45.95	50.98	53.49
Cho	14.59	30.54	58.31	113.24	202.25	113.45	132.57	123.23	107.18
LPC 16:0	0.84	0.54	0.55	0.70	1.17	1.07	2.40	2.38	2.12
LPC 18:2	0.69	0.44	0.37	0.24	0.32	0.14	0.33	0.25	0.87
LPC 18:1	1.35	0.57	0.51	0.42	0.57	0.35	0.80	0.74	1.63
LPC 18:0	0.38	0.26	0.12	0.28	0.96	0.62	0.83	0.91	0.64
LPC Total	3.26	1.81	1.56	1.64	3.02	2.17	4.35	4.28	5.27
PtCho 16:0/16:1	3.87	3.56	1.96	4.67	5.36	14.39	48.24	44.70	31.41
PtCho 16:0/22:6,18:1/22:5,18:2/20:4	0.64	0.89	0.37	0.46	0.58	1.02	1.41	1.35	0.90
PtCho 18:1/20:4,18:0/20:5,16:0/22:5	3.15	2.71	2.07	1.34	1.37	2.48	5.53	5.16	3.39
PtCho 18:0/20:4	1.27	1.11	0.54	0.97	1.31	2.58	5.57	5.91	3.52
PtCho 18:0/20:3	0.52	0.45	0.19	0.47	0.73	1.37	3.18	2.85	2.00
PtCho 18:1/22:6	0.20	0.25	0.10	0.15	0.22	0.35	0.49	0.39	0.18
PtCho 18:0/22:6,18:1/22:5	0.55	0.66	0.29	0.26	0.35	0.43	0.90	0.72	0.40
PtCho 18:0/22:5	0.70	0.58	0.40	0.25	0.25	0.24	0.82	0.69	0.44
PtCho 16:0/16:0	20.31	22.41	12.80	24.52	25.83	61.90	152.52	141.50	101.74
PtCho 16:0/18:2	17.87	16.29	8.21	19.45	20.75	48.83	89.46	87.43	55.39
PtCho 16:0/18:1	50.64	45.28	22.69	57.37	49.82	108.34	249.63	248.50	157.10
PtCho 16:0/20:5,16:1/20:4	0.42	0.42	0.23	0.31	0.41	0.92	1.92	1.78	1.09
PtCho 16:0/20:4	3.36	4.79	2.13	4.80	4.69	8.93	13.49	13.38	8.19
PtCho 16:0/20:3	13.43	14.49	6.83	16.29	14.65	30.41	45.80	44.19	28.39
PtCho 18:0/18:2,18:1/18:1	32.14	29.42	13.01	30.41	26.25	51.92	86.55	79.97	52.29
PtCho 18:0/18:1	17.05	14.36	6.27	14.85	13.73	27.96	53.02	51.63	32.03
PtCho Total	166.12	157.68	78.10	176.55	166.29	362.10	758.53	730.14	478.48
Pcho	520.96	575.07	524.05	132.94	113.72	69.25	70.05	59.70	62.32
SM	42.46	41.41	24.29	38.92	38.75	40.27	32.06	28.26	25.76
GPC	87.91	71.21	70.57	65.31	429.65	418.06	194.39	81.55	180.07

Average of triplicate injections

Table 24B: Cow #7 Fold Change in Milk Used in Figure 7

	Fold Change Relative to Week 1								
	Week of Lactation								
Lipid	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**
AcCho	0.00	0.00	0.00	1.00	0.00	0.00	0.20	0.17	0.11
Bet	1.00	0.64	0.49	0.20	0.23	0.14	0.52	0.57	0.60
Cho	1.00	2.09	4.00	7.76	13.86	7.78	9.09	8.45	7.35
LPC 16:0	1.00	0.65	0.66	0.84	1.40	1.28	2.86	2.85	2.54
LPC 18:2	1.00	0.63	0.54	0.34	0.46	0.20	0.48	0.36	1.26
LPC 18:1	1.00	0.42	0.38	0.31	0.42	0.26	0.59	0.54	1.21
LPC 18:0	1.00	0.69	0.33	0.73	2.53	1.63	2.17	2.38	1.68
LPC Total	1.00	0.55	0.48	0.50	0.92	0.67	1.33	1.31	1.61
PtCho 16:0/16:1	1.00	0.92	0.51	1.20	1.38	3.72	12.45	11.54	8.11
PtCho 16:0/22:6,18:1/22:5,18:2/20:4	1.00	1.39	0.57	0.72	0.90	1.59	2.20	2.11	1.39
PtCho 18:1/20:4,18:0/20:5,16:0/22:5	1.00	0.86	0.66	0.43	0.43	0.79	1.76	1.64	1.08
PtCho 18:0/20:4	1.00	0.87	0.43	0.76	1.03	2.03	4.38	4.65	2.77
PtCho 18:0/20:3	1.00	0.86	0.36	0.90	1.40	2.64	6.12	5.47	3.85
PtCho 18:1/22:6	1.00	1.25	0.52	0.77	1.10	1.75	2.43	1.96	0.89
PtCho 18:0/22:6,18:1/22:5	1.00	1.19	0.52	0.46	0.63	0.78	1.63	1.30	0.73
PtCho 18:0/22:5	1.00	0.83	0.58	0.36	0.36	0.35	1.18	0.98	0.63
PtCho 16:0/16:0	1.00	1.10	0.63	1.21	1.27	3.05	7.51	6.97	5.01
PtCho 16:0/18:2	1.00	0.91	0.46	1.09	1.16	2.73	5.01	4.89	3.10
PtCho 16:0/18:1	1.00	0.89	0.45	1.13	0.98	2.14	4.93	4.91	3.10
PtCho 16:0/20:5,16:1/20:4	1.00	1.00	0.56	0.73	0.98	2.19	4.60	4.26	2.61
PtCho 16:0/20:4	1.00	1.43	0.63	1.43	1.40	2.66	4.02	3.99	2.44
PtCho 16:0/20:3	1.00	1.08	0.51	1.21	1.09	2.26	3.41	3.29	2.11
PtCho 18:0/18:2,18:1/18:1	1.00	0.92	0.40	0.95	0.82	1.62	2.69	2.49	1.63
PtCho 18:0/18:1	1.00	0.84	0.37	0.87	0.81	1.64	3.11	3.03	1.88
PtCho Total	1.00	0.95	0.47	1.06	1.00	2.18	4.57	4.40	2.88
Pcho	1.00	1.10	1.01	0.26	0.22	0.13	0.13	0.11	0.12
SM	1.00	0.98	0.57	0.92	0.91	0.95	0.76	0.67	0.61
GPC	1.00	0.81	0.80	0.74	4.89	4.76	2.21	0.93	2.05

Table 25A: Cow #8 Average µM in Milk Used in Figure 7

	Average µM								
	Week of Lactation								
Lipid	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**
AcCho	0.00	0.00	0.00	0.03	0.04	0.02	0.01	0.01	0.01
Bet	265.91	81.08	46.79	24.24	15.64	14.33	30.43	32.18	67.53
Cho	9.73	28.31	40.07	59.92	79.79	51.63	65.69	127.19	227.72
LPC 16:0	1.07	1.06	1.25	1.08	1.00	1.00	1.81	1.70	0.94
LPC 18:2	0.13	0.67	0.71	0.32	0.34	0.32	0.55	0.50	0.39
LPC 18:1	0.28	1.06	1.32	0.61	0.61	0.42	1.00	0.94	0.96
LPC 18:0	0.28	0.28	0.53	0.21	0.23	0.33	0.58	0.76	0.41
LPC Total	1.74	3.07	3.81	2.21	2.17	2.06	3.93	3.90	2.70
PtCho 16:0/16:1	7.78	3.95	6.37	10.00	7.99	9.82	34.52	44.29	37.30
PtCho 16:0/22:6,18:1/22:5,18:2/20:4	1.02	0.52	0.69	0.58	0.45	0.56	1.49	1.49	1.05
PtCho 18:1/20:4,18:0/20:5,16:0/22:5	4.97	2.77	3.52	2.13	1.71	1.95	4.17	5.10	4.30
PtCho 18:0/20:4	3.23	0.83	1.19	1.53	1.23	1.36	3.30	4.16	4.43
PtCho 18:0/20:3	1.73	0.29	0.51	0.80	0.63	0.77	1.77	2.51	2.38
PtCho 18:1/22:6	0.31	0.15	0.16	0.25	0.17	0.18	0.55	0.57	0.37
PtCho 18:0/22:6,18:1/22:5	0.80	0.45	0.47	0.47	0.32	0.38	0.68	0.88	0.84
PtCho 18:0/22:5	1.18	0.57	0.81	0.40	0.27	0.50	0.49	0.86	0.61
PtCho 16:0/16:0	31.53	15.43	23.75	24.96	20.14	15.43	47.21	86.51	86.32
PtCho 16:0/18:2	21.17	11.82	22.95	28.22	18.46	28.42	98.06	81.10	110.23
PtCho 16:0/18:1	61.89	36.04	60.73	76.34	49.03	54.89	223.19	234.90	325.04
PtCho 16:0/20:5,16:1/20:4	0.87	0.35	0.54	0.42	0.34	0.43	1.49	2.02	1.53
PtCho 16:0/20:4	4.94	2.30	3.98	4.18	3.02	3.56	11.49	13.76	11.99
PtCho 16:0/20:3	13.87	9.24	14.72	17.82	11.16	14.59	41.36	42.68	55.54
PtCho 18:0/18:2,18:1/18:1	33.66	21.79	34.20	39.46	23.73	26.02	75.41	89.34	120.60
PtCho 18:0/18:1	19.88	10.83	18.14	19.95	13.25	12.71	36.58	44.34	69.09
PtCho Total	208.82	117.32	192.76	227.49	151.89	171.60	581.75	654.52	831.62
Pcho	660.40	519.24	472.54	139.12	104.34	20.20	19.09	74.92	117.77
SM	56.34	36.00	57.23	35.73	25.30	22.87	30.46	29.80	45.81
GPC	182.38	48.33	67.16	85.52	71.90	85.67	220.95	171.65	130.58

Average of triplicate injections

Table 25B: Cow #8 Fold Change in Milk Used in Figure 7

	Fold Change Relative to Week 1								
	Week of Lactation								
Lipid	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**
AcCho	0.00	0.00	0.00	1.00	1.29	0.70	0.34	0.19	0.18
Bet	1.00	0.30	0.18	0.09	0.06	0.05	0.11	0.12	0.25
Cho	1.00	2.91	4.12	6.16	8.20	5.31	6.75	13.08	23.41
LPC 16:0	1.00	0.99	1.18	1.01	0.94	0.94	1.69	1.60	0.88
LPC 18:2	1.00	5.24	5.53	2.49	2.63	2.47	4.29	3.94	3.04
LPC 18:1	1.00	3.85	4.80	2.21	2.20	1.51	3.61	3.39	3.48
LPC 18:0	1.00	1.03	1.93	0.76	0.83	1.20	2.12	2.74	1.50
LPC Total	1.00	1.76	2.19	1.27	1.24	1.18	2.25	2.24	1.55
PtCho 16:0/16:1	1.00	0.51	0.82	1.29	1.03	1.26	4.44	5.69	4.80
PtCho 16:0/22:6,18:1/22:5,18:2/20:4	1.00	0.52	0.68	0.57	0.44	0.55	1.46	1.47	1.03
PtCho 18:1/20:4,18:0/20:5,16:0/22:5	1.00	0.56	0.71	0.43	0.34	0.39	0.84	1.03	0.87
PtCho 18:0/20:4	1.00	0.26	0.37	0.47	0.38	0.42	1.02	1.29	1.37
PtCho 18:0/20:3	1.00	0.17	0.29	0.46	0.36	0.44	1.02	1.45	1.38
PtCho 18:1/22:6	1.00	0.48	0.53	0.81	0.56	0.57	1.78	1.83	1.20
PtCho 18:0/22:6,18:1/22:5	1.00	0.57	0.59	0.59	0.41	0.48	0.86	1.10	1.06
PtCho 18:0/22:5	1.00	0.49	0.69	0.34	0.23	0.43	0.41	0.73	0.52
PtCho 16:0/16:0	1.00	0.49	0.75	0.79	0.64	0.49	1.50	2.74	2.74
PtCho 16:0/18:2	1.00	0.56	1.08	1.33	0.87	1.34	4.63	3.83	5.21
PtCho 16:0/18:1	1.00	0.58	0.98	1.23	0.79	0.89	3.61	3.80	5.25
PtCho 16:0/20:5,16:1/20:4	1.00	0.41	0.63	0.48	0.39	0.50	1.71	2.34	1.76
PtCho 16:0/20:4	1.00	0.46	0.81	0.85	0.61	0.72	2.33	2.79	2.43
PtCho 16:0/20:3	1.00	0.67	1.06	1.28	0.80	1.05	2.98	3.08	4.00
PtCho 18:0/18:2,18:1/18:1	1.00	0.65	1.02	1.17	0.70	0.77	2.24	2.65	3.58
PtCho 18:0/18:1	1.00	0.54	0.91	1.00	0.67	0.64	1.84	2.23	3.48
PtCho Total	1.00	0.56	0.92	1.09	0.73	0.82	2.79	3.13	3.98
Pcho	1.00	0.79	0.72	0.21	0.16	0.03	0.03	0.11	0.18
SM	1.00	0.64	1.02	0.63	0.45	0.41	0.54	0.53	0.81
GPC	1.00	0.26	0.37	0.47	0.39	0.47	1.21	0.94	0.72

Table 26A: Cow #9 Average µM in Milk Used in Figure 7

	Average µM								
	Week of Lactation								
Lipid	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**
AcCho	0.00	0.00	0.00	0.05	0.07	0.08	0.00	0.01	0.01
Bet	143.86	58.51	33.91	19.66	30.06	34.74	58.31	77.38	89.38
Cho	26.39	39.35	68.82	105.56	159.58	171.55	107.30	155.72	129.83
LPC 16:0	0.23	1.37	0.67	0.45	0.41	0.55	1.23	1.42	1.45
LPC 18:2	0.08	2.30	0.41	0.13	0.14	0.27	0.20	0.22	0.21
LPC 18:1	0.11	4.21	0.73	0.21	0.23	0.43	0.51	0.69	0.62
LPC 18:0	0.08	0.42	0.23	0.12	0.12	0.20	0.42	0.70	0.55
LPC Total	0.49	8.30	2.04	0.90	0.90	1.45	2.36	3.02	2.83
PtCho 16:0/16:1	3.64	4.87	5.31	6.76	6.47	6.36	35.91	52.42	35.88
PtCho 16:0/22:6,18:1/22:5,18:2/20:4	0.69	0.90	0.74	0.48	0.45	0.49	1.53	2.38	1.96
PtCho 18:1/20:4,18:0/20:5,16:0/22:5	3.04	3.00	2.35	1.43	1.37	1.47	4.79	8.07	6.38
PtCho 18:0/20:4	1.36	1.88	1.53	1.29	1.18	1.31	5.56	8.37	6.95
PtCho 18:0/20:3	0.63	1.01	0.80	0.72	0.64	0.74	3.08	5.23	4.33
PtCho 18:1/22:6	0.18	0.35	0.28	0.20	0.17	0.20	0.62	0.95	0.67
PtCho 18:0/22:6,18:1/22:5	0.49	0.60	0.43	0.26	0.28	0.35	0.80	1.56	1.18
PtCho 18:0/22:5	0.70	0.70	0.45	0.18	0.22	0.25	0.56	1.28	1.03
PtCho 16:0/16:0	16.19	22.19	19.79	21.34	19.18	19.67	98.40	130.50	97.76
PtCho 16:0/18:2	10.38	19.45	16.84	18.60	15.60	17.41	73.58	89.12	68.56
PtCho 16:0/18:1	25.81	49.67	39.78	40.53	35.97	40.73	183.69	269.46	186.21
PtCho 16:0/20:5,16:1/20:4	0.54	0.64	0.53	0.39	0.33	0.32	1.75	2.62	1.91
PtCho 16:0/20:4	2.51	5.88	4.34	3.65	3.19	3.36	12.34	17.13	13.14
PtCho 16:0/20:3	7.27	19.04	14.64	12.59	10.47	11.81	39.85	53.53	40.35
PtCho 18:0/18:2,18:1/18:1	15.26	36.17	26.42	21.68	18.62	20.94	78.64	106.30	76.36
PtCho 18:0/18:1	8.25	20.05	14.46	11.63	10.14	12.45	45.27	62.44	50.57
PtCho Total	96.95	186.40	148.68	141.70	124.27	137.86	586.37	811.36	593.24
Pcho	629.92	575.52	438.25	90.33	31.67	26.13	14.61	18.96	16.42
SM	44.47	46.75	39.64	23.79	23.67	27.83	30.43	37.06	32.17
GPC	49.97	80.08	69.68	49.47	31.42	50.49	133.11	78.75	133.71

Average of triplicate injections

Table 26B: Cow #9 Fold Change in Milk Used in Figure 7

	Fold Change Relative to Week 1								
	Week of Lactation								
Lipid	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**
AcCho	0.00	0.00	0.00	1.00	1.41	1.80	0.04	0.28	0.19
Bet	1.00	0.41	0.24	0.14	0.21	0.24	0.41	0.54	0.62
Cho	1.00	1.49	2.61	4.00	6.05	6.50	4.07	5.90	4.92
LPC 16:0	1.00	5.97	2.95	1.98	1.81	2.41	5.39	6.21	6.36
LPC 18:2	1.00	29.54	5.26	1.61	1.78	3.44	2.51	2.80	2.69
LPC 18:1	1.00	38.90	6.70	1.92	2.11	4.01	4.69	6.36	5.76
LPC 18:0	1.00	5.43	2.95	1.50	1.55	2.60	5.48	9.00	7.07
LPC Total	1.00	16.86	4.14	1.83	1.83	2.96	4.79	6.14	5.76
PtCho 16:0/16:1	1.00	1.34	1.46	1.86	1.78	1.75	9.86	14.39	9.85
PtCho 16:0/22:6,18:1/22:5,18:2/20:4	1.00	1.30	1.06	0.69	0.65	0.70	2.21	3.43	2.82
PtCho 18:1/20:4,18:0/20:5,16:0/22:5	1.00	0.98	0.77	0.47	0.45	0.48	1.57	2.65	2.10
PtCho 18:0/20:4	1.00	1.38	1.12	0.94	0.87	0.96	4.07	6.14	5.09
PtCho 18:0/20:3	1.00	1.61	1.28	1.15	1.01	1.18	4.91	8.34	6.90
PtCho 18:1/22:6	1.00	1.88	1.51	1.07	0.92	1.06	3.38	5.16	3.64
PtCho 18:0/22:6,18:1/22:5	1.00	1.24	0.88	0.54	0.57	0.72	1.64	3.20	2.41
PtCho 18:0/22:5	1.00	1.00	0.64	0.25	0.31	0.35	0.81	1.83	1.47
PtCho 16:0/16:0	1.00	1.37	1.22	1.32	1.18	1.21	6.08	8.06	6.04
PtCho 16:0/18:2	1.00	1.87	1.62	1.79	1.50	1.68	7.09	8.58	6.60
PtCho 16:0/18:1	1.00	1.92	1.54	1.57	1.39	1.58	7.12	10.44	7.22
PtCho 16:0/20:5,16:1/20:4	1.00	1.19	0.98	0.72	0.61	0.60	3.25	4.86	3.54
PtCho 16:0/20:4	1.00	2.35	1.73	1.45	1.27	1.34	4.92	6.83	5.24
PtCho 16:0/20:3	1.00	2.62	2.01	1.73	1.44	1.62	5.48	7.36	5.55
PtCho 18:0/18:2,18:1/18:1	1.00	2.37	1.73	1.42	1.22	1.37	5.15	6.96	5.00
PtCho 18:0/18:1	1.00	2.43	1.75	1.41	1.23	1.51	5.49	7.57	6.13
PtCho Total	1.00	1.92	1.53	1.46	1.28	1.42	6.05	8.37	6.12
Pcho	1.00	0.91	0.70	0.14	0.05	0.04	0.02	0.03	0.03
SM	1.00	1.05	0.89	0.53	0.53	0.63	0.68	0.83	0.72
GPC	1.00	1.60	1.39	0.99	0.63	1.01	2.66	1.58	2.68

Table 27A: Cow #10 Average µM in Milk Used in Figure 7

	Average µM								
	Week of Lactation								
Lipid	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**
AcCho	0.00	0.00	0.00	0.08	0.08	0.09	0.03	0.01	0.01
Bet	80.70	52.83	44.23	17.17	17.72	25.60	55.63	68.79	69.73
Cho	60.26	84.33	92.17	120.47	150.37	158.80	157.98	151.18	171.23
LPC 16:0	0.98	1.04	0.76	0.65	0.61	0.92	1.49	1.91	1.81
LPC 18:2	0.29	0.27	0.27	0.26	0.17	0.16	0.29	0.26	0.29
LPC 18:1	1.05	0.83	0.85	0.46	0.30	0.28	0.56	0.65	0.76
LPC 18:0	0.39	0.36	0.31	0.23	0.24	0.35	0.67	0.46	0.45
LPC Total	2.71	2.49	2.19	1.60	1.32	1.71	3.02	3.27	3.32
PtCho 16:0/16:1	4.82	4.58	4.50	5.98	6.16	6.44	26.40	55.77	53.97
PtCho 16:0/22:6,18:1/22:5,18:2/20:4	0.57	0.46	0.57	0.53	0.42	0.43	1.35	2.14	1.80
PtCho 18:1/20:4,18:0/20:5,16:0/22:5	2.46	2.23	2.41	1.49	1.35	1.25	3.79	6.19	5.90
PtCho 18:0/20:4	1.43	1.17	1.39	1.42	1.42	1.18	3.72	5.61	6.05
PtCho 18:0/20:3	0.68	0.57	0.67	0.76	0.83	0.79	2.20	2.69	2.95
PtCho 18:1/22:6	0.19	0.20	0.20	0.18	0.15	0.16	0.58	0.77	0.54
PtCho 18:0/22:6,18:1/22:5	0.53	0.47	0.55	0.31	0.24	0.26	0.65	0.96	0.80
PtCho 18:0/22:5	0.64	0.60	0.57	0.28	0.23	0.21	0.55	0.75	0.63
PtCho 16:0/16:0	15.28	15.24	16.52	23.97	24.62	25.97	94.14	175.01	183.31
PtCho 16:0/18:2	16.51	12.70	14.52	18.97	17.97	18.24	80.74	116.15	112.74
PtCho 16:0/18:1	51.75	41.38	47.46	51.65	44.50	42.55	202.44	298.32	314.78
PtCho 16:0/20:5,16:1/20:4	0.52	0.37	0.43	0.41	0.38	0.37	1.23	2.00	2.39
PtCho 16:0/20:4	3.48	2.53	3.10	3.78	3.58	3.15	9.86	17.65	16.92
PtCho 16:0/20:3	13.61	10.76	13.08	14.34	12.73	10.33	36.60	51.67	50.87
PtCho 18:0/18:2,18:1/18:1	34.88	28.26	31.90	29.55	25.32	21.20	72.51	94.06	96.21
PtCho 18:0/18:1	18.91	15.29	16.86	15.49	14.28	12.97	41.09	49.23	50.09
PtCho Total	166.25	136.79	154.73	169.10	154.16	145.52	577.85	878.97	899.96
Pcho	819.63	690.67	637.94	240.00	139.79	131.56	22.84	12.43	38.10
SM	56.77	54.24	50.38	35.77	30.26	30.86	38.66	37.74	41.18
GPC	72.75	49.61	69.84	37.81	53.55	32.26	99.52	133.32	148.59

Average of triplicate injections

Table 27B: Cow #10 Fold Change in Milk Used in Figure 7

	Fold Change Relative to Week 1								
	Week of Lactation								
Lipid	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**
AcCho	0.00	0.00	0.00	1.00	1.02	1.10	0.37	0.14	0.08
Bet	1.00	0.65	0.55	0.21	0.22	0.32	0.69	0.85	0.86
Cho	1.00	1.40	1.53	2.00	2.50	2.64	2.62	2.51	2.84
LPC 16:0	1.00	1.07	0.78	0.67	0.63	0.94	1.53	1.96	1.86
LPC 18:2	1.00	0.91	0.93	0.90	0.58	0.55	1.00	0.88	1.00
LPC 18:1	1.00	0.79	0.80	0.44	0.29	0.26	0.53	0.61	0.72
LPC 18:0	1.00	0.92	0.79	0.58	0.61	0.90	1.72	1.18	1.16
LPC Total	1.00	0.92	0.81	0.59	0.49	0.63	1.11	1.21	1.23
PtCho 16:0/16:1	1.00	0.95	0.93	1.24	1.28	1.34	5.48	11.57	11.20
PtCho 16:0/22:6,18:1/22:5,18:2/20:4	1.00	0.81	1.01	0.93	0.75	0.76	2.39	3.78	3.19
PtCho 18:1/20:4,18:0/20:5,16:0/22:5	1.00	0.91	0.98	0.61	0.55	0.51	1.54	2.51	2.40
PtCho 18:0/20:4	1.00	0.82	0.97	0.99	0.99	0.83	2.60	3.92	4.23
PtCho 18:0/20:3	1.00	0.83	0.98	1.11	1.22	1.16	3.23	3.95	4.33
PtCho 18:1/22:6	1.00	1.06	1.09	0.98	0.80	0.84	3.09	4.12	2.91
PtCho 18:0/22:6,18:1/22:5	1.00	0.87	1.02	0.59	0.45	0.49	1.22	1.80	1.50
PtCho 18:0/22:5	1.00	0.95	0.90	0.43	0.36	0.33	0.86	1.17	0.99
PtCho 16:0/16:0	1.00	1.00	1.08	1.57	1.61	1.70	6.16	11.45	12.00
PtCho 16:0/18:2	1.00	0.77	0.88	1.15	1.09	1.11	4.89	7.04	6.83
PtCho 16:0/18:1	1.00	0.80	0.92	1.00	0.86	0.82	3.91	5.76	6.08
PtCho 16:0/20:5,16:1/20:4	1.00	0.72	0.83	0.80	0.73	0.71	2.38	3.88	4.62
PtCho 16:0/20:4	1.00	0.73	0.89	1.09	1.03	0.91	2.84	5.08	4.87
PtCho 16:0/20:3	1.00	0.79	0.96	1.05	0.94	0.76	2.69	3.80	3.74
PtCho 18:0/18:2,18:1/18:1	1.00	0.81	0.91	0.85	0.73	0.61	2.08	2.70	2.76
PtCho 18:0/18:1	1.00	0.81	0.89	0.82	0.76	0.69	2.17	2.60	2.65
PtCho Total	1.00	0.82	0.93	1.02	0.93	0.88	3.48	5.29	5.41
Pcho	1.00	0.84	0.78	0.29	0.17	0.16	0.03	0.02	0.05
SM	1.00	0.96	0.89	0.63	0.53	0.54	0.68	0.66	0.73
GPC	1.00	0.68	0.96	0.52	0.74	0.44	1.37	1.83	2.04

Table 28A: Cow #11 Average µM in Milk Used in Figure 7

	Average µM					
	Week of Lactation					
Lipid	**1**	**2**	**3**	**4**	**5**	**6**
AcCho	0.00	0.00	0.00	0.03	0.16	0.13
Bet	24.41	45.55	23.06	27.16	38.47	29.33
Cho	16.08	31.18	38.63	81.80	69.39	65.77
LPC 16:0	0.57	0.89	0.83	0.52	0.75	0.61
LPC 18:2	0.20	0.34	0.32	0.14	0.21	0.10
LPC 18:1	0.46	0.83	0.65	0.27	0.41	0.28
LPC 18:0	0.24	0.25	0.26	0.23	0.41	0.21
LPC Total	1.47	2.31	2.05	1.16	1.79	1.20
PtCho 16:0/16:1	6.97	4.86	8.97	7.16	8.30	5.90
PtCho 16:0/22:6,18:1/22:5,18:2/20:4	0.78	0.52	0.93	0.29	0.74	0.37
PtCho 18:1/20:4,18:0/20:5,16:0/22:5	3.74	2.36	3.50	1.08	2.50	1.30
PtCho 18:0/20:4	1.78	1.18	2.20	1.24	2.23	1.38
PtCho 18:0/20:3	0.73	0.51	0.97	0.73	1.33	0.93
PtCho 18:1/22:6	0.35	0.19	0.34	0.12	0.27	0.15
PtCho 18:0/22:6,18:1/22:5	0.80	0.47	0.75	0.21	0.58	0.26
PtCho 18:0/22:5	0.96	0.59	0.73	0.20	0.63	0.23
PtCho 16:0/16:0	22.91	16.19	31.85	24.77	30.85	21.04
PtCho 16:0/18:2	22.71	15.43	33.08	21.16	24.71	16.07
PtCho 16:0/18:1	67.07	45.84	93.62	50.99	57.84	36.92
PtCho 16:0/20:5,16:1/20:4	0.50	0.37	0.69	0.26	0.48	0.28
PtCho 16:0/20:4	4.94	3.32	7.09	3.19	4.29	2.74
PtCho 16:0/20:3	19.90	13.45	26.44	13.42	16.54	10.23
PtCho 18:0/18:2,18:1/18:1	46.03	30.47	58.29	29.32	34.17	21.24
PtCho 18:0/18:1	23.68	15.54	32.48	17.90	22.03	13.51
PtCho Total	223.85	151.28	301.91	172.04	207.49	132.53
Pcho	270.34	541.90	331.67	130.56	108.61	67.11
SM	53.08	40.37	49.96	29.45	34.68	24.16
GPC	29.37	121.96	95.78	43.25	73.01	51.85

Average of triplicate injections

Table 28B: Cow #11 Fold Change in Milk Used in Figure 7

	Fold Change Relative to Week 1					
	Week of Lactation					
Lipid	**1**	**2**	**3**	**4**	**5**	**6**
AcCho	0.00	0.00	0.00	1.00	4.69	3.90
Bet	1.00	1.87	0.94	1.11	1.58	1.20
Cho	1.00	1.94	2.40	5.09	4.31	4.09
LPC 16:0	1.00	1.57	1.47	0.91	1.33	1.07
LPC 18:2	1.00	1.70	1.58	0.72	1.06	0.52
LPC 18:1	1.00	1.80	1.40	0.59	0.90	0.60
LPC 18:0	1.00	1.04	1.07	0.96	1.72	0.89
LPC Total	1.00	1.57	1.40	0.79	1.22	0.82
PtCho 16:0/16:1	1.00	0.70	1.29	1.03	1.19	0.85
PtCho 16:0/22:6,18:1/22:5,18:2/20:4	1.00	0.67	1.20	0.37	0.94	0.47
PtCho 18:1/20:4,18:0/20:5,16:0/22:5	1.00	0.63	0.94	0.29	0.67	0.35
PtCho 18:0/20:4	1.00	0.66	1.23	0.70	1.25	0.77
PtCho 18:0/20:3	1.00	0.69	1.32	1.00	1.81	1.27
PtCho 18:1/22:6	1.00	0.55	0.96	0.35	0.76	0.44
PtCho 18:0/22:6,18:1/22:5	1.00	0.59	0.95	0.27	0.73	0.32
PtCho 18:0/22:5	1.00	0.61	0.76	0.21	0.66	0.24
PtCho 16:0/16:0	1.00	0.71	1.39	1.08	1.35	0.92
PtCho 16:0/18:2	1.00	0.68	1.46	0.93	1.09	0.71
PtCho 16:0/18:1	1.00	0.68	1.40	0.76	0.86	0.55
PtCho 16:0/20:5,16:1/20:4	1.00	0.74	1.36	0.51	0.96	0.56
PtCho 16:0/20:4	1.00	0.67	1.44	0.65	0.87	0.56
PtCho 16:0/20:3	1.00	0.68	1.33	0.67	0.83	0.51
PtCho 18:0/18:2,18:1/18:1	1.00	0.66	1.27	0.64	0.74	0.46
PtCho 18:0/18:1	1.00	0.66	1.37	0.76	0.93	0.57
PtCho Total	1.00	0.68	1.35	0.77	0.93	0.59
Pcho	1.00	2.00	1.23	0.48	0.40	0.25
SM	1.00	0.76	0.94	0.55	0.65	0.46
GPC	1.00	4.15	3.26	1.47	2.49	1.77

Table 29A: Cow #12 Average µM in Milk Used in Figure 7

	Average µM					
	Week of Lactation					
Lipid	**1**	**2**	**3**	**4**	**5**	**6**
AcCho	0.00	0.00	0.00	0.09	0.09	0.14
Bet	108.32	41.08	22.76	13.68	17.78	12.65
Cho	26.80	61.97	83.11	153.99	129.56	225.94
LPC 16:0	0.98	1.03	1.21	1.17	0.72	0.87
LPC 18:2	0.24	0.36	0.64	0.97	0.37	0.35
LPC 18:1	0.73	0.84	1.13	1.17	0.49	0.48
LPC 18:0	0.43	0.48	0.59	0.48	0.35	0.41
LPC Total	2.38	2.71	3.58	3.80	1.93	2.11
PtCho 16:0/16:1	5.50	6.36	8.95	9.90	7.84	9.33
PtCho 16:0/22:6,18:1/22:5,18:2/20:4	0.68	0.80	1.31	0.89	0.95	0.79
PtCho 18:1/20:4,18:0/20:5,16:0/22:5	2.66	2.69	4.58	2.35	2.89	2.25
PtCho 18:0/20:4	1.65	1.59	2.52	2.15	1.91	1.89
PtCho 18:0/20:3	0.85	0.74	1.25	1.25	1.08	1.18
PtCho 18:1/22:6	0.25	0.32	0.51	0.36	0.47	0.28
PtCho 18:0/22:6,18:1/22:5	0.57	0.72	1.05	0.48	0.98	0.40
PtCho 18:0/22:5	0.69	0.70	1.17	0.50	0.77	0.32
PtCho 16:0/16:0	20.31	21.57	30.80	34.12	31.99	30.79
PtCho 16:0/18:2	26.76	22.30	31.83	28.37	25.01	27.97
PtCho 16:0/18:1	71.59	63.77	84.30	63.70	68.83	55.96
PtCho 16:0/20:5,16:1/20:4	0.69	0.70	0.94	0.70	0.57	0.62
PtCho 16:0/20:4	5.46	6.02	8.59	6.95	4.70	6.21
PtCho 16:0/20:3	21.69	21.13	30.27	22.74	18.99	18.73
PtCho 18:0/18:2,18:1/18:1	51.27	47.02	61.88	42.92	39.48	34.76
PtCho 18:0/18:1	28.05	25.51	32.12	23.20	21.06	18.19
PtCho Total	238.66	221.94	302.07	240.57	227.55	209.67
Pcho	608.80	659.05	508.15	237.88	249.31	60.33
SM	58.88	52.94	52.18	37.32	37.55	29.75
GPC	132.65	112.53	112.19	64.48	36.59	39.29

Average of triplicate injections

Table 29B: Cow #12 Fold Change in Milk Used in Figure 7

	Fold Change Relative to Week 1					
	Week of Lactation					
Lipid	**1**	**2**	**3**	**4**	**5**	**6**
AcCho	0.00	0.00	0.00	1.00	1.02	1.60
Bet	1.00	0.38	0.21	0.13	0.16	0.12
Cho	1.00	2.31	3.10	5.75	4.84	8.43
LPC 16:0	1.00	1.04	1.23	1.19	0.73	0.89
LPC 18:2	1.00	1.53	2.71	4.10	1.56	1.46
LPC 18:1	1.00	1.15	1.56	1.61	0.68	0.66
LPC 18:0	1.00	1.11	1.36	1.11	0.82	0.95
LPC Total	1.00	1.14	1.50	1.59	0.81	0.89
PtCho 16:0/16:1	1.00	1.16	1.63	1.80	1.43	1.70
PtCho 16:0/22:6,18:1/22:5,18:2/20:4	1.00	1.17	1.93	1.31	1.40	1.16
PtCho 18:1/20:4,18:0/20:5,16:0/22:5	1.00	1.01	1.72	0.88	1.09	0.85
PtCho 18:0/20:4	1.00	0.97	1.52	1.30	1.15	1.14
PtCho 18:0/20:3	1.00	0.87	1.47	1.47	1.27	1.39
PtCho 18:1/22:6	1.00	1.26	2.05	1.44	1.89	1.10
PtCho 18:0/22:6,18:1/22:5	1.00	1.26	1.85	0.85	1.73	0.71
PtCho 18:0/22:5	1.00	1.01	1.70	0.72	1.12	0.46
PtCho 16:0/16:0	1.00	1.06	1.52	1.68	1.58	1.52
PtCho 16:0/18:2	1.00	0.83	1.19	1.06	0.93	1.05
PtCho 16:0/18:1	1.00	0.89	1.18	0.89	0.96	0.78
PtCho 16:0/20:5,16:1/20:4	1.00	1.01	1.35	1.02	0.83	0.90
PtCho 16:0/20:4	1.00	1.10	1.57	1.27	0.86	1.14
PtCho 16:0/20:3	1.00	0.97	1.40	1.05	0.88	0.86
PtCho 18:0/18:2,18:1/18:1	1.00	0.92	1.21	0.84	0.77	0.68
PtCho 18:0/18:1	1.00	0.91	1.14	0.83	0.75	0.65
PtCho Total	1.00	0.93	1.27	1.01	0.95	0.88
Pcho	1.00	1.08	0.83	0.39	0.41	0.10
SM	1.00	0.90	0.89	0.63	0.64	0.51
GPC	1.00	0.85	0.85	0.49	0.28	0.30

Table 30A: Cow #1 Average µM in Plasma Used in Figure 8

	Average µM								
	Week of Lactation								
Lipid	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**
Bet	22.07	16.05	13.10	12.81	14.08	15.85	26.86	23.76	36.14
Cho	2.23	3.79	4.48	3.82	4.05	2.26	4.15	4.29	3.31
LPC 16:0	22.50	26.13	59.41	55.59	118.14	45.91	32.67	19.03	31.15
LPC 18:2	58.39	73.36	154.15	181.13	443.43	154.22	109.94	67.67	128.84
LPC 18:1	26.58	32.97	70.39	57.20	135.33	47.85	43.30	25.47	43.11
LPC 18:0	22.95	28.85	65.68	69.29	163.18	56.85	59.57	35.50	67.89
LPC Total	130.42	161.31	349.63	363.21	860.07	304.83	245.48	147.68	270.99
PtCho 16:0/16:1	8.07	25.36	19.90	42.58	52.35	46.54	53.89	84.27	63.81
PtCho 16:0/22:6,18:1/22:5,18:2/20:4	6.82	25.85	16.67	34.05	41.66	36.48	47.96	63.46	52.00
PtCho 18:1/20:4,18:0/20:5,16:0/22:5	25.16	90.20	56.40	118.91	145.76	127.06	171.92	227.82	190.76
PtCho 18:0/20:4	41.40	198.49	115.87	309.20	384.10	334.33	496.30	705.37	649.73
PtCho 18:0/20:3	42.49	188.60	118.75	329.40	451.46	374.52	542.86	744.33	748.50
PtCho 18:1/22:6	0.95	4.82	2.69	6.63	9.41	8.33	9.46	10.87	9.15
PtCho 18:0/22:6,18:1/22:5	5.00	29.12	15.11	37.24	50.34	43.25	58.25	80.47	59.75
PtCho 18:0/22:5	11.47	62.93	34.37	76.82	99.02	86.08	112.14	163.88	141.58
PtCho 16:0/16:0	7.74	29.21	21.21	57.44	70.28	63.38	79.48	123.26	83.91
PtCho 16:0/18:2	287.97	760.42	635.38	1498.72	1661.95	1547.56	1624.76	2281.37	2222.20
PtCho 16:0/18:1	140.90	333.85	275.60	465.31	493.50	454.88	513.70	773.62	628.20
PtCho 16:0/20:5,16:1/20:4	9.30	23.99	19.40	43.00	53.47	46.19	57.29	86.13	78.41
PtCho 16:0/20:4	36.34	118.59	96.75	216.93	247.51	232.61	272.65	355.74	340.29
PtCho 16:0/20:3	98.67	239.71	199.92	471.69	573.83	554.80	511.45	682.19	744.72
PtCho 18:0/18:2,18:1/18:1	264.55	641.88	519.95	1481.34	1685.39	1560.55	1634.29	2303.34	2448.04
PtCho 18:0/18:1	110.92	275.84	216.19	468.75	508.12	469.03	533.38	859.75	723.73
PtCho Total	1097.75	3048.86	2364.15	5658.03	6528.15	5985.59	6719.79	9545.89	9184.78
Pcho	0.55	0.98	0.55	1.62	3.46	1.43	0.00	0.00	0.00
SM	244.63	365.87	381.13	621.85	762.34	604.09	416.23	472.39	332.02
GPC	2.75	0.81	6.95	2.92	14.15	1.40	2.72	4.36	3.97

Average of triplicate injections

Table 30B: Cow #1 Fold Change in Plasma Used in Figure 8

	Fold Change Relative to Week 1								
	Week of Lactation								
Lipid	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**
Bet	1.00	0.73	0.59	0.58	0.64	0.72	1.22	1.08	1.64
Cho	1.00	1.70	2.01	1.71	1.82	1.02	1.86	1.92	1.48
LPC 16:0	1.00	1.16	2.64	2.47	5.25	2.04	1.45	0.85	1.38
LPC 18:2	1.00	1.26	2.64	3.10	7.59	2.64	1.88	1.16	2.21
LPC 18:1	1.00	1.24	2.65	2.15	5.09	1.80	1.63	0.96	1.62
LPC 18:0	1.00	1.26	2.86	3.02	7.11	2.48	2.60	1.55	2.96
LPC Total	1.00	1.24	2.68	2.78	6.59	2.34	1.88	1.13	2.08
PtCho 16:0/16:1	1.00	3.14	2.46	5.27	6.48	5.76	6.68	10.44	7.90
PtCho 16:0/22:6,18:1/22:5,18:2/20:4	1.00	3.79	2.44	4.99	6.11	5.35	7.03	9.30	7.62
PtCho 18:1/20:4,18:0/20:5,16:0/22:5	1.00	3.58	2.24	4.73	5.79	5.05	6.83	9.05	7.58
PtCho 18:0/20:4	1.00	4.79	2.80	7.47	9.28	8.08	11.99	17.04	15.70
PtCho 18:0/20:3	1.00	4.44	2.79	7.75	10.63	8.82	12.78	17.52	17.62
PtCho 18:1/22:6	1.00	5.06	2.82	6.95	9.86	8.74	9.92	11.40	9.59
PtCho 18:0/22:6,18:1/22:5	1.00	5.82	3.02	7.45	10.07	8.65	11.65	16.09	11.95
PtCho 18:0/22:5	1.00	5.49	3.00	6.70	8.64	7.51	9.78	14.29	12.35
PtCho 16:0/16:0	1.00	3.78	2.74	7.43	9.08	8.19	10.27	15.93	10.85
PtCho 16:0/18:2	1.00	2.64	2.21	5.20	5.77	5.37	5.64	7.92	7.72
PtCho 16:0/18:1	1.00	2.37	1.96	3.30	3.50	3.23	3.65	5.49	4.46
PtCho 16:0/20:5,16:1/20:4	1.00	2.58	2.09	4.62	5.75	4.97	6.16	9.26	8.43
PtCho 16:0/20:4	1.00	3.26	2.66	5.97	6.81	6.40	7.50	9.79	9.36
PtCho 16:0/20:3	1.00	2.43	2.03	4.78	5.82	5.62	5.18	6.91	7.55
PtCho 18:0/18:2,18:1/18:1	1.00	2.43	1.97	5.60	6.37	5.90	6.18	8.71	9.25
PtCho 18:0/18:1	1.00	2.49	1.95	4.23	4.58	4.23	4.81	7.75	6.52
PtCho Total	1.00	2.78	2.15	5.15	5.95	5.45	6.12	8.70	8.37
Pcho	1.00	1.78	1.00	2.96	6.31	2.61	0.00	0.00	0.00
SM	1.00	1.50	1.56	2.54	3.12	2.47	1.70	1.93	1.36
GPC	1.00	0.29	2.53	1.06	5.14	0.51	0.99	1.59	1.44

Table 31A: Cow #2 Average µM in Plasma Used in Figure 8

	Average µM Week of Lactation								
Lipid	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**
Bet	19.38	14.63	11.88	50.55	28.00	16.37	20.72	23.13	34.50
Cho	2.73	2.31	1.96	4.51	3.32	2.04	3.42	2.49	3.25
LPC 16:0	8.68	14.36	29.89	37.90	41.39	41.99	34.01	54.65	32.81
LPC 18:2	21.50	30.50	73.75	137.74	141.20	130.89	122.18	211.26	119.06
LPC 18:1	11.71	13.63	32.55	40.33	40.67	46.18	45.42	76.35	41.79
LPC 18:0	8.91	12.96	30.05	51.31	57.30	46.89	61.91	94.01	67.07
LPC Total	50.81	71.46	166.24	267.28	280.57	265.95	263.52	436.27	260.73
PtCho 16:0/16:1	12.46	5.73	16.02	39.02	41.09	48.61	154.37	102.40	107.38
PtCho 16:0/22:6,18:1/22:5,18:2/20:4	12.84	6.91	16.98	29.35	28.21	32.32	116.43	72.00	62.88
PtCho 18:1/20:4,18:0/20:5,16:0/22:5	43.54	20.76	51.77	101.74	94.72	109.28	372.75	246.46	237.17
PtCho 18:0/20:4	72.40	29.00	88.52	264.06	248.39	248.06	928.54	643.87	723.09
PtCho 18:0/20:3	35.60	15.47	74.12	289.81	246.36	244.10	1081.53	875.27	911.22
PtCho 18:1/22:6	1.94	0.71	2.29	5.07	4.61	5.76	21.62	11.96	12.62
PtCho 18:0/22:6,18:1/22:5	11.83	5.43	14.51	33.76	31.52	34.61	148.76	79.93	86.06
PtCho 18:0/22:5	25.50	11.30	29.14	70.90	64.34	70.04	234.68	154.80	163.63
PtCho 16:0/16:0	12.30	5.50	14.04	46.98	55.70	49.52	167.23	94.52	120.00
PtCho 16:0/18:2	339.08	262.87	588.39	1260.77	1296.12	1343.99	3551.43	3221.07	2604.69
PtCho 16:0/18:1	202.51	135.95	266.93	375.37	392.69	470.10	1002.86	973.84	719.76
PtCho 16:0/20:5,16:1/20:4	12.11	6.41	16.32	37.38	34.32	38.39	130.71	95.39	90.83
PtCho 16:0/20:4	49.62	33.64	83.16	177.76	173.91	185.52	522.68	415.29	355.50
PtCho 16:0/20:3	113.94	70.06	171.70	393.48	363.88	423.84	1029.59	984.61	833.99
PtCho 18:0/18:2,18:1/18:1	361.99	222.92	493.47	1437.87	1472.68	1327.49	3376.44	3309.32	2740.90
PtCho 18:0/18:1	159.98	89.45	197.36	450.47	463.64	455.94	1034.83	1010.11	831.95
PtCho Total	1467.65	922.10	2124.71	5013.77	5012.18	5087.58	13874.47	12290.83	10601.67
Pcho	0.97	0.00	0.30	1.16	0.50	0.36	0.00	0.00	0.00
SM	233.57	166.75	338.97	579.76	576.95	655.86	482.30	412.51	453.20
GPC	0.39	0.03	0.20	1.12	1.21	0.99	2.01	6.96	2.66

Average of triplicate injections

Table 31B: Cow #2 Fold Change in Plasma Used in Figure 8

	Fold Change Relative to Week 1								
	Week of Lactation								
Lipid	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**
Bet	1.00	0.76	0.61	2.61	1.44	0.84	1.07	1.19	1.78
Cho	1.00	0.85	0.72	1.65	1.22	0.75	1.25	0.91	1.19
LPC 16:0	1.00	1.65	3.44	4.36	4.77	4.84	3.92	6.29	3.78
LPC 18:2	1.00	1.42	3.43	6.41	6.57	6.09	5.68	9.82	5.54
LPC 18:1	1.00	1.16	2.78	3.44	3.47	3.94	3.88	6.52	3.57
LPC 18:0	1.00	1.46	3.37	5.76	6.43	5.26	6.95	10.55	7.53
LPC Total	1.00	1.41	3.27	5.26	5.52	5.23	5.19	8.59	5.13
PtCho 16:0/16:1	1.00	0.46	1.29	3.13	3.30	3.90	12.39	8.22	8.62
PtCho 16:0/22:6,18:1/22:5,18:2/20:4	1.00	0.54	1.32	2.28	2.20	2.52	9.06	5.61	4.90
PtCho 18:1/20:4,18:0/20:5,16:0/22:5	1.00	0.48	1.19	2.34	2.18	2.51	8.56	5.66	5.45
PtCho 18:0/20:4	1.00	0.40	1.22	3.65	3.43	3.43	12.82	8.89	9.99
PtCho 18:0/20:3	1.00	0.43	2.08	8.14	6.92	6.86	30.38	24.59	25.60
PtCho 18:1/22:6	1.00	0.37	1.18	2.62	2.38	2.97	11.16	6.17	6.51
PtCho 18:0/22:6,18:1/22:5	1.00	0.46	1.23	2.85	2.66	2.93	12.57	6.76	7.27
PtCho 18:0/22:5	1.00	0.44	1.14	2.78	2.52	2.75	9.20	6.07	6.42
PtCho 16:0/16:0	1.00	0.45	1.14	3.82	4.53	4.03	13.60	7.68	9.76
PtCho 16:0/18:2	1.00	0.78	1.74	3.72	3.82	3.96	10.47	9.50	7.68
PtCho 16:0/18:1	1.00	0.67	1.32	1.85	1.94	2.32	4.95	4.81	3.55
PtCho 16:0/20:5,16:1/20:4	1.00	0.53	1.35	3.09	2.83	3.17	10.79	7.88	7.50
PtCho 16:0/20:4	1.00	0.68	1.68	3.58	3.50	3.74	10.53	8.37	7.16
PtCho 16:0/20:3	1.00	0.61	1.51	3.45	3.19	3.72	9.04	8.64	7.32
PtCho 18:0/18:2,18:1/18:1	1.00	0.62	1.36	3.97	4.07	3.67	9.33	9.14	7.57
PtCho 18:0/18:1	1.00	0.56	1.23	2.82	2.90	2.85	6.47	6.31	5.20
PtCho Total	1.00	0.63	1.45	3.42	3.42	3.47	9.45	8.37	7.22
Pcho	1.00	0.00	0.31	1.20	0.51	0.37	0.00	0.00	0.00
SM	1.00	0.71	1.45	2.48	2.47	2.81	2.06	1.77	1.94
GPC	1.00	0.08	0.52	2.86	3.10	2.55	5.14	17.82	6.83

Table 32A: Cow #3 Average µM in Plasma Used in Figure 8

	Average µM Week of Lactation								
Lipid	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**
Bet	23.34	22.85	18.19	11.84	13.15	13.62	37.03	33.98	37.18
Cho	3.90	5.60	6.54	2.40	5.98	3.82	5.96	2.43	1.78
LPC 16:0	14.99	27.70	24.70	32.00	27.16	36.32	31.56	30.57	24.71
LPC 18:2	27.29	62.90	62.89	98.28	63.69	112.46	116.31	106.35	67.59
LPC 18:1	15.81	34.41	34.26	39.35	31.42	43.34	57.02	51.13	30.99
LPC 18:0	14.06	30.21	29.59	40.57	50.30	66.25	68.79	66.36	42.05
LPC Total	72.15	155.23	151.45	210.20	172.57	258.38	273.68	254.41	165.33
PtCho 16:0/16:1	4.51	9.39	15.68	48.16	48.80	82.93	156.44	214.19	199.66
PtCho 16:0/22:6,18:1/22:5,18:2/20:4	3.52	8.83	10.09	38.22	32.03	70.44	70.19	93.31	87.68
PtCho 18:1/20:4,18:0/20:5,16:0/22:5	10.75	26.86	38.42	144.89	105.32	248.20	344.98	462.42	417.69
PtCho 18:0/20:4	17.35	46.71	78.90	372.85	240.31	539.36	992.71	1322.74	1282.06
PtCho 18:0/20:3	11.91	37.67	75.46	350.54	258.19	695.11	1285.81	1779.35	1679.89
PtCho 18:1/22:6	0.42	1.62	1.54	7.55	4.81	12.85	13.29	19.37	15.39
PtCho 18:0/22:6,18:1/22:5	3.44	8.92	9.60	48.25	26.10	68.31	53.69	79.69	64.13
PtCho 18:0/22:5	6.37	15.84	21.46	111.50	51.07	136.55	278.38	347.23	321.21
PtCho 16:0/16:0	4.19	12.20	13.26	52.75	51.46	83.06	163.41	239.15	203.18
PtCho 16:0/18:2	167.26	326.83	492.80	1368.46	1630.42	3153.70	3252.94	4067.13	3951.00
PtCho 16:0/18:1	97.53	179.10	253.80	529.97	514.83	928.50	1170.13	1413.01	1362.69
PtCho 16:0/20:5,16:1/20:4	3.15	6.88	11.20	35.93	39.07	83.98	139.69	189.06	165.34
PtCho 16:0/20:4	18.83	44.05	69.44	201.97	225.86	462.98	501.41	672.73	638.59
PtCho 16:0/20:3	55.76	115.13	183.80	455.97	469.72	1093.04	1262.48	1558.98	1489.91
PtCho 18:0/18:2,18:1/18:1	168.41	358.56	516.78	1394.79	1585.59	3465.67	3636.49	4565.53	4479.44
PtCho 18:0/18:1	80.02	170.12	238.96	561.57	491.87	1021.48	1446.54	1690.68	1658.13
PtCho Total	653.43	1368.71	2031.19	5723.37	5775.46	12146.17	14768.56	18714.55	18015.99
Pcho	0.13	0.92	0.16	1.11	3.31	1.88	0.00	0.00	0.00
SM	110.91	230.09	265.90	654.36	502.72	527.82	412.52	424.40	497.76
GPC	0.15	0.32	0.13	3.07	1.55	5.06	4.05	1.89	0.42

Average of triplicate injections

Table 32B: Cow #3 Fold Change in Plasma Used in Figure 8

	Fold Change Relative to Week 1								
	Week of Lactation								
Lipid	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**
Bet	1.00	0.98	0.78	0.51	0.56	0.58	1.59	1.46	1.59
Cho	1.00	1.44	1.68	0.62	1.53	0.98	1.53	0.62	0.46
LPC 16:0	1.00	1.85	1.65	2.13	1.81	2.42	2.11	2.04	1.65
LPC 18:2	1.00	2.30	2.30	3.60	2.33	4.12	4.26	3.90	2.48
LPC 18:1	1.00	2.18	2.17	2.49	1.99	2.74	3.61	3.23	1.96
LPC 18:0	1.00	2.15	2.10	2.89	3.58	4.71	4.89	4.72	2.99
LPC Total	1.00	2.15	2.10	2.91	2.39	3.58	3.79	3.53	2.29
PtCho 16:0/16:1	1.00	2.08	3.48	10.69	10.83	18.40	34.71	47.52	44.30
PtCho 16:0/22:6,18:1/22:5,18:2/20:4	1.00	2.51	2.87	10.87	9.11	20.04	19.97	26.54	24.94
PtCho 18:1/20:4,18:0/20:5,16:0/22:5	1.00	2.50	3.57	13.47	9.79	23.08	32.08	43.00	38.84
PtCho 18:0/20:4	1.00	2.69	4.55	21.49	13.85	31.09	57.22	76.24	73.89
PtCho 18:0/20:3	1.00	3.16	6.34	29.43	21.68	58.36	107.95	149.39	141.03
PtCho 18:1/22:6	1.00	3.86	3.68	18.02	11.47	30.67	31.72	46.21	36.73
PtCho 18:0/22:6,18:1/22:5	1.00	2.59	2.79	14.04	7.60	19.88	15.62	23.19	18.66
PtCho 18:0/22:5	1.00	2.49	3.37	17.49	8.01	21.42	43.68	54.48	50.40
PtCho 16:0/16:0	1.00	2.91	3.16	12.58	12.27	19.80	38.96	57.02	48.44
PtCho 16:0/18:2	1.00	1.95	2.95	8.18	9.75	18.86	19.45	24.32	23.62
PtCho 16:0/18:1	1.00	1.84	2.60	5.43	5.28	9.52	12.00	14.49	13.97
PtCho 16:0/20:5,16:1/20:4	1.00	2.18	3.55	11.39	12.39	26.63	44.29	59.94	52.42
PtCho 16:0/20:4	1.00	2.34	3.69	10.73	11.99	24.59	26.63	35.73	33.91
PtCho 16:0/20:3	1.00	2.06	3.30	8.18	8.42	19.60	22.64	27.96	26.72
PtCho 18:0/18:2,18:1/18:1	1.00	2.13	3.07	8.28	9.41	20.58	21.59	27.11	26.60
PtCho 18:0/18:1	1.00	2.13	2.99	7.02	6.15	12.77	18.08	21.13	20.72
PtCho Total	1.00	2.09	3.11	8.76	8.84	18.59	22.60	28.64	27.57
Pcho	1.00	7.24	1.27	8.76	26.13	14.85	0.00	0.00	0.00
SM	1.00	2.07	2.40	5.90	4.53	4.76	3.72	3.83	4.49
GPC	1.00	2.19	0.92	20.98	10.59	34.57	27.68	12.93	2.88

Table 33A: Cow #4 Average µM in Plasma Used in Figure 8

	Average µM Week of Lactation								
Lipid	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**
Bet	25.77	14.06	7.66	18.21	8.75	17.62	17.13	24.05	45.59
Cho	2.70	4.62	2.56	5.59	2.40	4.93	2.80	2.21	4.86
LPC 16:0	18.31	19.63	22.80	103.88	32.53	52.57	37.06	30.31	33.51
LPC 18:2	35.55	42.48	55.18	430.04	117.63	187.10	141.65	128.21	125.77
LPC 18:1	18.22	17.13	23.16	127.03	36.32	51.55	47.25	44.35	45.33
LPC 18:0	14.93	20.15	24.95	176.56	44.08	70.21	70.65	73.81	77.10
LPC Total	87.01	99.39	126.08	837.50	230.56	361.43	296.61	276.68	281.71
PtCho 16:0/16:1	11.88	7.13	8.34	38.20	33.03	26.65	107.91	72.93	140.85
PtCho 16:0/22:6,18:1/22:5,18:2/20:4	11.23	6.56	7.04	28.26	28.02	23.17	99.40	56.66	81.37
PtCho 18:1/20:4,18:0/20:5,16:0/22:5	37.27	21.01	22.03	100.96	95.50	79.08	336.00	216.56	336.50
PtCho 18:0/20:4	62.94	34.01	38.50	273.37	231.23	212.52	835.45	627.04	1044.66
PtCho 18:0/20:3	41.49	29.28	36.68	271.61	251.20	211.00	949.90	748.24	1194.03
PtCho 18:1/22:6	1.76	1.06	1.13	5.63	5.89	4.40	23.07	11.93	17.68
PtCho 18:0/22:6,18:1/22:5	12.55	6.79	7.29	32.91	31.42	24.40	133.06	71.59	88.42
PtCho 18:0/22:5	28.17	14.65	14.86	70.77	67.76	55.57	212.36	138.92	248.97
PtCho 16:0/16:0	16.32	8.69	9.06	60.66	39.63	39.83	152.51	99.82	198.88
PtCho 16:0/18:2	322.56	295.23	330.87	1182.17	1210.42	1086.51	3184.13	2137.23	3068.55
PtCho 16:0/18:1	174.55	125.02	143.10	363.85	359.65	311.94	786.07	561.57	891.46
PtCho 16:0/20:5,16:1/20:4	10.97	7.02	7.15	38.41	35.88	31.77	121.87	79.53	143.49
PtCho 16:0/20:4	45.44	38.55	42.17	183.59	189.78	166.26	547.35	344.96	527.54
PtCho 16:0/20:3	95.59	101.99	116.90	393.80	455.32	350.40	935.02	786.77	1123.92
PtCho 18:0/18:2,18:1/18:1	277.34	334.00	378.94	1474.00	1449.71	1214.74	3202.85	2513.75	3442.45
PtCho 18:0/18:1	132.38	128.07	149.37	452.07	436.65	360.99	966.23	730.88	1072.56
PtCho Total	1282.44	1159.05	1313.44	4970.28	4921.10	4199.23	12593.20	9198.38	13621.31
Pcho	1.11	0.00	0.20	2.26	0.33	0.94	0.00	0.00	0.00
SM	229.58	220.63	209.67	486.74	358.45	438.08	375.47	309.35	415.02
GPC	1.38	0.04	0.39	14.50	0.56	18.67	3.26	4.44	2.58

Average of triplicate injections

Table 33B: Cow #4 Fold Change in Plasma Used in Figure 8

	Fold Change Relative to Week 1								
	Week of Lactation								
Lipid	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**
Bet	1.00	0.55	0.30	0.71	0.34	0.68	0.66	0.93	1.77
Cho	1.00	1.71	0.95	2.07	0.89	1.83	1.04	0.82	1.80
LPC 16:0	1.00	1.07	1.24	5.67	1.78	2.87	2.02	1.65	1.83
LPC 18:2	1.00	1.19	1.55	12.10	3.31	5.26	3.98	3.61	3.54
LPC 18:1	1.00	0.94	1.27	6.97	1.99	2.83	2.59	2.43	2.49
LPC 18:0	1.00	1.35	1.67	11.83	2.95	4.70	4.73	4.94	5.16
LPC Total	1.00	1.14	1.45	9.62	2.65	4.15	3.41	3.18	3.24
PtCho 16:0/16:1	1.00	0.60	0.70	3.22	2.78	2.24	9.09	6.14	11.86
PtCho 16:0/22:6,18:1/22:5,18:2/20:4	1.00	0.58	0.63	2.52	2.49	2.06	8.85	5.04	7.24
PtCho 18:1/20:4,18:0/20:5,16:0/22:5	1.00	0.56	0.59	2.71	2.56	2.12	9.01	5.81	9.03
PtCho 18:0/20:4	1.00	0.54	0.61	4.34	3.67	3.38	13.27	9.96	16.60
PtCho 18:0/20:3	1.00	0.71	0.88	6.55	6.05	5.09	22.90	18.04	28.78
PtCho 18:1/22:6	1.00	0.60	0.64	3.19	3.34	2.49	13.08	6.76	10.02
PtCho 18:0/22:6,18:1/22:5	1.00	0.54	0.58	2.62	2.50	1.95	10.61	5.71	7.05
PtCho 18:0/22:5	1.00	0.52	0.53	2.51	2.40	1.97	7.54	4.93	8.84
PtCho 16:0/16:0	1.00	0.53	0.56	3.72	2.43	2.44	9.35	6.12	12.19
PtCho 16:0/18:2	1.00	0.92	1.03	3.66	3.75	3.37	9.87	6.63	9.51
PtCho 16:0/18:1	1.00	0.72	0.82	2.08	2.06	1.79	4.50	3.22	5.11
PtCho 16:0/20:5,16:1/20:4	1.00	0.64	0.65	3.50	3.27	2.90	11.11	7.25	13.08
PtCho 16:0/20:4	1.00	0.85	0.93	4.04	4.18	3.66	12.04	7.59	11.61
PtCho 16:0/20:3	1.00	1.07	1.22	4.12	4.76	3.67	9.78	8.23	11.76
PtCho 18:0/18:2,18:1/18:1	1.00	1.20	1.37	5.31	5.23	4.38	11.55	9.06	12.41
PtCho 18:0/18:1	1.00	0.97	1.13	3.41	3.30	2.73	7.30	5.52	8.10
PtCho Total	1.00	0.90	1.02	3.88	3.84	3.27	9.82	7.17	10.62
Pcho	1.00	0.00	0.18	2.04	0.29	0.84	0.00	0.00	0.00
SM	1.00	0.96	0.91	2.12	1.56	1.91	1.64	1.35	1.81
GPC	1.00	0.03	0.28	10.51	0.41	13.53	2.37	3.22	1.87

Table 34A: Cow #5 Average µM in Plasma Used in Figure 8

	Average µM Week of Lactation								
Lipid	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**
Bet	19.56	11.83	10.85	10.99	13.24	25.86	28.19	30.84	40.75
Cho	2.94	3.44	5.21	4.28	3.12	3.92	2.17	3.82	2.85
LPC 16:0	20.12	20.16	62.94	67.80	39.81	63.33	29.03	28.30	21.64
LPC 18:2	43.47	56.81	188.35	236.58	135.13	250.91	115.28	116.64	90.54
LPC 18:1	25.45	27.87	92.75	81.61	45.46	78.61	45.58	47.91	38.38
LPC 18:0	21.09	24.73	82.70	91.15	52.88	98.63	56.15	71.29	50.79
LPC Total	110.13	129.57	426.74	477.15	273.29	491.48	246.04	264.13	201.36
PtCho 16:0/16:1	8.12	9.84	16.34	48.25	47.56	57.61	105.96	114.09	74.89
PtCho 16:0/22:6,18:1/22:5,18:2/20:4	4.80	7.44	12.25	34.66	33.99	40.12	87.42	74.84	44.19
PtCho 18:1/20:4,18:0/20:5,16:0/22:5	18.44	27.43	45.16	118.47	115.61	137.12	276.99	255.31	175.39
PtCho 18:0/20:4	24.74	43.61	77.43	274.93	263.26	349.06	663.22	712.72	509.52
PtCho 18:0/20:3	18.12	44.97	72.95	297.44	265.09	383.97	732.53	855.92	681.12
PtCho 18:1/22:6	0.81	1.37	2.12	7.22	7.27	9.33	18.24	14.02	8.67
PtCho 18:0/22:6,18:1/22:5	4.42	7.32	12.23	44.08	40.90	49.86	105.47	106.84	49.14
PtCho 18:0/22:5	12.64	18.22	33.37	102.64	92.09	109.39	176.37	190.98	123.01
PtCho 16:0/16:0	6.68	10.16	16.81	56.46	53.91	65.46	112.99	128.98	81.05
PtCho 16:0/18:2	217.64	316.93	548.54	1545.16	1688.11	1833.97	2991.79	2793.08	2014.05
PtCho 16:0/18:1	124.33	163.48	259.76	501.91	514.54	554.01	933.52	890.86	678.33
PtCho 16:0/20:5,16:1/20:4	7.05	8.30	13.92	40.01	40.02	49.77	89.45	93.39	68.22
PtCho 16:0/20:4	24.49	38.01	69.09	200.44	223.39	262.27	431.75	386.49	276.46
PtCho 16:0/20:3	79.01	120.70	180.81	512.43	617.72	689.50	960.46	1011.76	720.14
PtCho 18:0/18:2,18:1/18:1	206.53	367.35	569.20	1637.87	1908.31	2260.48	3072.45	3530.87	2424.81
PtCho 18:0/18:1	99.20	160.49	254.93	565.02	609.78	696.90	1112.18	1252.63	883.02
PtCho Total	857.03	1345.62	2184.90	5987.00	6521.56	7548.82	11870.79	12412.79	8812.01
Pcho	0.11	0.66	0.97	2.76	0.58	1.66	0.00	0.00	0.00
SM	145.25	229.58	298.58	723.43	675.67	746.99	446.12	356.67	290.70
GPC	1.76	1.10	5.93	34.39	1.02	22.52	1.77	2.98	2.63

Average of triplicate injections

Table 34B: Cow #5 Fold Change in Plasma Used in Figure 8

	Fold Change Relative to Week 1								
	Week of Lactation								
Lipid	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**
Bet	1.00	0.60	0.55	0.56	0.68	1.32	1.44	1.58	2.08
Cho	1.00	1.17	1.77	1.46	1.06	1.33	0.74	1.30	0.97
LPC 16:0	1.00	1.00	3.13	3.37	1.98	3.15	1.44	1.41	1.08
LPC 18:2	1.00	1.31	4.33	5.44	3.11	5.77	2.65	2.68	2.08
LPC 18:1	1.00	1.10	3.64	3.21	1.79	3.09	1.79	1.88	1.51
LPC 18:0	1.00	1.17	3.92	4.32	2.51	4.68	2.66	3.38	2.41
LPC Total	1.00	1.18	3.87	4.33	2.48	4.46	2.23	2.40	1.83
PtCho 16:0/16:1	1.00	1.21	2.01	5.94	5.86	7.10	13.05	14.05	9.22
PtCho 16:0/22:6,18:1/22:5,18:2/20:4	1.00	1.55	2.55	7.23	7.09	8.36	18.23	15.60	9.21
PtCho 18:1/20:4,18:0/20:5,16:0/22:5	1.00	1.49	2.45	6.42	6.27	7.43	15.02	13.84	9.51
PtCho 18:0/20:4	1.00	1.76	3.13	11.11	10.64	14.11	26.80	28.81	20.59
PtCho 18:0/20:3	1.00	2.48	4.02	16.41	14.63	21.18	40.42	47.22	37.58
PtCho 18:1/22:6	1.00	1.70	2.63	8.95	9.02	11.57	22.62	17.38	10.75
PtCho 18:0/22:6,18:1/22:5	1.00	1.66	2.77	9.98	9.26	11.28	23.87	24.18	11.12
PtCho 18:0/22:5	1.00	1.44	2.64	8.12	7.29	8.65	13.95	15.11	9.73
PtCho 16:0/16:0	1.00	1.52	2.52	8.45	8.07	9.80	16.92	19.31	12.13
PtCho 16:0/18:2	1.00	1.46	2.52	7.10	7.76	8.43	13.75	12.83	9.25
PtCho 16:0/18:1	1.00	1.31	2.09	4.04	4.14	4.46	7.51	7.17	5.46
PtCho 16:0/20:5,16:1/20:4	1.00	1.18	1.97	5.67	5.67	7.06	12.68	13.24	9.67
PtCho 16:0/20:4	1.00	1.55	2.82	8.19	9.12	10.71	17.63	15.78	11.29
PtCho 16:0/20:3	1.00	1.53	2.29	6.49	7.82	8.73	12.16	12.81	9.11
PtCho 18:0/18:2,18:1/18:1	1.00	1.78	2.76	7.93	9.24	10.95	14.88	17.10	11.74
PtCho 18:0/18:1	1.00	1.62	2.57	5.70	6.15	7.03	11.21	12.63	8.90
PtCho Total	1.00	1.57	2.55	6.99	7.61	8.81	13.85	14.48	10.28
Pcho	1.00	5.82	8.56	24.41	5.11	14.71	0.00	0.00	0.00
SM	1.00	1.58	2.06	4.98	4.65	5.14	3.07	2.46	2.00
GPC	1.00	0.62	3.36	19.52	0.58	12.78	1.00	1.69	1.49

Table 35A: Cow #6 Average µM in Plasma Used in Figure 8

	Average µM Week of Lactation								
Lipid	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**
Bet	35.77	19.60	11.92	14.61	14.20	12.91	28.70	45.88	84.16
Cho	3.40	2.91	4.45	2.31	4.68	3.28	2.38	2.57	3.61
LPC 16:0	22.07	19.30	25.82	45.81	20.99	50.87	34.14	30.15	20.46
LPC 18:2	49.33	44.69	69.64	161.90	72.16	195.21	136.98	116.21	40.88
LPC 18:1	20.40	23.30	30.67	46.78	21.67	53.72	41.83	35.34	18.44
LPC 18:0	17.23	20.50	30.83	56.80	19.66	72.01	72.68	61.50	27.83
LPC Total	109.03	107.78	156.97	311.29	134.47	371.82	285.64	243.21	107.59
PtCho 16:0/16:1	5.06	6.02	8.97	29.63	31.65	25.92	64.00	103.71	44.76
PtCho 16:0/22:6,18:1/22:5,18:2/20:4	4.16	6.01	8.89	26.79	31.40	22.39	54.38	80.66	42.86
PtCho 18:1/20:4,18:0/20:5,16:0/22:5	12.15	17.83	27.99	89.45	99.82	78.05	184.02	290.50	162.88
PtCho 18:0/20:4	19.08	32.73	58.57	224.75	232.93	206.61	525.90	899.39	488.87
PtCho 18:0/20:3	12.76	25.27	63.08	232.45	228.51	239.75	622.96	1026.29	456.57
PtCho 18:1/22:6	0.63	1.03	1.45	4.96	5.54	4.39	12.64	15.99	7.05
PtCho 18:0/22:6,18:1/22:5	3.28	5.81	8.72	31.30	37.64	25.08	95.50	106.37	61.37
PtCho 18:0/22:5	6.15	11.07	16.46	59.92	74.29	49.96	145.77	225.94	134.02
PtCho 16:0/16:0	6.36	7.22	9.80	33.22	37.66	32.19	90.04	143.37	59.40
PtCho 16:0/18:2	260.15	257.86	413.56	1184.50	1246.03	1093.28	1936.70	3045.52	1013.72
PtCho 16:0/18:1	115.27	134.00	181.81	351.27	416.09	311.25	501.07	807.91	408.38
PtCho 16:0/20:5,16:1/20:4	4.33	4.83	8.52	29.27	28.39	26.02	60.56	104.16	56.92
PtCho 16:0/20:4	25.88	30.61	53.77	162.63	167.81	151.97	280.75	444.36	233.52
PtCho 16:0/20:3	75.71	87.35	140.53	392.62	374.98	395.63	642.40	1063.12	361.95
PtCho 18:0/18:2,18:1/18:1	200.38	255.46	435.45	1256.31	1072.98	1288.50	2187.35	3447.39	890.15
PtCho 18:0/18:1	72.64	115.17	174.59	369.24	370.85	360.07	591.93	931.74	324.54
PtCho Total	824.00	998.27	1612.16	4478.34	4456.55	4311.07	7995.95	12736.42	4746.96
Pcho	0.50	0.09	0.54	0.55	0.70	0.98	0.00	0.00	0.00
SM	162.53	166.13	253.82	581.60	609.13	531.58	366.15	303.72	325.30
GPC	2.62	1.37	1.98	17.87	0.37	12.16	5.95	4.34	0.49

Average of triplicate injections

Table 35B: Cow #6 Fold Change in Plasma Used in Figure 8

	Fold Change Relative to Week 1								
	Week of Lactation								
Lipid	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**
Bet	1.00	0.55	0.33	0.41	0.40	0.36	0.80	1.28	2.35
Cho	1.00	0.86	1.31	0.68	1.38	0.96	0.70	0.76	1.06
LPC 16:0	1.00	0.87	1.17	2.08	0.95	2.31	1.55	1.37	0.93
LPC 18:2	1.00	0.91	1.41	3.28	1.46	3.96	2.78	2.36	0.83
LPC 18:1	1.00	1.14	1.50	2.29	1.06	2.63	2.05	1.73	0.90
LPC 18:0	1.00	1.19	1.79	3.30	1.14	4.18	4.22	3.57	1.61
LPC Total	1.00	0.99	1.44	2.86	1.23	3.41	2.62	2.23	0.99
PtCho 16:0/16:1	1.00	1.19	1.77	5.85	6.25	5.12	12.65	20.49	8.84
PtCho 16:0/22:6,18:1/22:5,18:2/20:4	1.00	1.44	2.14	6.44	7.54	5.38	13.06	19.38	10.30
PtCho 18:1/20:4,18:0/20:5,16:0/22:5	1.00	1.47	2.30	7.36	8.22	6.42	15.15	23.91	13.41
PtCho 18:0/20:4	1.00	1.72	3.07	11.78	12.21	10.83	27.57	47.15	25.63
PtCho 18:0/20:3	1.00	1.98	4.94	18.21	17.90	18.79	48.81	80.41	35.77
PtCho 18:1/22:6	1.00	1.63	2.29	7.84	8.75	6.94	19.97	25.26	11.13
PtCho 18:0/22:6,18:1/22:5	1.00	1.77	2.66	9.55	11.48	7.65	29.13	32.45	18.72
PtCho 18:0/22:5	1.00	1.80	2.68	9.75	12.08	8.13	23.71	36.75	21.80
PtCho 16:0/16:0	1.00	1.13	1.54	5.22	5.92	5.06	14.15	22.53	9.33
PtCho 16:0/18:2	1.00	0.99	1.59	4.55	4.79	4.20	7.44	11.71	3.90
PtCho 16:0/18:1	1.00	1.16	1.58	3.05	3.61	2.70	4.35	7.01	3.54
PtCho 16:0/20:5,16:1/20:4	1.00	1.11	1.97	6.76	6.55	6.01	13.98	24.04	13.14
PtCho 16:0/20:4	1.00	1.18	2.08	6.28	6.48	5.87	10.85	17.17	9.02
PtCho 16:0/20:3	1.00	1.15	1.86	5.19	4.95	5.23	8.48	14.04	4.78
PtCho 18:0/18:2,18:1/18:1	1.00	1.27	2.17	6.27	5.35	6.43	10.92	17.20	4.44
PtCho 18:0/18:1	1.00	1.59	2.40	5.08	5.11	4.96	8.15	12.83	4.47
PtCho Total	1.00	1.21	1.96	5.43	5.41	5.23	9.70	15.46	5.76
Pcho	1.00	0.17	1.07	1.10	1.41	1.96	0.00	0.00	0.00
SM	1.00	1.02	1.56	3.58	3.75	3.27	2.25	1.87	2.00
GPC	1.00	0.52	0.76	6.82	0.14	4.64	2.27	1.66	0.19

Table 36A: Cow #7 Average µM in Plasma Used in Figure 8

	Average µM Week of Lactation								
Lipid	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**
Bet	18.85	16.83	13.41	16.41	19.49	14.42	42.20	47.13	56.08
Cho	4.70	5.78	4.76	2.78	4.25	4.04	3.64	3.02	3.44
LPC 16:0	21.41	29.87	41.02	57.73	38.39	38.97	56.79	37.72	28.03
LPC 18:2	48.75	78.36	97.31	142.84	100.53	94.60	224.26	137.14	74.16
LPC 18:1	24.76	33.78	39.53	54.87	42.90	45.22	108.10	57.48	33.51
LPC 18:0	23.95	32.69	42.98	55.99	49.71	73.52	143.32	88.44	51.49
LPC Total	118.87	174.71	220.85	311.44	231.52	252.32	532.47	320.78	187.18
PtCho 16:0/16:1	7.72	9.88	9.83	38.80	52.78	160.50	239.58	115.20	192.18
PtCho 16:0/22:6,18:1/22:5,18:2/20:4	6.91	10.28	10.42	46.53	41.77	132.93	114.93	55.40	95.50
PtCho 18:1/20:4,18:0/20:5,16:0/22:5	25.95	36.43	38.94	153.22	123.83	446.23	589.16	293.24	490.30
PtCho 18:0/20:4	54.22	83.37	86.15	396.80	255.22	1020.56	1691.26	879.35	1476.86
PtCho 18:0/20:3	29.67	47.32	43.69	259.15	235.15	1129.22	2172.36	1174.61	1907.81
PtCho 18:1/22:6	1.05	1.42	1.45	6.80	5.96	26.71	21.98	11.00	17.97
PtCho 18:0/22:6,18:1/22:5	6.27	8.94	9.03	49.90	33.03	179.73	112.10	51.35	84.50
PtCho 18:0/22:5	14.59	20.63	23.19	120.11	63.35	262.61	431.17	205.42	350.79
PtCho 16:0/16:0	9.92	14.77	13.06	55.83	67.86	256.70	314.90	156.77	251.88
PtCho 16:0/18:2	332.52	465.24	559.11	1845.79	1758.32	4411.23	4450.93	2445.42	4220.76
PtCho 16:0/18:1	176.42	218.03	223.77	692.10	628.88	1386.92	1535.81	791.86	1455.35
PtCho 16:0/20:5,16:1/20:4	6.69	8.80	10.90	39.62	44.52	142.88	193.55	99.43	155.66
PtCho 16:0/20:4	47.22	67.80	84.73	276.32	265.69	696.94	683.93	380.26	648.48
PtCho 16:0/20:3	100.74	119.16	138.19	421.17	431.14	1346.47	1691.31	940.95	1642.59
PtCho 18:0/18:2,18:1/18:1	346.84	430.97	457.09	1422.80	1474.78	4578.13	5306.64	2865.96	5109.72
PtCho 18:0/18:1	143.85	166.90	160.53	537.88	480.66	1433.49	1917.79	948.46	1738.58
PtCho Total	1310.57	1709.94	1870.08	6362.82	5962.93	17611.27	21467.41	11414.67	19838.92
Pcho	0.48	1.12	0.93	0.84	2.90	2.45	0.00	0.00	0.00
SM	193.63	218.60	253.47	806.07	571.10	526.52	393.89	393.38	584.86
GPC	1.91	1.60	5.77	9.42	5.04	4.50	19.51	6.56	0.50

Average of triplicate injections

Table 36B: Cow #7 Fold Change in Plasma Used in Figure 8

	Fold Change Relative to Week 1								
	Week of Lactation								
Lipid	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**
Bet	1.00	0.89	0.71	0.87	1.03	0.77	2.24	2.50	2.98
Cho	1.00	1.23	1.01	0.59	0.90	0.86	0.77	0.64	0.73
LPC 16:0	1.00	1.40	1.92	2.70	1.79	1.82	2.65	1.76	1.31
LPC 18:2	1.00	1.61	2.00	2.93	2.06	1.94	4.60	2.81	1.52
LPC 18:1	1.00	1.36	1.60	2.22	1.73	1.83	4.37	2.32	1.35
LPC 18:0	1.00	1.36	1.79	2.34	2.08	3.07	5.98	3.69	2.15
LPC Total	1.00	1.47	1.86	2.62	1.95	2.12	4.48	2.70	1.57
PtCho 16:0/16:1	1.00	1.28	1.27	5.02	6.83	20.78	31.01	14.91	24.88
PtCho 16:0/22:6,18:1/22:5,18:2/20:4	1.00	1.49	1.51	6.74	6.05	19.25	16.64	8.02	13.83
PtCho 18:1/20:4,18:0/20:5,16:0/22:5	1.00	1.40	1.50	5.90	4.77	17.19	22.70	11.30	18.89
PtCho 18:0/20:4	1.00	1.54	1.59	7.32	4.71	18.82	31.19	16.22	27.24
PtCho 18:0/20:3	1.00	1.59	1.47	8.74	7.93	38.06	73.22	39.59	64.30
PtCho 18:1/22:6	1.00	1.35	1.38	6.47	5.67	25.40	20.90	10.46	17.09
PtCho 18:0/22:6,18:1/22:5	1.00	1.43	1.44	7.96	5.27	28.68	17.89	8.19	13.48
PtCho 18:0/22:5	1.00	1.41	1.59	8.23	4.34	18.00	29.55	14.08	24.04
PtCho 16:0/16:0	1.00	1.49	1.32	5.63	6.84	25.88	31.75	15.81	25.40
PtCho 16:0/18:2	1.00	1.40	1.68	5.55	5.29	13.27	13.39	7.35	12.69
PtCho 16:0/18:1	1.00	1.24	1.27	3.92	3.56	7.86	8.71	4.49	8.25
PtCho 16:0/20:5,16:1/20:4	1.00	1.31	1.63	5.92	6.65	21.36	28.93	14.86	23.27
PtCho 16:0/20:4	1.00	1.44	1.79	5.85	5.63	14.76	14.48	8.05	13.73
PtCho 16:0/20:3	1.00	1.18	1.37	4.18	4.28	13.37	16.79	9.34	16.31
PtCho 18:0/18:2,18:1/18:1	1.00	1.24	1.32	4.10	4.25	13.20	15.30	8.26	14.73
PtCho 18:0/18:1	1.00	1.16	1.12	3.74	3.34	9.97	13.33	6.59	12.09
PtCho Total	1.00	1.30	1.43	4.86	4.55	13.44	16.38	8.71	15.14
Pcho	1.00	2.33	1.93	1.74	6.03	5.09	0.00	0.00	0.00
SM	1.00	1.13	1.31	4.16	2.95	2.72	2.03	2.03	3.02
GPC	1.00	0.84	3.01	4.92	2.63	2.35	10.20	3.43	0.26

Table 37A: Cow #8 Average µM in Plasma Used in Figure 8

	Average µM Week of Lactation								
Lipid	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**
Bet	30.73	27.41	20.02	17.22	15.45	11.40	14.37	18.37	29.37
Cho	2.84	4.59	6.73	3.55	4.24	1.92	2.30	2.34	2.34
LPC 16:0	8.68	25.92	15.13	49.59	43.70	50.04	25.13	24.34	24.71
LPC 18:2	14.84	57.39	31.86	126.92	131.00	146.56	56.24	80.13	53.89
LPC 18:1	7.78	30.39	16.89	58.28	56.01	62.87	25.36	32.36	35.14
LPC 18:0	6.49	22.75	15.69	42.93	48.57	58.46	39.19	45.31	36.74
LPC Total	37.79	136.46	79.57	277.72	279.28	317.93	145.92	182.15	150.49
PtCho 16:0/16:1	3.08	5.29	5.63	40.63	44.64	41.05	155.65	120.79	93.83
PtCho 16:0/22:6,18:1/22:5,18:2/20:4	3.36	4.76	5.72	28.70	32.65	32.68	117.86	86.97	61.37
PtCho 18:1/20:4,18:0/20:5,16:0/22:5	8.40	13.42	18.70	94.65	113.22	118.84	412.67	318.02	239.94
PtCho 18:0/20:4	12.32	21.59	31.87	205.53	270.62	285.44	1036.05	872.61	606.92
PtCho 18:0/20:3	10.35	13.47	21.60	196.42	301.86	298.76	1007.48	995.16	707.54
PtCho 18:1/22:6	0.36	0.74	0.75	4.97	6.87	7.66	26.75	16.83	8.95
PtCho 18:0/22:6,18:1/22:5	2.51	3.62	4.16	25.67	33.90	40.19	177.84	94.43	64.75
PtCho 18:0/22:5	4.26	5.88	8.17	56.29	74.42	84.94	297.93	224.95	181.26
PtCho 16:0/16:0	3.32	7.91	8.16	36.75	43.69	37.25	161.32	128.50	102.03
PtCho 16:0/18:2	128.25	210.94	245.08	1104.24	1265.77	1078.53	3326.69	2971.68	1666.66
PtCho 16:0/18:1	63.37	120.30	132.43	479.36	502.75	413.08	1065.66	914.01	1003.73
PtCho 16:0/20:5,16:1/20:4	2.56	3.88	5.60	28.79	35.15	32.82	132.25	133.34	78.08
PtCho 16:0/20:4	13.78	25.76	35.95	152.00	189.82	169.29	554.81	519.08	277.55
PtCho 16:0/20:3	47.18	71.73	88.22	360.55	475.10	444.03	1186.20	1073.68	520.34
PtCho 18:0/18:2,18:1/18:1	110.71	197.43	245.03	841.07	1161.10	1103.91	3430.63	3121.33	1665.63
PtCho 18:0/18:1	43.79	83.57	102.04	353.85	438.37	417.02	1168.35	1011.41	803.59
PtCho Total	457.61	790.30	959.14	4009.46	4989.92	4605.48	14258.13	12602.80	8082.18
Pcho	0.00	0.36	0.00	1.11	0.54	1.53	0.00	0.00	0.00
SM	91.62	150.29	180.94	606.99	516.86	608.75	490.45	307.81	387.77
GPC	0.05	0.45	0.03	9.83	0.74	7.11	0.39	1.07	1.52

Average of triplicate injections

Table 37B: Cow #8 Fold Change in Plasma Used in Figure 8

	Fold Change Relative to Week 1								
	Week of Lactation								
Lipid	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**
Bet	1.00	0.89	0.65	0.56	0.50	0.37	0.47	0.60	0.96
Cho	1.00	1.61	2.37	1.25	1.49	0.67	0.81	0.82	0.82
LPC 16:0	1.00	2.99	1.74	5.71	5.04	5.77	2.90	2.80	2.85
LPC 18:2	1.00	3.87	2.15	8.55	8.83	9.87	3.79	5.40	3.63
LPC 18:1	1.00	3.91	2.17	7.49	7.20	8.08	3.26	4.16	4.52
LPC 18:0	1.00	3.51	2.42	6.62	7.49	9.01	6.04	6.99	5.66
LPC Total	1.00	3.61	2.11	7.35	7.39	8.41	3.86	4.82	3.98
PtCho 16:0/16:1	1.00	1.71	1.83	13.18	14.48	13.32	50.49	39.18	30.44
PtCho 16:0/22:6,18:1/22:5,18:2/20:4	1.00	1.42	1.70	8.53	9.71	9.72	35.04	25.86	18.25
PtCho 18:1/20:4,18:0/20:5,16:0/22:5	1.00	1.60	2.23	11.27	13.49	14.16	49.15	37.88	28.58
PtCho 18:0/20:4	1.00	1.75	2.59	16.68	21.96	23.16	84.06	70.80	49.25
PtCho 18:0/20:3	1.00	1.30	2.09	18.97	29.16	28.86	97.31	96.12	68.34
PtCho 18:1/22:6	1.00	2.07	2.09	13.81	19.09	21.29	74.36	46.77	24.87
PtCho 18:0/22:6,18:1/22:5	1.00	1.44	1.66	10.24	13.52	16.03	70.95	37.67	25.83
PtCho 18:0/22:5	1.00	1.38	1.92	13.21	17.46	19.93	69.91	52.79	42.53
PtCho 16:0/16:0	1.00	2.38	2.46	11.07	13.16	11.22	48.59	38.70	30.73
PtCho 16:0/18:2	1.00	1.64	1.91	8.61	9.87	8.41	25.94	23.17	13.00
PtCho 16:0/18:1	1.00	1.90	2.09	7.56	7.93	6.52	16.82	14.42	15.84
PtCho 16:0/20:5,16:1/20:4	1.00	1.51	2.19	11.23	13.71	12.80	51.58	52.01	30.45
PtCho 16:0/20:4	1.00	1.87	2.61	11.03	13.78	12.29	40.26	37.67	20.14
PtCho 16:0/20:3	1.00	1.52	1.87	7.64	10.07	9.41	25.14	22.76	11.03
PtCho 18:0/18:2,18:1/18:1	1.00	1.78	2.21	7.60	10.49	9.97	30.99	28.19	15.04
PtCho 18:0/18:1	1.00	1.91	2.33	8.08	10.01	9.52	26.68	23.10	18.35
PtCho Total	1.00	1.73	2.10	8.76	10.90	10.06	31.16	27.54	17.66
Pcho	0.00	1.00	0.00	3.06	1.50	4.20	0.00	0.00	0.00
SM	1.00	1.64	1.97	6.63	5.64	6.64	5.35	3.36	4.23
GPC	1.00	8.90	0.61	193.10	14.62	139.63	7.62	20.94	29.91

Table 38A: Cow #9 Average µM in Plasma Used in Figure 8

	Average µM Week of Lactation								
Lipid	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**
Bet	32.67	26.98	13.72	20.48	31.74	42.64	53.84	77.63	56.22
Cho	5.77	2.72	3.07	3.98	5.66	7.46	3.99	4.39	3.08
LPC 16:0	27.58	26.17	32.05	46.93	56.98	86.88	28.70	21.30	36.65
LPC 18:2	55.17	75.37	96.36	175.25	205.15	347.95	106.80	59.28	142.46
LPC 18:1	32.09	32.61	41.07	59.77	69.42	110.95	38.17	23.20	56.52
LPC 18:0	25.36	26.31	43.87	76.86	86.06	151.01	68.42	39.33	85.29
LPC Total	140.21	160.46	213.35	358.81	417.60	696.79	242.09	143.11	320.92
PtCho 16:0/16:1	10.78	18.92	14.78	45.16	49.52	59.00	188.01	112.36	101.73
PtCho 16:0/22:6,18:1/22:5,18:2/20:4	6.21	14.75	9.87	35.43	41.07	51.83	130.73	72.77	62.03
PtCho 18:1/20:4,18:0/20:5,16:0/22:5	21.25	50.96	34.12	127.49	134.27	186.41	469.39	304.56	268.74
PtCho 18:0/20:4	33.08	90.84	66.64	304.21	329.37	500.20	1439.08	1014.00	893.23
PtCho 18:0/20:3	29.80	75.47	80.04	341.35	358.00	544.81	1958.46	1399.89	1202.59
PtCho 18:1/22:6	1.06	2.44	1.84	7.46	9.54	12.13	27.72	14.76	12.17
PtCho 18:0/22:6,18:1/22:5	5.02	12.72	9.13	38.41	46.21	60.76	215.62	97.57	69.79
PtCho 18:0/22:5	10.71	28.25	18.03	79.72	80.88	123.97	349.71	237.16	225.14
PtCho 16:0/16:0	8.92	17.86	12.27	51.36	58.54	77.21	253.96	138.47	129.66
PtCho 16:0/18:2	295.65	547.36	478.50	1449.26	1607.99	1919.10	4218.00	2826.42	2391.08
PtCho 16:0/18:1	158.62	220.92	188.74	443.25	510.86	571.14	1214.97	937.32	785.04
PtCho 16:0/20:5,16:1/20:4	8.72	18.58	13.46	44.98	47.14	61.57	170.67	120.72	109.40
PtCho 16:0/20:4	34.32	81.63	66.30	221.50	243.51	307.66	653.31	450.73	379.75
PtCho 16:0/20:3	102.74	183.64	184.57	558.42	585.74	691.87	1475.20	1011.31	852.61
PtCho 18:0/18:2,18:1/18:1	251.70	485.92	532.12	1709.17	1912.59	2348.45	4994.55	3386.50	2755.61
PtCho 18:0/18:1	119.44	194.30	201.68	540.91	631.04	735.40	1582.63	1088.06	910.85
PtCho Total	1098.01	2044.57	1912.10	5998.07	6646.26	8251.54	19342.02	13212.63	11149.40
Pcho	1.07	0.86	0.52	1.25	1.59	2.75	0.00	0.00	0.00
SM	204.88	268.94	236.89	537.31	572.58	747.95	444.48	458.86	300.41
GPC	3.04	0.21	2.92	1.01	1.79	4.09	2.97	0.44	11.64

Average of triplicate injections

Table 38B: Cow #9 Fold Change in Plasma Used in Figure 8

	Fold Change Relative to Week 1								
	Week of Lactation								
Lipid	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**
Bet	1.00	0.83	0.42	0.63	0.97	1.30	1.65	2.38	1.72
Cho	1.00	0.47	0.53	0.69	0.98	1.29	0.69	0.76	0.53
LPC 16:0	1.00	0.95	1.16	1.70	2.07	3.15	1.04	0.77	1.33
LPC 18:2	1.00	1.37	1.75	3.18	3.72	6.31	1.94	1.07	2.58
LPC 18:1	1.00	1.02	1.28	1.86	2.16	3.46	1.19	0.72	1.76
LPC 18:0	1.00	1.04	1.73	3.03	3.39	5.95	2.70	1.55	3.36
LPC Total	1.00	1.14	1.52	2.56	2.98	4.97	1.73	1.02	2.29
PtCho 16:0/16:1	1.00	1.76	1.37	4.19	4.59	5.47	17.44	10.42	9.44
PtCho 16:0/22:6,18:1/22:5,18:2/20:4	1.00	2.38	1.59	5.71	6.62	8.35	21.06	11.72	9.99
PtCho 18:1/20:4,18:0/20:5,16:0/22:5	1.00	2.40	1.61	6.00	6.32	8.77	22.09	14.33	12.65
PtCho 18:0/20:4	1.00	2.75	2.01	9.20	9.96	15.12	43.51	30.66	27.00
PtCho 18:0/20:3	1.00	2.53	2.69	11.45	12.01	18.28	65.71	46.97	40.35
PtCho 18:1/22:6	1.00	2.31	1.74	7.05	9.02	11.46	26.19	13.94	11.49
PtCho 18:0/22:6,18:1/22:5	1.00	2.53	1.82	7.65	9.20	12.10	42.92	19.42	13.89
PtCho 18:0/22:5	1.00	2.64	1.68	7.44	7.55	11.58	32.65	22.14	21.02
PtCho 16:0/16:0	1.00	2.00	1.38	5.76	6.56	8.66	28.47	15.52	14.53
PtCho 16:0/18:2	1.00	1.85	1.62	4.90	5.44	6.49	14.27	9.56	8.09
PtCho 16:0/18:1	1.00	1.39	1.19	2.79	3.22	3.60	7.66	5.91	4.95
PtCho 16:0/20:5,16:1/20:4	1.00	2.13	1.54	5.16	5.41	7.06	19.58	13.85	12.55
PtCho 16:0/20:4	1.00	2.38	1.93	6.45	7.10	8.96	19.04	13.13	11.07
PtCho 16:0/20:3	1.00	1.79	1.80	5.44	5.70	6.73	14.36	9.84	8.30
PtCho 18:0/18:2,18:1/18:1	1.00	1.93	2.11	6.79	7.60	9.33	19.84	13.45	10.95
PtCho 18:0/18:1	1.00	1.63	1.69	4.53	5.28	6.16	13.25	9.11	7.63
PtCho Total	1.00	1.86	1.74	5.46	6.05	7.51	17.62	12.03	10.15
Pcho	1.00	0.80	0.49	1.16	1.49	2.57	0.00	0.00	0.00
SM	1.00	1.31	1.16	2.62	2.79	3.65	2.17	2.24	1.47
GPC	1.00	0.07	0.96	0.33	0.59	1.34	0.98	0.15	3.82

Table 39A: Cow #10 Average µM in Plasma Used in Figure 8

	Average µM Week of Lactation								
Lipid	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**
Bet	22.02	19.66	20.55	16.65	18.93	24.11	45.06	56.38	66.89
Cho	6.01	4.15	5.18	4.18	3.43	3.79	3.94	2.62	4.06
LPC 16:0	22.16	27.35	28.14	65.34	34.80	49.11	29.68	24.78	30.02
LPC 18:2	41.97	62.94	64.00	163.71	79.17	136.54	76.09	54.20	85.88
LPC 18:1	28.63	34.99	32.52	78.69	32.82	53.26	32.48	23.63	43.17
LPC 18:0	23.98	27.34	30.46	76.87	39.37	66.81	48.35	45.09	62.38
LPC Total	116.75	152.61	155.12	384.61	186.16	305.72	186.60	147.71	221.45
PtCho 16:0/16:1	8.14	10.97	13.62	61.61	30.96	69.51	218.04	132.63	127.09
PtCho 16:0/22:6,18:1/22:5,18:2/20:4	8.65	8.98	12.36	50.88	19.48	55.82	161.61	88.37	73.54
PtCho 18:1/20:4,18:0/20:5,16:0/22:5	31.36	30.63	44.53	183.13	72.31	218.20	537.40	357.45	309.92
PtCho 18:0/20:4	42.25	47.47	76.60	398.72	155.73	524.69	1435.77	1041.15	961.16
PtCho 18:0/20:3	29.36	43.99	64.64	322.32	180.54	504.68	1533.81	1240.86	1058.84
PtCho 18:1/22:6	1.21	1.51	2.00	8.87	2.57	10.36	32.00	16.28	14.92
PtCho 18:0/22:6,18:1/22:5	7.84	8.39	11.72	63.77	18.20	68.85	221.80	109.26	81.66
PtCho 18:0/22:5	19.11	17.85	27.37	137.30	41.88	156.15	370.07	226.53	219.09
PtCho 16:0/16:0	8.52	10.80	14.78	69.79	35.48	83.18	292.50	158.78	133.11
PtCho 16:0/18:2	268.24	359.99	475.15	1500.32	1145.92	1932.27	4211.84	3417.46	2555.70
PtCho 16:0/18:1	189.00	210.04	244.67	652.49	491.93	695.69	1512.97	1094.71	1028.83
PtCho 16:0/20:5,16:1/20:4	10.44	11.98	16.73	58.35	30.06	68.68	198.37	135.37	132.15
PtCho 16:0/20:4	40.67	50.40	72.89	241.84	152.89	298.49	721.23	542.06	468.12
PtCho 16:0/20:3	81.90	116.37	142.31	420.40	345.74	600.61	1388.08	1213.40	937.15
PtCho 18:0/18:2,18:1/18:1	256.36	345.98	429.20	1274.83	1262.16	1999.35	4679.70	4022.69	2843.38
PtCho 18:0/18:1	139.77	165.68	195.22	557.74	502.97	741.46	1873.85	1337.24	1120.92
PtCho Total	1142.82	1441.03	1843.80	6002.35	4488.82	8027.99	19389.04	15134.25	12065.58
Pcho	0.42	0.62	0.57	0.65	1.11	1.43	0.00	0.00	0.00
SM	188.00	251.01	285.41	565.74	710.89	799.23	550.67	510.08	396.41
GPC	0.23	0.95	0.35	1.42	0.26	0.72	1.27	0.48	1.44

Average of triplicate injections

Table 39B: Cow #10 Fold Change in Plasma Used in Figure 8

	Fold Change Relative to Week 1								
	Week of Lactation								
Lipid	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**
Bet	1.00	0.89	0.93	0.76	0.86	1.09	2.05	2.56	3.04
Cho	1.00	0.69	0.86	0.70	0.57	0.63	0.66	0.44	0.68
LPC 16:0	1.00	1.23	1.27	2.95	1.57	2.22	1.34	1.12	1.35
LPC 18:2	1.00	1.50	1.52	3.90	1.89	3.25	1.81	1.29	2.05
LPC 18:1	1.00	1.22	1.14	2.75	1.15	1.86	1.13	0.83	1.51
LPC 18:0	1.00	1.14	1.27	3.21	1.64	2.79	2.02	1.88	2.60
LPC Total	1.00	1.31	1.33	3.29	1.59	2.62	1.60	1.27	1.90
PtCho 16:0/16:1	1.00	1.35	1.67	7.57	3.80	8.54	26.79	16.29	15.61
PtCho 16:0/22:6,18:1/22:5,18:2/20:4	1.00	1.04	1.43	5.88	2.25	6.45	18.68	10.21	8.50
PtCho 18:1/20:4,18:0/20:5,16:0/22:5	1.00	0.98	1.42	5.84	2.31	6.96	17.14	11.40	9.88
PtCho 18:0/20:4	1.00	1.12	1.81	9.44	3.69	12.42	33.98	24.64	22.75
PtCho 18:0/20:3	1.00	1.50	2.20	10.98	6.15	17.19	52.25	42.27	36.07
PtCho 18:1/22:6	1.00	1.25	1.65	7.36	2.13	8.59	26.54	13.50	12.38
PtCho 18:0/22:6,18:1/22:5	1.00	1.07	1.50	8.14	2.32	8.78	28.30	13.94	10.42
PtCho 18:0/22:5	1.00	0.93	1.43	7.18	2.19	8.17	19.36	11.85	11.46
PtCho 16:0/16:0	1.00	1.27	1.73	8.19	4.17	9.77	34.34	18.64	15.63
PtCho 16:0/18:2	1.00	1.34	1.77	5.59	4.27	7.20	15.70	12.74	9.53
PtCho 16:0/18:1	1.00	1.11	1.29	3.45	2.60	3.68	8.01	5.79	5.44
PtCho 16:0/20:5,16:1/20:4	1.00	1.15	1.60	5.59	2.88	6.58	19.00	12.96	12.66
PtCho 16:0/20:4	1.00	1.24	1.79	5.95	3.76	7.34	17.74	13.33	11.51
PtCho 16:0/20:3	1.00	1.42	1.74	5.13	4.22	7.33	16.95	14.82	11.44
PtCho 18:0/18:2,18:1/18:1	1.00	1.35	1.67	4.97	4.92	7.80	18.25	15.69	11.09
PtCho 18:0/18:1	1.00	1.19	1.40	3.99	3.60	5.30	13.41	9.57	8.02
PtCho Total	1.00	1.26	1.61	5.25	3.93	7.02	16.97	13.24	10.56
Pcho	1.00	1.47	1.35	1.54	2.61	3.39	0.00	0.00	0.00
SM	1.00	1.34	1.52	3.01	3.78	4.25	2.93	2.71	2.11
GPC	1.00	4.17	1.55	6.26	1.14	3.18	5.58	2.12	6.34

Table 40A: Cow #11 Average µM in Plasma Used in Figure 8

	Average µM Week of Lactation					
Lipid	**1**	**2**	**3**	**4**	**5**	**6**
Bet	42.29	18.67	13.15	21.45	32.90	37.71
Cho	5.18	2.57	4.89	4.83	3.06	5.14
LPC 16:0	53.19	19.88	27.03	73.06	37.00	57.24
LPC 18:2	137.37	54.97	59.77	175.76	100.42	186.87
LPC 18:1	68.69	28.95	27.92	76.04	32.59	61.62
LPC 18:0	56.58	21.26	24.40	83.83	47.38	84.55
LPC Total	315.82	125.05	139.12	408.68	217.39	390.28
PtCho 16:0/16:1	16.61	20.10	11.62	65.19	48.66	52.47
PtCho 16:0/22:6,18:1/22:5,18:2/20:4	12.19	15.87	14.20	60.51	41.19	42.63
PtCho 18:1/20:4,18:0/20:5,16:0/22:5	43.75	56.07	33.95	190.31	145.95	149.83
PtCho 18:0/20:4	90.24	129.47	62.10	502.29	430.36	455.56
PtCho 18:0/20:3	65.13	88.93	52.59	385.26	443.79	469.10
PtCho 18:1/22:6	2.05	2.91	2.66	9.91	8.41	8.98
PtCho 18:0/22:6,18:1/22:5	11.46	18.03	13.89	77.13	56.07	58.61
PtCho 18:0/22:5	26.44	40.84	21.29	161.56	111.48	114.41
PtCho 16:0/16:0	16.50	20.83	12.45	90.72	67.06	74.09
PtCho 16:0/18:2	562.33	576.51	456.59	2141.18	1694.69	1742.78
PtCho 16:0/18:1	257.67	283.33	223.37	882.42	513.77	540.70
PtCho 16:0/20:5,16:1/20:4	14.49	14.13	9.69	51.84	45.55	49.37
PtCho 16:0/20:4	80.86	84.35	63.89	285.42	245.26	254.28
PtCho 16:0/20:3	161.60	166.95	146.44	505.61	545.22	582.47
PtCho 18:0/18:2,18:1/18:1	458.84	493.81	466.87	1851.60	1756.89	1960.67
PtCho 18:0/18:1	195.40	232.83	204.91	853.25	558.74	614.94
PtCho Total	2015.54	2244.94	1796.50	8114.20	6713.10	7170.87
Pcho	0.96	1.15	0.57	2.64	2.16	2.57
SM	313.55	293.04	354.35	1041.48	703.55	771.51
GPC	10.74	0.75	0.24	13.12	0.42	3.70

Average of triplicate injections

Table 40B: Cow #11 Fold Change in Plasma Used in Figure 8

	Fold Change Relative to Week 1					
	Week of Lactation					
Lipid	**1**	**2**	**3**	**4**	**5**	**6**
Bet	1.00	0.44	0.31	0.51	0.78	0.89
Cho	1.00	0.50	0.94	0.93	0.59	0.99
LPC 16:0	1.00	0.37	0.51	1.37	0.70	1.08
LPC 18:2	1.00	0.40	0.44	1.28	0.73	1.36
LPC 18:1	1.00	0.42	0.41	1.11	0.47	0.90
LPC 18:0	1.00	0.38	0.43	1.48	0.84	1.49
LPC Total	1.00	0.40	0.44	1.29	0.69	1.24
PtCho 16:0/16:1	1.00	1.21	0.70	3.93	2.93	3.16
PtCho 16:0/22:6,18:1/22:5,18:2/20:4	1.00	1.30	1.17	4.97	3.38	3.50
PtCho 18:1/20:4,18:0/20:5,16:0/22:5	1.00	1.28	0.78	4.35	3.34	3.42
PtCho 18:0/20:4	1.00	1.43	0.69	5.57	4.77	5.05
PtCho 18:0/20:3	1.00	1.37	0.81	5.92	6.81	7.20
PtCho 18:1/22:6	1.00	1.43	1.30	4.84	4.11	4.39
PtCho 18:0/22:6,18:1/22:5	1.00	1.57	1.21	6.73	4.89	5.11
PtCho 18:0/22:5	1.00	1.54	0.81	6.11	4.22	4.33
PtCho 16:0/16:0	1.00	1.26	0.75	5.50	4.06	4.49
PtCho 16:0/18:2	1.00	1.03	0.81	3.81	3.01	3.10
PtCho 16:0/18:1	1.00	1.10	0.87	3.42	1.99	2.10
PtCho 16:0/20:5,16:1/20:4	1.00	0.97	0.67	3.58	3.14	3.41
PtCho 16:0/20:4	1.00	1.04	0.79	3.53	3.03	3.14
PtCho 16:0/20:3	1.00	1.03	0.91	3.13	3.37	3.60
PtCho 18:0/18:2,18:1/18:1	1.00	1.08	1.02	4.04	3.83	4.27
PtCho 18:0/18:1	1.00	1.19	1.05	4.37	2.86	3.15
PtCho Total	1.00	1.11	0.89	4.03	3.33	3.56
Pcho	1.00	1.20	0.60	2.75	2.24	2.67
SM	1.00	0.93	1.13	3.32	2.24	2.46
GPC	1.00	0.07	0.02	1.22	0.04	0.34

Table 41A: Cow #12 Average µM in Plasma Used in Figure 8

	Average µM Week of Lactation					
Lipid	**1**	**2**	**3**	**4**	**5**	**6**
Bet	13.12	12.43	9.69	8.22	13.25	11.58
Cho	2.49	4.47	3.77	2.70	5.64	5.45
LPC 16:0	14.16	21.39	28.74	30.59	52.35	51.25
LPC 18:2	29.74	53.87	75.56	87.88	158.46	162.47
LPC 18:1	15.65	25.24	33.59	31.84	59.18	53.14
LPC 18:0	14.52	23.47	33.14	32.82	66.58	72.86
LPC Total	74.07	123.97	171.03	183.14	336.58	339.72
PtCho 16:0/16:1	6.27	9.09	10.81	40.86	36.92	38.01
PtCho 16:0/22:6,18:1/22:5,18:2/20:4	4.32	9.05	9.65	33.14	26.65	31.09
PtCho 18:1/20:4,18:0/20:5,16:0/22:5	17.04	32.99	36.09	120.15	92.11	106.86
PtCho 18:0/20:4	25.30	51.04	65.01	256.82	212.75	270.20
PtCho 18:0/20:3	14.22	34.69	53.75	227.61	177.07	259.99
PtCho 18:1/22:6	0.52	1.48	1.66	5.86	5.49	5.75
PtCho 18:0/22:6,18:1/22:5	3.39	9.04	10.46	39.82	28.85	34.45
PtCho 18:0/22:5	9.04	23.30	26.70	91.34	67.34	74.19
PtCho 16:0/16:0	7.80	9.91	12.21	49.81	60.86	56.69
PtCho 16:0/18:2	219.15	386.49	423.29	1299.41	1184.80	1266.00
PtCho 16:0/18:1	115.72	179.55	186.67	452.33	434.38	406.45
PtCho 16:0/20:5,16:1/20:4	7.78	10.50	11.78	37.81	30.42	36.51
PtCho 16:0/20:4	27.60	49.31	57.25	175.74	156.15	183.75
PtCho 16:0/20:3	68.16	120.28	128.06	380.97	372.96	384.20
PtCho 18:0/18:2,18:1/18:1	204.19	364.16	415.25	1241.61	1291.88	1411.68
PtCho 18:0/18:1	86.58	157.28	168.82	455.77	486.91	483.13
PtCho Total	817.07	1448.18	1617.44	4909.06	4665.54	5048.94
Pcho	0.45	1.08	0.68	0.66	3.12	3.52
SM	155.31	215.23	223.80	568.74	561.01	558.46
GPC	1.09	2.07	4.02	0.79	18.70	17.23

Average of triplicate injections

Table 41B: Cow #12 Fold Change in Plasma Used in Figure 8

	Fold Change Relative to Week 1					
	Week of Lactation					
Lipid	**1**	**2**	**3**	**4**	**5**	**6**
Bet	1.00	0.95	0.74	0.63	1.01	0.88
Cho	1.00	1.79	1.51	1.08	2.26	2.19
LPC 16:0	1.00	1.51	2.03	2.16	3.70	3.62
LPC 18:2	1.00	1.81	2.54	2.96	5.33	5.46
LPC 18:1	1.00	1.61	2.15	2.03	3.78	3.39
LPC 18:0	1.00	1.62	2.28	2.26	4.59	5.02
LPC Total	1.00	1.67	2.31	2.47	4.54	4.59
PtCho 16:0/16:1	1.00	1.45	1.72	6.52	5.89	6.07
PtCho 16:0/22:6,18:1/22:5,18:2/20:4	1.00	2.09	2.23	7.67	6.17	7.20
PtCho 18:1/20:4,18:0/20:5,16:0/22:5	1.00	1.94	2.12	7.05	5.41	6.27
PtCho 18:0/20:4	1.00	2.02	2.57	10.15	8.41	10.68
PtCho 18:0/20:3	1.00	2.44	3.78	16.00	12.45	18.28
PtCho 18:1/22:6	1.00	2.84	3.18	11.23	10.53	11.03
PtCho 18:0/22:6,18:1/22:5	1.00	2.67	3.09	11.75	8.51	10.16
PtCho 18:0/22:5	1.00	2.58	2.96	10.11	7.45	8.21
PtCho 16:0/16:0	1.00	1.27	1.57	6.39	7.80	7.27
PtCho 16:0/18:2	1.00	1.76	1.93	5.93	5.41	5.78
PtCho 16:0/18:1	1.00	1.55	1.61	3.91	3.75	3.51
PtCho 16:0/20:5,16:1/20:4	1.00	1.35	1.51	4.86	3.91	4.69
PtCho 16:0/20:4	1.00	1.79	2.07	6.37	5.66	6.66
PtCho 16:0/20:3	1.00	1.76	1.88	5.59	5.47	5.64
PtCho 18:0/18:2,18:1/18:1	1.00	1.78	2.03	6.08	6.33	6.91
PtCho 18:0/18:1	1.00	1.82	1.95	5.26	5.62	5.58
PtCho Total	1.00	1.77	1.98	6.01	5.71	6.18
Pcho	1.00	2.41	1.52	1.49	6.99	7.88
SM	1.00	1.39	1.44	3.66	3.61	3.60
GPC	1.00	1.89	3.68	0.72	17.09	15.74

Table 42A: Average µM in Milk Used in Figure 9

	Average µM					
	Sample #1		Sample #2		Sample #3	
Lipid	Extraction 1	Extraction 2	Extraction 1	Extraction 2	Extraction 1	Extraction 2
AcCho	0.00	0.00	0.00	0.00	0.01	0.02
Bet	50.29	43.99	20.64	18.68	14.47	14.99
Cho	37.48	32.25	202.25	178.59	62.02	62.08
LPC 16:0	2.04	1.44	1.17	1.01	0.65	0.60
LPC 18:2	3.63	2.59	0.32	0.32	0.29	0.33
LPC 18:1	4.33	3.68	0.57	0.47	0.45	0.33
LPC 18:0	1.11	0.59	0.96	0.92	0.32	0.38
LPC Total	11.11	8.29	3.02	2.73	1.72	1.64
PtCho 16:0/16:1	8.19	8.13	5.36	5.00	5.81	5.76
PtCho 16:0/22:6,18:1/22:5,18:2/20:4	2.96	1.80	0.58	0.72	0.51	0.49
PtCho 18:1/20:4,18:0/20:5,16:0/22:5	5.88	3.68	1.37	1.38	1.60	1.52
PtCho 18:0/20:4	5.91	3.57	1.31	1.24	1.52	1.62
PtCho 18:0/20:3	5.14	2.40	0.73	0.70	0.87	1.03
PtCho 18:1/22:6	0.94	0.61	0.22	0.27	0.18	0.18
PtCho 18:0/22:6,18:1/22:5	3.08	1.45	0.35	0.54	0.28	0.27
PtCho 18:0/22:5	2.68	1.14	0.25	0.31	0.24	0.25
PtCho 16:0/16:0	36.95	37.26	25.83	24.51	18.30	20.19
PtCho 16:0/18:2	76.99	47.81	20.75	18.93	17.92	18.39
PtCho 16:0/18:1	120.99	114.69	49.82	44.52	40.87	39.14
PtCho 16:0/20:5,16:1/20:4	1.55	1.05	0.41	0.37	0.36	0.40
PtCho 16:0/20:4	13.53	11.38	4.69	4.20	4.13	4.09
PtCho 16:0/20:3	47.52	41.52	14.65	13.22	14.16	14.01
PtCho 18:0/18:2,18:1/18:1	141.22	91.61	26.25	25.15	26.79	27.29
PtCho 18:0/18:1	72.01	53.40	13.73	12.79	13.99	14.34
PtCho Total	545.54	421.51	166.29	153.86	147.52	148.97
Pcho	642.96	564.68	113.72	99.09	164.91	160.64
SM	45.79	33.76	38.75	37.87	30.28	29.57
GPC	212.12	224.23	429.65	410.09	71.10	44.31

Average of triplicate injections

Table 42B: Fold Change in Milk Used in Figure 9

	Fold Change Relative to Extraction 1					
	Sample #1		Sample #2		Sample #3	
Lipid	Extraction 1	Extraction 2	Extraction 1	Extraction 2	Extraction 1	Extraction 2
AcCho	0.00	0.00	0.00	0.00	1.00	1.35
Bet	1.00	0.87	1.00	0.91	1.00	1.04
Cho	1.00	0.86	1.00	0.88	1.00	1.00
LPC 16:0	1.00	0.70	1.00	0.86	1.00	0.92
LPC 18:2	1.00	0.71	1.00	1.01	1.00	1.12
LPC 18:1	1.00	0.85	1.00	0.84	1.00	0.73
LPC 18:0	1.00	0.53	1.00	0.96	1.00	1.18
LPC Total	1.00	0.75	1.00	0.90	1.00	0.95
PtCho 16:0/16:1	1.00	0.99	1.00	0.93	1.00	0.99
PtCho 16:0/22:6,18:1/22:5,18:2/20:4	1.00	0.61	1.00	1.25	1.00	0.95
PtCho 18:1/20:4,18:0/20:5,16:0/22:5	1.00	0.63	1.00	1.01	1.00	0.95
PtCho 18:0/20:4	1.00	0.60	1.00	0.95	1.00	1.06
PtCho 18:0/20:3	1.00	0.47	1.00	0.96	1.00	1.18
PtCho 18:1/22:6	1.00	0.65	1.00	1.21	1.00	0.99
PtCho 18:0/22:6,18:1/22:5	1.00	0.47	1.00	1.54	1.00	0.95
PtCho 18:0/22:5	1.00	0.42	1.00	1.22	1.00	1.04
PtCho 16:0/16:0	1.00	1.01	1.00	0.95	1.00	1.10
PtCho 16:0/18:2	1.00	0.62	1.00	0.91	1.00	1.03
PtCho 16:0/18:1	1.00	0.95	1.00	0.89	1.00	0.96
PtCho 16:0/20:5,16:1/20:4	1.00	0.68	1.00	0.92	1.00	1.10
PtCho 16:0/20:4	1.00	0.84	1.00	0.90	1.00	0.99
PtCho 16:0/20:3	1.00	0.87	1.00	0.90	1.00	0.99
PtCho 18:0/18:2,18:1/18:1	1.00	0.65	1.00	0.96	1.00	1.02
PtCho 18:0/18:1	1.00	0.74	1.00	0.93	1.00	1.03
PtCho Total	1.00	0.77	1.00	0.93	1.00	1.01
Pcho	1.00	0.88	1.00	0.87	1.00	0.97
SM	1.00	0.74	1.00	0.98	1.00	0.98
GPC	1.00	1.06	1.00	0.95	1.00	0.62

Table 43A: Average µM in Plasma Used in Figure 10

	Average µM					
	Sample #1		Sample #2		Sample #3	
Lipid	Extraction 1	Extraction 2	Extraction 1	Extraction 2	Extraction 1	Extraction 2
Bet	16.05	15.18	15.09	11.84	12.24	13.15
Cho	3.79	3.80	2.98	2.40	6.00	5.98
LPC 16:0	26.13	26.39	47.29	32.00	29.60	27.16
LPC 18:2	73.36	77.37	148.70	98.28	73.43	63.69
LPC 18:1	32.97	34.42	60.47	39.35	32.75	31.42
LPC 18:0	28.85	29.23	60.26	40.57	44.92	50.30
LPC Total	161.31	167.42	316.73	210.20	180.69	172.57
PtCho 16:0/16:1	25.36	19.92	61.65	48.16	54.75	48.80
PtCho 16:0/22:6,18:1/22:5,18:2/20:4	25.85	19.66	47.95	38.22	36.98	32.03
PtCho 18:1/20:4,18:0/20:5,16:0/22:5	90.20	69.01	185.79	144.89	122.89	105.32
PtCho 18:0/20:4	198.49	152.03	480.17	372.85	268.25	240.31
PtCho 18:0/20:3	188.60	142.53	447.67	350.54	269.70	258.19
PtCho 18:1/22:6	4.82	3.68	9.62	7.55	5.80	4.81
PtCho 18:0/22:6,18:1/22:5	29.12	22.06	63.19	48.25	32.31	26.10
PtCho 18:0/22:5	62.93	48.38	146.74	111.50	61.44	51.07
PtCho 16:0/16:0	29.21	22.96	69.63	52.75	66.11	51.46
PtCho 16:0/18:2	760.42	580.80	1713.90	1368.46	1695.37	1630.42
PtCho 16:0/18:1	333.85	257.52	678.88	529.97	543.14	514.83
PtCho 16:0/20:5,16:1/20:4	23.99	18.35	46.38	35.93	42.98	39.07
PtCho 16:0/20:4	118.59	90.58	255.23	201.97	238.12	225.86
PtCho 16:0/20:3	239.71	184.51	574.45	455.97	471.58	469.72
PtCho 18:0/18:2,18:1/18:1	641.88	489.90	1763.36	1394.79	1564.10	1585.59
PtCho 18:0/18:1	275.84	211.58	704.78	561.57	494.84	491.87
PtCho Total	3048.86	2333.48	7249.42	5723.37	5968.38	5775.46
Pcho	0.98	1.28	1.32	1.11	2.48	3.31
SM	365.87	358.26	925.85	654.36	451.18	502.72
GPC	0.81	1.01	6.38	3.07	3.66	1.55

Average of triplicate injections

Table 43B: Fold Change in Plasma Used in Figure 10

	Fold Change Relative to Extraction 1					
	Sample #1		Sample #2		Sample #3	
Lipid	Extraction 1	Extraction 2	Extraction 1	Extraction 2	Extraction 1	Extraction 2
Bet	1.00	0.95	1.00	0.78	1.00	1.07
Cho	1.00	1.00	1.00	0.81	1.00	1.00
LPC 16:0	1.00	1.01	1.00	0.68	1.00	0.92
LPC 18:2	1.00	1.05	1.00	0.66	1.00	0.87
LPC 18:1	1.00	1.04	1.00	0.65	1.00	0.96
LPC 18:0	1.00	1.01	1.00	0.67	1.00	1.12
LPC Total	1.00	1.04	1.00	0.66	1.00	0.96
PtCho 16:0/16:1	1.00	0.79	1.00	0.78	1.00	0.89
PtCho 16:0/22:6,18:1/22:5,18:2/20:4	1.00	0.76	1.00	0.80	1.00	0.87
PtCho 18:1/20:4,18:0/20:5,16:0/22:5	1.00	0.77	1.00	0.78	1.00	0.86
PtCho 18:0/20:4	1.00	0.77	1.00	0.78	1.00	0.90
PtCho 18:0/20:3	1.00	0.76	1.00	0.78	1.00	0.96
PtCho 18:1/22:6	1.00	0.76	1.00	0.78	1.00	0.83
PtCho 18:0/22:6,18:1/22:5	1.00	0.76	1.00	0.76	1.00	0.81
PtCho 18:0/22:5	1.00	0.77	1.00	0.76	1.00	0.83
PtCho 16:0/16:0	1.00	0.79	1.00	0.76	1.00	0.78
PtCho 16:0/18:2	1.00	0.76	1.00	0.80	1.00	0.96
PtCho 16:0/18:1	1.00	0.77	1.00	0.78	1.00	0.95
PtCho 16:0/20:5,16:1/20:4	1.00	0.77	1.00	0.77	1.00	0.91
PtCho 16:0/20:4	1.00	0.76	1.00	0.79	1.00	0.95
PtCho 16:0/20:3	1.00	0.77	1.00	0.79	1.00	1.00
PtCho 18:0/18:2,18:1/18:1	1.00	0.76	1.00	0.79	1.00	1.01
PtCho 18:0/18:1	1.00	0.77	1.00	0.80	1.00	0.99
PtCho Total	1.00	0.77	1.00	0.79	1.00	0.97
Pcho	1.00	1.31	1.00	0.84	1.00	1.34
SM	1.00	0.98	1.00	0.71	1.00	1.11
GPC	1.00	1.25	1.00	0.48	1.00	0.42

Table 44: GPC External Calibration Curve Used in Figure 11

Calibration Level	M	Injection #	Area
		1	3.88E+09
1	1.19E-02	2	3.93E+09
		3	3.85E+09
		1	2.73E+09
2	3.75E-03	2	2.74E+09
		3	2.82E+09
		1	1.86E+09
3	1.18E-03	2	1.90E+09
		3	1.89E+09
		1	1.17E+09
4	3.74E-04	2	1.19E+09
		3	1.13E+09
		1	6.38E+08
5	1.18E-04	2	6.28E+08
		3	6.28E+08
		1	3.49E+08
6	3.74E-05	2	3.28E+08
		3	3.59E+08
		1	1.40E+08
7	1.18E-05	2	1.50E+08
		3	1.55E+08
		1	5.56E+07
8	3.73E-06	2	6.23E+07
		3	5.58E+07
		1	1.73E+07
9	1.18E-06	2	1.74E+07
		3	1.63E+07
		1	4.67E+06
10	3.73E-07	2	4.49E+06
		3	5.29E+06
		1	1.61E+06
11	1.18E-07	2	1.52E+06
		3	1.48E+06
		1	2.39E+05
12	3.72E-08	2	2.38E+05
		3	3.93E+05
		1	1.40E+05
13	1.18E-08	2	1.40E+05
		3	1.25E+05
		1	2.94E+04
14	3.71E-09	2	4.45E+04
		3	5.37E+04
		1	1.96E+04
15	1.17E-09	2	2.30E+04
		3	2.49E+04

Vita

Jesse Lee Middleton was born on June 28, 1985 in Jellico, TN to the parents of Christine Stephens and Jesse Lee Middleton Sr. He attended Whitley County High School in Williamsburg, KY and later graduated from Eastern Kentucky University in Richmond, KY where he received his Bachelor of Science in Chemistry in the spring of 2010. He began his graduate studies in chemistry at the University of Tennessee in Knoxville, TN and received his Master of Science in Chemistry in May 2013.