HORMONAL ALGAE:
A SOURCE OF FUNCTIONAL FATTY ACIDS

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Abstract

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Based on an endogenous hormone study, three cytokinin type phytohormones; benzyladenine (BA), trans-zeatin (tZ) and methylthiol trans-zeatin (MeSZ), as well as abscisic acid (ABA) were exogenously added at three concentrations (10^{-7}, 10^{-6} and 10^{-5} M) to cultures of *Chlorella vulgaris* in an attempt to alter growth rate, total lipid and fatty acid yields and fatty acid profile. Growth stimulation was highest at 10^{-6} M for BA, MeSZ and ABA and 10^{-5} M for tZ. All treatments caused changes in total lipid and fatty acid content, with BA causing an increase to lipid content. The most significant change in the fatty acid profile was observed with the addition of MeSZ at 10^{-7} and 10^{-6} M causing increases of 204% and 457% in linolenic acid respectively above the control. These results are novel and potentially highly impactful, as MeSZ has never been added exogenously to algae and may be used to stimulate overproduction of linolenic acid for pharmaceutical or industrial purposes.

Keywords: *Chlorella vulgaris*, benzyladenine, *trans*-zeatin, methylthiol *trans*-zeatin, abscisic acid, fatty acid, linolenic acid

Preface

All of the work presented henceforth was conducted in either the Plant Physiology Laboratory or the Trent Centre for Biomaterials Research Laboratory at Trent University, Peterborough Campus, Ontario, Canada. I was the lead investigator, responsible for all major areas of concept formation, data collection and analysis, as well as manuscript composition. All data presented in the Results Chapter is original and unpublished to date. Dr. Suresh Narine and Dr. Neil Emery were the supervisory authors on this project and were involved throughout the project in concept formation, methods development and manuscript edits, as was Dr. Eric Sager, as third committee member.

Acknowledgements

Foremost, I would like to express my gratitude to my supervisors Dr. Suresh Narine and Dr. Neil Emery for allowing me the opportunity to work with them in my Master's program, and for being steadfast and consistent in their support of me throughout my time as their student. Dr. Narine's expertise in lipid biochemistry and Dr. Emery's expertise in plant physiology and phytohormones have been indispensible to the completion of this project and their guidance, motivation and enthusiasm has pushed me to become a better student. I would also like to thank my committee member, Dr. Eric Sager, for his encouragement, knowledge and insightful comments.

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List of Abbreviations and Symbols

AA - Arachidonic acid
ABA - Abscisic acid
ADP - Adenosine diphosphate
ALA - Alpha-linolenic acid
AMP- Adenosine monophosphate
ATP - Adenosine triphosphate
BA - Benzyladenine
BAP - Benzylaminopurine
C. vulgaris- Chlorella vulgaris
CK - Cytokinin
CKX - Cytokinin oxidase-dehydrogenase
CPPU - *N*-(2-chloro-4-pyridyl)-*N*'-phenylurea
cZ - cis-Zeatin
DGDG - Digalactosyldiacylglycerol
DGTS - Diacylglyceryltrimethylhomoserine
DHA - Docosahexaenoic acid
DPU - Diphenylurea
DZ - Dihydrozeatin
EPA - Eicosapentaenoic acid
FA - Fatty acid
FAE - Fatty acid elongase
FAME - Fatty acid methyl ester
FAS - Fatty acid synthase
FB - Free base
GA - Gibberellin
GC-FID - Gas Chromatography Flame Ionization detection
HGT - Horizontal gene transfer
HPLC-(ESI) MS/MS - Electrospray-ionization, liquid chromatography-tandem mass spectrometry
IAA - Indole-3-acetic acid
iP - Isopentenyladenine
IPT - Isopentenyltransferase
LA - Linoleic acid
LOG - "Lonely Guy" enzyme
M - Moles/ Litre

MemT - Meta-methoxytopolin
MeoT - Ortho-methoxytopolin
MEP - Methylerythritol phosphate pathway
MeS - Methylthiol
MeSiP - Methylthiol isopentenyladenine
MeSZ - Methylthiol trans-zeatin
MeSZR - Methylthiol trans-zeatin riboside
MGDG - monogalacto-syldiacylglycerol
mT - Meta-topolin
MVA - Mevalonate pathway
MW - Molecular weight
NMR - Nuclear Magnetic Resonance
NT - Nucleotide
OD - Optical Density
oT - Ortho-topolin
PA - Phaesic acid
PC - Phosphatidylcholine
PE -Phosphatidylethanolamine
PG - Phosphatidylglycerol
Ppm - Parts per million
PS - Phosphatidylserine
PUFA - Polyunsaturated fatty acid
R - Riboside
RR - Response regulators
SQDG - Sulfoquinovosyldiacylglycerol
TAG - Triacylglycerides
TCS - Two-component system
TDZ - Thidiazuron
TLC - Thin Layer Chromatography
tZ - trans-Zeatin
UTEX - University of Texas

1. Literature Review

1.1 Algae Classification, Evolution and Compounds:

Algae are defined as eukaryotic, usually autotrophic/photosynthetic organisms, not including higher plants (Bhattacharya and Medlin, 1998; Lee, 2008). The term "microalgae" refers solely to unicellular algae (Tarakhovskaya *et al*., 2007). According to Bhattacharya and Medlin (1998), the main lineages of algae break down into: Dinoflagellates, Glaucophyta, Heterokonta, Haptophyta, Euglenophyta, Cryptophyta, Chlorarachniophyta, Rhodophyta and Chlorophyta. The lineage Chlorophyta encompasses all green algae, of which we shall be focusing on the class *Chlorophyceae* (Bhattacharya and Medlin, 1998).

Chlorophyta emerged in the Precambrian period in the Proterozoic era, approximately 3000 million years ago (Lee, 2008). Green algae share features such as the inclusion of chlorophyll a and b as well as some pigments like xanthophylls and carotenes. The most common polysaccharide found is starch, commonly inside the chloroplast, and the cell walls are composed of a cellulose fibre matrix while the cell membrane is composed of a lipid bilayer (Leliaert *et al*., 2012).

Algae have been used in many ways and for many different purposes by humans throughout history. Today components from algae are commonly integrated into human foods as a supplement or a colorant (Gong *et al*., 2011) and are used widely as a feedstock in aquaculture (Spolaore *et al*., 2006). However, we have learned that algae are not only healthy in the diet but are also ideal organisms for the production of high value compounds for pharmaceutical or industrial uses (Gong *et al*., 2011). This is because their metabolisms can be easily manipulated by changes in abiotic factors to overproduce a wide range of substances (Gong *et al*., 2011).

There are a few species of algae which are currently being used to produce pharmaceutical products on an industrial scale for the fatty acids (FA's) eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), various proteins (Gong *et al*., 2011), carotenoids such as β-carotene, lutein, xanthins, polyphenols, tocopherols, vitamins and minerals (Priyadarshani and Rath, 2012). Other useful compounds include certain toxins, sterols, amino acids, anti-microbial, anti-fungal and anti-viral agents (Priyadarshani and Rath, 2012).

It has also been found that most microalgae have the ability to produce large quantities of lipids, especially triacylglycerols (TAG's), under suboptimal growth conditions, particularly nitrogen deprivation (Fuentes-Grunewald *et al*., 2012; Gardner *et al*., 2011). Under abiotic stress, growth slows and the algal cell switches to a metabolism that enhances carbon storage in lipid bodies instead of synthesising proteins and carbohydrates (Xin *et al*., 2010). This TAG accumulation makes algae attractive as a source of biofuel in addition to other advantages over other plant based biofuel sources such as: their ability to be grown on marginal lands, use of untreated water, rapid growth rate, and the ability for year-round harvest (Gouveia and Oliveira, 2009). Moreover, algae are extremely efficient fixers of carbon dioxide, making them good candidates for the removal of flue gases released from industrial operations (Lopes da Silva *et al*., 2009).

Although the phenomenon of lipid accumulation occurs under stressful conditions, it is important to note that during favorable growth conditions with high nutrient availability, lipid accumulation is low (Šoštarič *et al*., 2009). In a study to determine the optimal growth media for the microalgae *Chlorella vulgaris*, it was determined that when grown in nutrient-rich media the extractible lipid yield only reached a maximum of 1.69% of the algae dry weight (Šoštarič *et al*.,

2009). Another study using the same species under nitrogen deprived conditions produced a lipid yield of ~53% (Widjaja *et al*., 2009).

Algae grown under favourable conditions follow a sigmoidal growth pattern characterised by three major phases: the lag, exponential and stationary phases. Each phase has characteristic effects on the nutrient level left in the media, cell reproduction rate and the production of different cellular compounds including carbohydrates, proteins and lipids. These phases and culture changes are depicted in Figure 1.

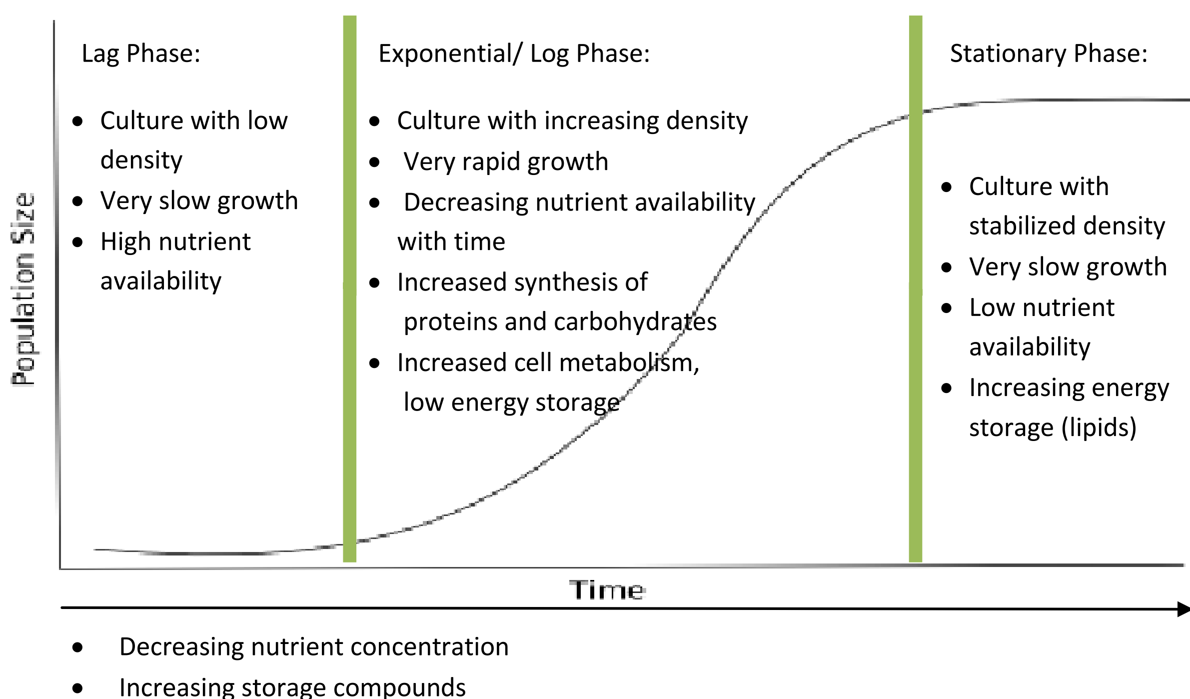

Figure 1: Sigmoidal growth of typical closed system algae culture showing growth phases and media changes associated with phases

1.2 Abiotic Manipulation of Algae:

Different combinations of growth conditions can trigger significant changes in the production of compounds such as pigments, carbohydrates, proteins and lipids within algae cells.

For example, with increasing photoperiod (light: dark; 16:8 hr) in combination with a light intensity of 100 μmol m^{-2} s^{-1} the highest growth rate for *Chlorella vulgaris*, along with a decrease in chlorophyll *a* content, was observed, concomitant with an increase in β-carotene and protein content (Seyfabadi *et al*., 2011). With decreasing photoperiod and light intensity the opposite was observed for chlorophyll *a*, β-carotene and protein content (Seyfabadi *et al*., 2011). An increase in chlorophyll *a*, total carbohydrates and protein content per cell can also be obtained by growing cultures of *C. vulgaris* in nitrogen deprived media and with a 10^{-7} M input of cadmium (Chia *et al*., 2015). These conditions produced the highest lipid content, and, notably, the greatest increase in TAG's with a corresponding decrease in polar lipids. Interestingly, these conditions also produced the lowest cell density but the highest dry weight when compared to sufficient nitrogen and decreased cadmium concentrations (Chia *et al*., 2015). In terms of lipid content, high nitrogen conditions produced a significantly reduced lipid content as well as a very low TAG accumulation of only 3% of the total lipid (Stephenson *et al*., 2010; Figure 2).

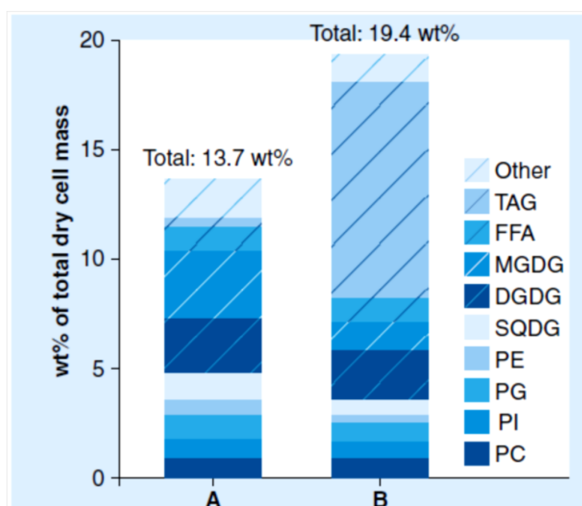

Figure 2: Lipid composition of *Chlorella vulgaris* after A) 20 days in nitrogen sufficient media and B) 12 days in nitrogen deficient media (Stephenson *et al*., 2010)

1.3 Phytohormones:

Algae, like any other living organism, rely on hormones to regulate biochemical processes within the cell (Tarakhovskaya *et al*., 2007). A hormone is generally defined as a compound of low molecular weight (~225 MW), which is excreted by organelles in the cell in low concentrations (usually <1nm) over time. The effects of hormones are far reaching within the organism and can dictate anything from metabolic reactions to gene transcription. Hormones found within plants are specifically termed phytohormones (Tarakhovskaya *et al*., 2007), although the same compounds are now known to be frequently found in many other organisms (Spichal 2012).

Microalgae have been known to naturally produce, and react to, the exogenous application of a mixture of cytokinins (CK's) (Piotrowska and Czerpak, 2009; Noble *et al*., 2014) as well as abscisic acid (ABA) (Noble *et al*., 2014) and were the two hormone groups studied in this thesis. Therefore most of the following discussion will be focused on CK's and ABA synthesis and degradation and their effects on microalgae. A brief summary of the effects of some other known phytohormones on both higher plants and algae can be viewed in Table 1.

1.3.1 Cytokinins - Most CK's are synthesised from adenine (Davies, 2004) by the replacement of the N^6-side chains (Spíchal, 2012), in developing seeds and roots and are transported through the xylem vessels (Davies, 2004). It is known that the enzyme isopentenyltransferase (IPT) is responsible for the initiation of CK synthesis, while cytokinin oxidase-dehydrogenase (CKX) is responsible for CK degradation (Spíchal, 2012). In between there are many interconversions among free base (FB), riboside (R), nucleotide (NT), glucosides (GLUC) and other forms (see Figure 5). These hormones are involved in many plant processes including cell division, the

delay of leaf senescence, cell enlargement and therefore leaf broadening, chloroplast formation, opening of the stomata, plant morphogenesis (differentiation of parts), growth of lateral buds (Davies, 2004) and response to abiotic and biotic stressors (Spíchal, 2012).

1.3.1.1 Cytokinins in Algae – Although now considered plant hormones, one theory of how CK's were obtained by plants of all types is through horizontal gene transfer (HGT) from bacteria during the endosymbiotic event which gave rise to chloroplasts from cyanobacteria (Spíchal, 2012). The genes involved in the synthesis of the CK enzymes isopentenyltransferase (IPT) and CKX have been identified in several species of cyanobacteria, along with corresponding CK's (Spíchal, 2012). Some studies have also found genes which code for proteins used in a "two-component system" (TCS) signal transduction pathway employed by prokaryotes and lower eukaryotes (Pils and Heyl, 2009). It was also found that algae contain Type B Response Regulator (RR) genes, which create essential members of the TCS, as well as Type C RR's, which are mostly shared by all plants (Pils and Heyl, 2009).

Though these RR genes were found in algae, it is interesting to note that no conventional CK receptors were found in some studies of algae (Frébort *et al*., 2011; Pils and Heyl, 2009) though CK's are known to be synthesised in brown (*Phaeophyta*), red (*Rhodophyta*), and green (*Chlorophyta*) algae (Tarakhovskaya *et al*., 2007). However, Blanc *et al* (2010) found orthologs of *Arabidopsis* genes coding for phytohormone receptors for CK's, ABA, auxins, brassinosteroids, jasmonic acid and polyamines in the genome of *Chlorella variabilis* NC64 A which is a very close relative of the green unicellular algae *Chlorella vulgaris* (Blanc *et al*., 2010). It is, therefore, likely that lower eukaryotes such as algae do, in fact, possess CK receptors though they may be of a more primitive form which would function with a TCS.

Cytokinins are common to almost all organisms, and can be split into two groups: adenine type and phenylurea type CK's (Spíchal, 2012). Adenine type CK's either have saturated or unsaturated side chains with two or more units of hydrocarbons. These types of adenine CK's are called isoprenoid adenine CK's (Figure 3) and include the hormones N^6-isopentenyladenine (iP), dihydrozeatin (DZ), *trans*-zeatin (tZ) and *cis*-zeatin (cZ). The second type of adenine CK's are the aromatic adenine CK's, named because of the substitution of the side chains for aromatic rings (Figure 3). These hormones include N^6- benzyladenine (BA), *meta*-topolin (mT) and *ortho*-topolin (oT). The phenylurea type CK's are synthesised from phenylurea and include nitrogen in their rings. These include compounds such as *N*-(2-chloro-4-pyridyl)-*N*'-phenylurea (CPPU) ($C_{12}H_{10}ClN_3O$) and thidiazuron (TDZ) ($C_9H_8N_4OS$) (Spíchal, 2012).

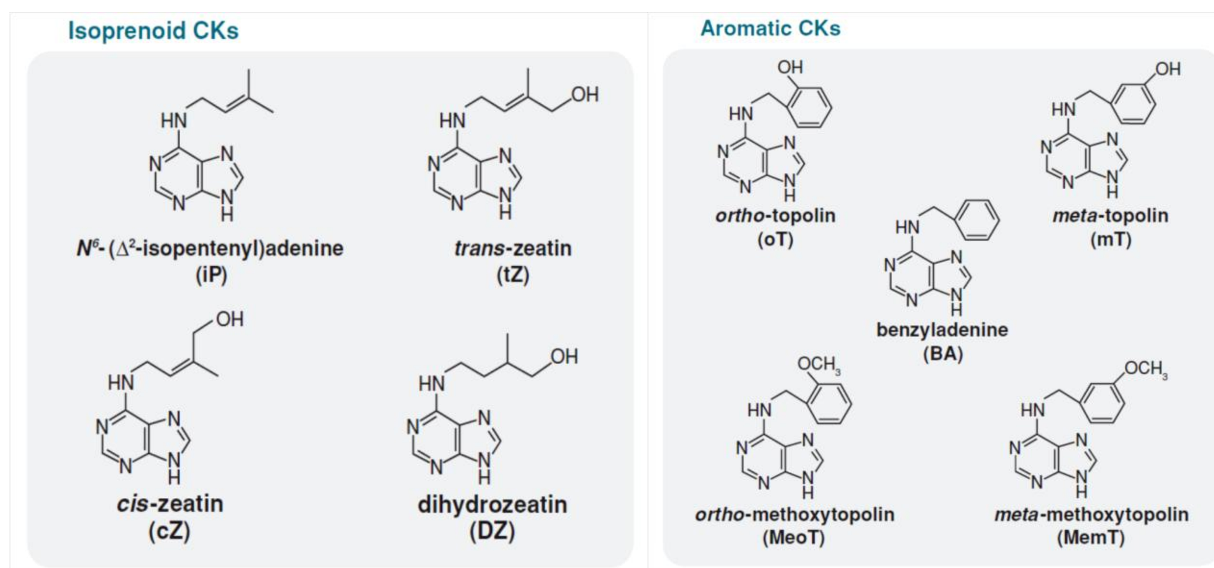

Figure 3: Diagrams of Isoprenoid and Aromatic CK types (Sakakibara, 2006)

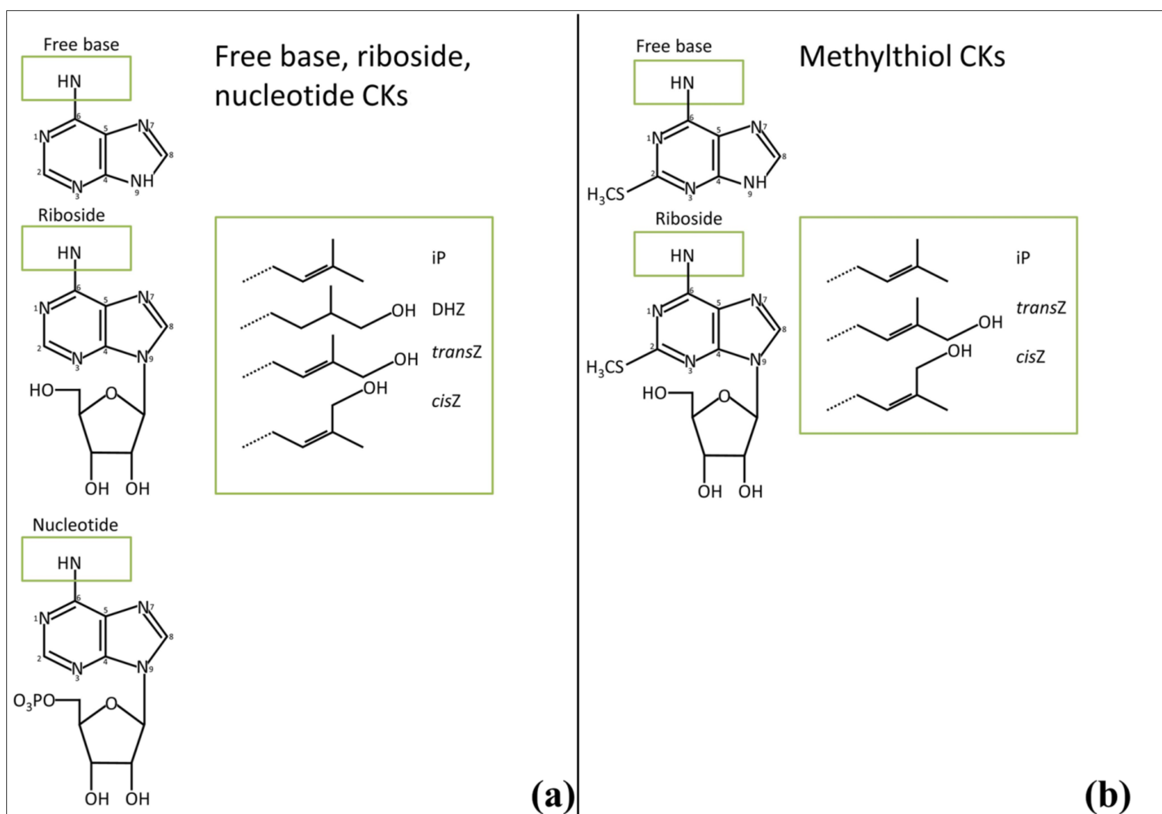

Figure 4: Diagram of freebase, riboside and nucleotide CK types and methylthiol conjugates (adapted from Morrison *et al*., 2015)

Another group of adenine type CK's, termed heterocyclic CK's, have recently been accepted which include the CK kinetin (Piotrowska and Czerpak, 2009). These hormones have long been considered to be synthetic; however, kinetin was isolated from both fresh human and plant tissue confirming its natural occurrence in both plants and animals (Piotrowska and Czerpak, 2009).

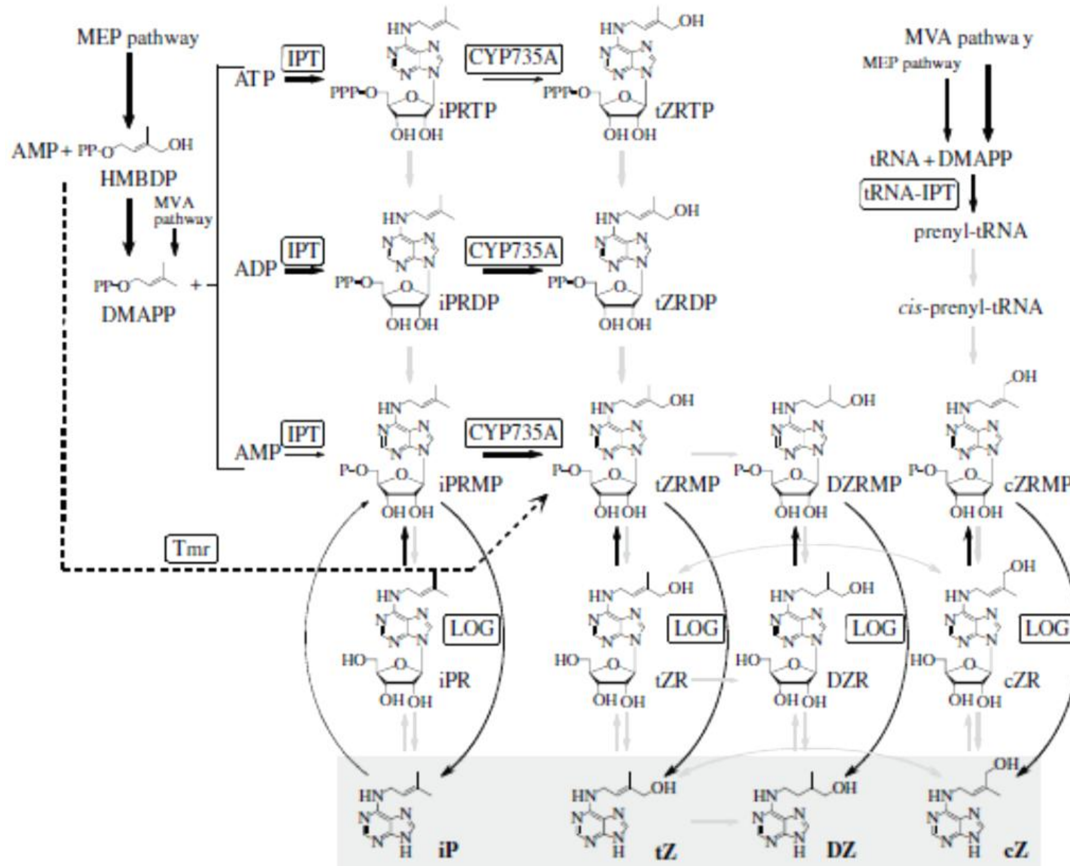

Figure 5: Cytokinin biosynthesis, interconversion and degradation scheme in plants showing action of "Lonely Guy" conversion enzyme (Kamada-Nobusada & Sakakibara, 2009)

1.3.1.2 Cytokinin Synthesis - Isoprenoid CK's are synthesised by one of two main pathways, the methylerythritol phosphate pathway (MEP) or the mevalonate pathway (MVA) (Sakakibara, 2006; Figure 5). These two pathways are responsible for the formation of the isoprenoid side chain CK's (ie. iP and tZ) and the cis isomer form (cZ) respectively (see Figure 5). In the MEP pathway adenosine phosphate-isopentyltransferases (IPT) uses either ATP, ADP or AMP to form iPRTP, iPRDP and iPRMP respectively. These molecules form the bases of the CK iP. To form the isoprenoid CK tZ, these three molecules can be hydroxylated, but only iPRMP can be further altered by the loss of a double bond to produce DZRMP (precursor for the CK DZ). Collectively

these tri-, di-, and monophosphate molecules are termed nucleotides (suffix NT) and are thought to be the least active forms, or storage forms, of the isoprenoid CK's. From there they can then be converted to riboside forms (suffix R) by the cleavage of the phosphate group to create iPR, tZR and DZR which are thought to be slightly more biologically active than the corresponding NT forms. These ribosides can then be converted to free base forms (no suffix) which are the most biologically active forms by the loss of the ribose attachment to create iP, tZ and DZ (Sakakibara, 2006). A shorter conversion has also been discovered whereby the inactive NT form can be directly converted to its fully active FB form by the action of a conversion enzyme encoded by the “Lonely Guy” (LOG) gene which is depicted in Figure 5 (Kamada-Nobusada & Sakakibara, 2009).

The MVA begins with the degradation of tRNA by tRNA IPT to produce cis-prenyl-tRNA which is then converted to cZNT (the structural isomer of tZNT). It is then converted to its R and FB forms by the same mechanisms as in the MEP pathway (Sakakibara, 2006).

Two other prevalent CK forms with slightly more elusive functions are glucosides (GLUC's) and methylthiols (MeSCK's). Glucosides are free base forms conjugated with glucose and are thought to be inactive storage forms of the different CK's, whereas MeSCK's are conjugated with a sulphur and methyl group whose function, until recently, was largely unknown (see Figure 4, 6) (Sakakibara, 2006).

A study by Morrison *et al*., (2015) on *Ustilago maydis* corn cob infected tissue saw low but accumulating amounts of methylthiol trans-zeatin FB (MeSZ) and R forms (MeSZR) in the later stages of infection with both the dikaryon and solopathogen form of *Ustilago maydis*, although none was found in uninfected cob tissue. It was suggested that these MeSCK forms may be an

accumulation based on the decreased ability for CKX degradation by the plant, and may have originated from *U. maydis* allowing for continued proliferation of cells and tumour formation. The CK GLUC storage forms remained relatively abundant with time in the control tissue, however in both infected tissue types GLUC's decreased significantly. This indicated that perhaps the fungus was liberating these forms for its own use, as infected tissue also showed increasing levels of FB, R and NT forms (Morrison *et al*., 2015).

Methylthiol type CK's were found to be abundantly produced by the bacteria *Sinorhizobium meliloti*, *S. fredii*, *S. medicae* and *Mesorhizobium loti* (Kisiala *et al*., 2013) as well as in the pathogen *Rhodococcus fasciens* (Pertry *et al*., 2009) and the photosynthetic protist *Euglena gracilis* (Noble *et al*., 2014)

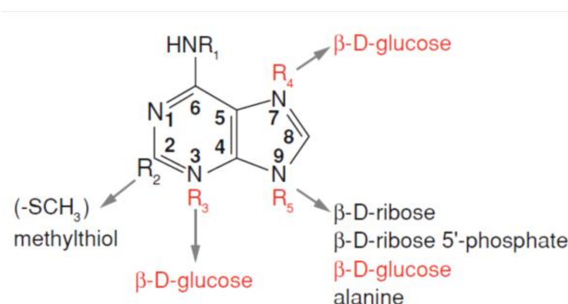

Figure 6: Diagram showing attachment sites of side chains for different CK types (Sakakibara, 2006)

Cytokinins have been shown to be sensitive to nitrogen levels, and their responses vary with changes in nitrogen sources (Sakakibara, 2006). Arabidopsis thaliana adenosine phosphates-isopentenyltransferase genes, specifically AtIPT3 and AtIPT5, responded differently in their rates of CK synthesis depending on the nitrogen source available; AtIPT3 responds well to NO_3^- under nitrogen limitation, while AtIPT5 responds to both NO_3^- and NH_4^+. This indicates that the

synthesis of CK's is nitrogen sensitive, and therefore the growth and differentiation of cells which occurs with high CK concentrations would not occur in nitrogen depleted conditions (Sakakibara, 2006).

1.3.1.3 Cytokinin Effects on Algae - Piotrowska and Czerpak (2009) conducted an exogenous hormone addition experiment on *Chlorella vulgaris* to examine the effects on growth rate and photoperiod as well as chlorophyll and carotenoid content. It was found that the highest growth rate was achieved with 10^{-6} M of diphenylurea (DPU), followed by tZ at 10^{-8} M, kinetin at 10^{-7} M and finally BA at 10^{-7} M, though all additions at 10^{-3} M had a cytotoxic effect on the cultures. All four CK's at these optimal concentrations also caused significant increases in chlorophyll (up to 226%) and carotenoids (up to 89%). It was found that DPU and tZ had positive effects on the cell division cycle during dark periods as their addition kept the light dependant enzyme NADH-hydroxypyruvate reductase active (Piotrowska and Czerpak, 2009).

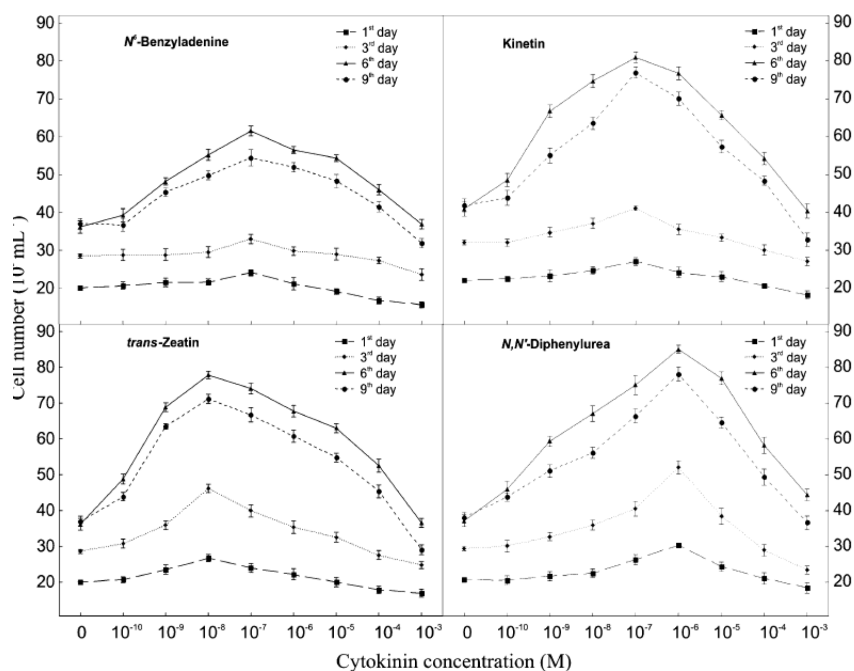

Figure 7: *C. vulgaris* cell counts in response to CK additions highlighting optimal concentrations (Piotrowska and Czerpak, 2009)

Contrary to the previous study's results, the optimal exogenous tZ concentrations for *Euglena gracilis* was found to be slightly more at 10^{-7} M, with 10^{-8} M having less of an effect on growth rate (Noble *et al*., 2014). However, 10^{-9} M was found to be the optimal concentration for all other hormones added exogenously (ABA, iP, BAP, IAA and GA_3) with concentrations of 10^{-5} M of tZ, ABA, IAA and iP being cytotoxic to the culture as shown by the dramatic decrease in cell concentration (Noble *et al*., 2014).

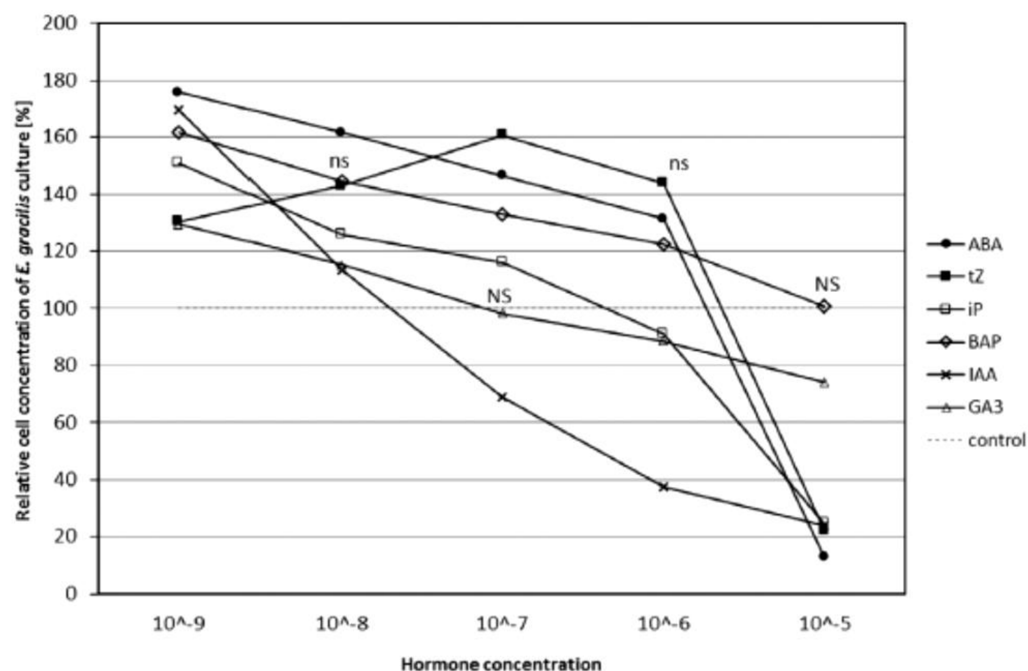

Figure 8: Growth response of *E. gracilis* to single hormone addition at multiple concentrations (Noble et al., 2014)

Twenty six different combinations of these five hormones at multiple concentrations were also tested and it was found that the combination of tZ at 10^{-7} M with ABA at 10^{-9} M produced the greatest increase in cell number by 140% above the control (Noble *et al*., 2014). This discovery partially informed the decision to use tZ and ABA as exogenous additions to *Chlorella vulgaris* in the present study. The combination with the least effect on the growth rate

was all five hormones at their optimum concentration as determined by the single hormone additions shown in Figure 8. Both single hormone treatments and multiple hormone treatments had significant effects on cell diameter as well, notably the cultures treated with optimal concentrations were larger while inhibitory concentrations caused reduced cell size. The cultures treated with combinations of hormones produced the largest cell diameter of any other treatment (Noble *et al*., 2014).

An endogenous hormone profile was also established which revealed that *E. gracilis* produced large amounts of cZR (39.43 pmol/g) and smaller amounts of MeSiP (2.37 pmol/g) which was retained in the pellet along with smaller concentrations of iP, tZNT, cZNT and iPNT and the aromatic benzylaminopurine (BAP). Larger amounts of MeSiP (3.52 pmol/ml) were secreted into the supernatant along with small amounts of tZ and iP, cZR, tZNT, cZNT, DZNT and iPNT, MeSZ, MeSZR, MeSiP and MeSiPA and BAP. Taken together these results indicate that the tRNA degradation pathway (cis/MVA pathway) is the predominant CK pathway used by *E. gracilis* (Noble *et al*., 2014).

Although much has been studied in terms of the effects of CK's on both algae growth rate and the production of different pigments and carbohydrates, surprisingly little has been studied on their effects on the fatty acid (FA) profile of the cells. As FA's are essential lipid molecules involved in many cellular functions and CK's have been shown to alter the production of similar lipid molecules, for example carotenoids (Piotrowska and Czerpak, 2009), it follows that they may have an effect on FA's as well. This link will be discussed further in a later section.

1.3.2 Abscisic Acid - Unlike the other phytohormones, ABA is not a group, but is a single compound (Davies, 2004). It is synthesised from glyceraldehyde-3-phosphate in mature leaves and roots and is transported in the xylem and phloem in higher plants. ABA is made usually in response to water shortage, and with its build up causes closing of the stomata in higher plants. It also triggers the synthesis of storage proteins, inhibits shoot growth, antagonises gibberellin in germinating grains and affects dormancy (Davies, 2004). It was originally thought that algae did not contain ABA, but instead a hormone called lunularic acid, which carried out the same roles as ABA in higher plants, triggering the stress response (Tarakhovskaya *et al.*, 2007). This theory however has been debunked by recent and growing evidence to the contrary, as ABA has been recognized in several green microalgal species as well as some brown algae. It is now accepted that algae do in fact contain ABA (Tarakhovskaya *et al.*, 2007; Noble *et al*., 2014).

1.3.2.1 Abscisic Acid Synthesis - The specific pathway which leads to ABA synthesis starts with the formation of glyceraldehyde-3-phosphate from 2-C-methyl-D-erythritol-4-phosphate (Nambara and Marion-Poll, 2005; Figure 9). This pathway is abbreviated by MEP, and is the pathway used by green algae and eubacteria, whereas another pathway is used in tandem with MEP in higher plants. The enzyme which has been shown to trigger ABA synthesis is zeaxanthin epoxidase (ZEP) in the plastid.

The second part of the synthesis, starting with xanthoxin, occurs in the cytosol, though the transport mechanism to the cytosol remains unknown. During this phase of synthesis, xanthoxin is converted to abscisic aldehyde by an alcohol dehydrogenase (ABA2) which in turns forms abscisic acid through oxidisation by abscisic aldehyde oxidase (AAO3) (Nambara and Marion-Poll, 2005).

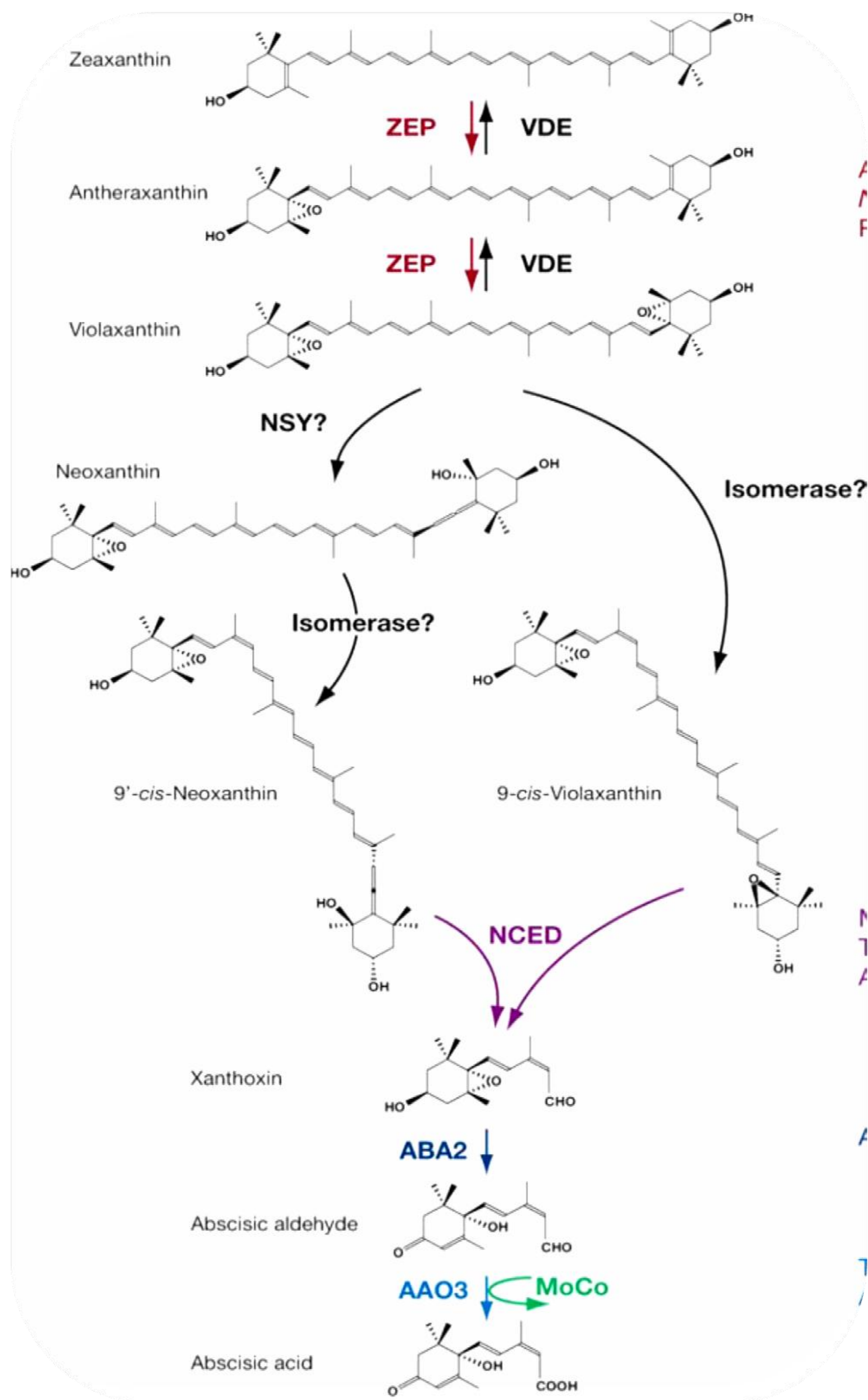

Figure 9: Diagram showing the biosynthesis pathway of ABA (Nambara and Marion-Poll, 2005)

ABA catabolism is caused by two pathways: hydroxylation and conjugation (Nambara and Marion-Poll, 2005). During hydroxylation, one of the methyl groups (usually at the C-8' position) is oxidised by cytochrome P450 monooxygenase which breaks ABA down into phaseic acid (PA) which is further catabolised to dihydrophaseic acid (DPA) by a reductase enzyme. During conjugation, the second pathway, the carboxyl and hydroxyl groups of ABA conjugate with glucose to form ABA glucosyl ester (ABA-GE) (Nambara and Marion-Poll, 2005).

The effects of abiotic stressors, especially increased salinity and decreased nitrogen, on ABA concentration has been fairly well documented (Cowan *et al.*, 1997; Tarakhovskaya *et al.*, 2007; Yoshida *et al.*, 2003). This is why, recently, ABA has become known as a stress phytohormone, because of its involvement in the stress response (Tarakhovskaya *et al.*, 2007). With an increase in salinity in some microalgal species, endogenous ABA concentrations have increased (Tarakhovskaya *et al.*, 2007). This was exemplified when algal species from *Dunaliella* and *Chlorella* were subjected to salt stress and an increase in ABA concentration was observed (Cowan *et al.*, 1997; Hartung, 2010). Increases in ABA concentration have also been noted during nitrogen deficiency in *Dunaliella*, as well as alkaline shock, heat stress, light stress, drought, oxidative stress and acid stress in several other algal species (Hartung, 2010).

Kobayashi *et al.* (1997) conducted a study on the effect of ABA on morphogenesis and carotenoid production in *Haematococcus pluvialis*, and found that ABA caused the cells to mature faster and caused an increase in carotenoid production (Kobayashi *et al.*, 1997). Therefore it can be seen that the addition of exogenous ABA can also create significant effects on algal development, as well as internal production of endogenous ABA.

Noble *et al*. (2014) found that the addition of ABA at 10^{-9} M to *Euglena gracilis* increased growth rate the most above the control when compared to other hormones from the auxin, gibberellin and CK families, especially when added in combination with tZ, as previously noted. It was also found that the addition of ABA at this optimal concentration produced an average cell diameter increase of 0.79 µm above the control (Noble *et al*., 2014).

As is the case with CK's, very little has been published on the link between FA's and ABA, though ABA is known to produce multiple effects on microalgae when added exogenously, as discussed. This specific hormone is known to be especially active when microalgae are subjected to sub-optimal growth conditions such as osmotic stress and nutrient deficiency (Hartung, 2010), which are also known to produce changes in membrane lipid composition (Kobayashi *et al*., 2006) and therefore likely have an effect on the FA composition of the cell. This link will be discussed further in a later section.

1.3.3 Other Phytohormones

It is known that hormones other than CK's and ABA are likely to play a role in lipid content, TAG accumulation and changes in fatty acid profiles. Therefore, I have summarized potential linkages with in Table 1. For pragmatic reasons I had to focus on two hormone groups, but perhaps future work may involve the study of more hormone groups.

Table 1: Table showing effects of other phytohormones (not covered in this thesis) on both higher plants and algae

Phytohormone	Effects In Higher Plants	Effects in Algae
Jasmonic Acid	○ important in defense from parasites and insects ○ promotes senescence ○ inhibits growth (Davies, 2004)	○ stimulates cell division and increases pigment production ○ increases monosaccharides and protein secretion in *Chlorella vulgaris* (Czerpak *et al*., 2006)
Salicylic Acid	○ pathogen resistance ○ inhibits ethylene synthesis ○ inhibits effects of ABA (Davies, 2004)	○ increased growth, protein content, RNA, sugars, chlorophylls, carotenoids and increased overall photosynthetic rate in *Chlorella vulgaris* (Czerpak *et al*., 2002)
Ethylene	○ fruit and leaf abscission ○ release from dormancy (Davies, 2004)	○ Unknown - effect is difficult to examine as ethylene is in gaseous form
Polyamines	○ involved in normal morphology ○ regulates cell division (Davies, 2004)	○ exogenous addition caused decreased synthesis of ornithine decarboxylase which causes increase in polyamine production in cells in *Chlamydomonas reinhartii* (Theiss *et al*., 2004)
Signal Peptides	○ defence from parasitic attack or herbivory ○ increase jasmonic acid ○ cell division, and reproduction (Davies, 2004)	○ Not Found

Auxins	○ delay of senescence and ripening ○ promotion of flowering (Davies, 2004). ○ seed development and growth ○ cell division, elongation and differentiation ○ apical dominance and tropism in response to light and gravity (Teale *et al*., 2006)	○ induce cell division and elongation, suppression of branching in some red algae (Kiseleva *et al*., 2012) ○ stimulate production of carotenoids in *Chlorella pyrenoidosa* (Czerpak and Bajguz, 1997) ○ increase biomass, chlorophyll and protein content (Czerpak *et al*., 1994). ○ increase production of proteins, pigments, phosphates, some carbohydrates, and glycolic acid (Piotrowska *et al*., 2008). ○ increased lipid content by ~12% in *Chlorella sorokiniana* with tZ (Hunt *et al*., 2010).
Gibberellins	○ control seed germination, flower and seed development ○ promote stem elongation and leaf expansion (Yamaguchi, 2008) ○ improve salt tolerance with nitrogen in *Brassica juncea* (Siddiqui *et al*., 2008).	○ increased biomass and chlorophyll *a* production in *Chlorella sorokiniana* ○ increased protein content by ~50%, lipid content by ~8% (with auxin) (Hunt *et al*., 2010). ○ break dormancy and increase germination of *Chara vulgaris* oospores (Sederias and Colman, 2007)
Brassino-steroids	○ essential for photomorphogenesis and cell elongation ○ seed germination, flowering, senescence,	○ increased growth rate ○ increased protein and nucleic acids synthesis in *Chlorella vulgaris*

	vascular differentiation, stomata formation, plant morphology and male fertility (Wang et al., 2012) o heat stress tolerance by rapeseed (*Brassica napus*) and tomatoes seedlings (Dhaubhadel *et al*., 1999) o salt stress tolerance in wheat (Ali *et al*., 2006) o fungicide for potato crops (Khripach *et al*., 2000)	(Tarakhovskaya *et al*., 2007)

1.4 Lipids:

Lipids in algae serve multiple purposes, including being the foundation for cell structures such as the cell membrane, as well as acting as a source of energy (Fuentes-Grünewald *et al.*, 2012). The major lipid classes generally found in algae are the phospholipids, galactolipids and sulfolipids (Goss and Wilhelm, 2010). Some phospholipids commonly encountered in algal cells are phosphatidylglycerol (PG), phosphatidylcholine (PC), phosphatidylserine (PS) and phosphatidylethanolamine (PE) (Goss and Wilhelm, 2010). Common galactolipids include digalactosyldiacylglycerol (DGDG) and monogalacto-syldiacylglycerol (MGDG) while the most common sulpholipid is sulfoquinovosyldiacylglycerol (SQDG) (Flaim *et al*., 2012).

In terms of percentages, the galactolipid MGDG accounts for approximately 50% of the lipids in the cell, followed by DGDG at ~30%, and SQDG and PG at ~5-12% (Flaim *et al*., 2012). In green algae, another lipid class has been identified called betaine lipids, mainly diacylglyceryltrimethylhomoserine (DGTS), which sometimes replaces PC in green marine algae (Goss and Wilhelm, 2010).

All algal lipids have specific roles within the cell and perform specific functions based on their physical and chemical properties. The thylakoid membrane of the chloroplast is generally composed of SQDG and the phospholipid PG, which both carry a negative charge (Flaim *et al*., 2012). The galactolipids DGDG and MGDG have no charge and fill the spaces between membrane proteins as well as provide added stability for the membrane, because of their linear acyl chains and therefore tight packing abilities (Flaim *et al*., 2012). Chemical structures of some of the main structural lipids found in algae are depicted in Figure 10.

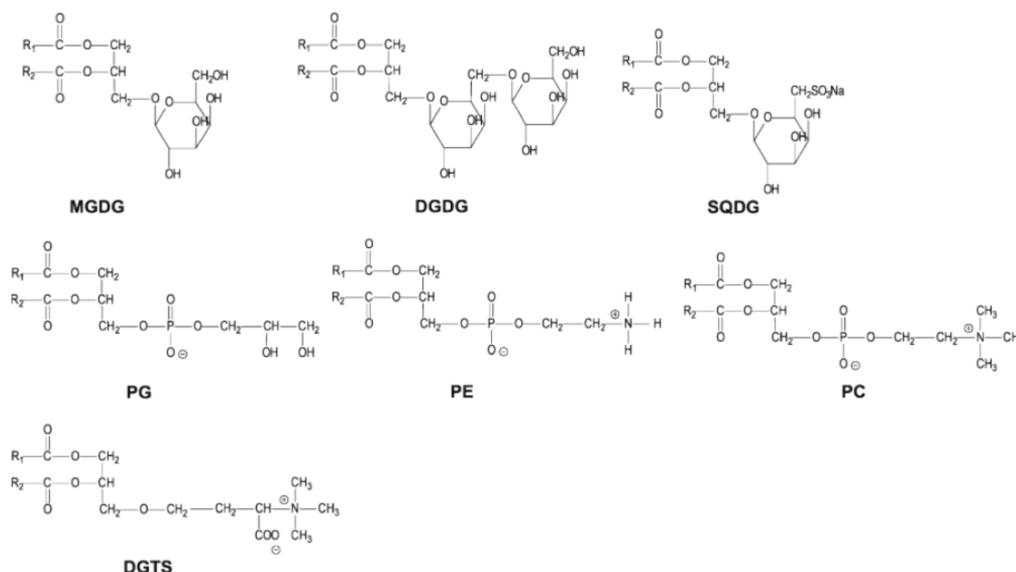

Figure 10: Chemical structures of major algal lipids (Goss and Wilhelm, 2010)

1.4.1 Lipid Manipulation in Algal Systems - When subjected to suboptimal or stressful conditions such as very high light intensity or nitrogen starvation, algae tend to accumulate lipids as a store of energy (Chen *et al*., 2011). These lipid stores can accumulate between 1-85% of the cells dry weight (Chen *et al*., 2011). The storage lipids are usually composed mainly of neutral lipids, including TAG's, which contain large amounts of carbon (eg. ~55 carbons per molecule) (Fuentes-Grünewald *et al.*, 2012). This high carbon content is what makes these algal oils especially good candidates for use as biofuel (Fuentes-Grünewald *et al.*, 2012). However this poses a problem, as high growth rates only occur when environmental conditions are favourable, and TAG accumulation only reaches a substantial amount when conditions are poor (Chen *et al*., 2011). These remarkable increases in lipid accumulation also occur in conjunction with significantly lowered biomass during nitrogen starvation (Gouveia *et al*., 2009).

Studies specifically conducted on the algae *Dunaliella tertiolecta* (Chen *et al*., 2011), *Coelastrella saipanensis*, *Scenedesmus obliquus* (Gardner *et al*., 2011), *Alexandrium minutum*, *Heterosigma akashiwo* (Fuentes-Grünewald *et al*., 2012) and *Neochloris oleoabundans* (Beal *et al*., 2010; Gouveia *et al*., 2009; Li *et al*., 2008) have all concluded that suboptimal growth conditions positively affect lipid accumulation in algal cells. Most of these studies have emphasised nitrogen limitation/starvation as being the main cause of lipid accumulation, including TAG's (Beal *et al*., 2010; Chen *et al*., 2011; Fuentes-Grünewald *et al.*, 2012; Gardner *et al*., 2011; Gouveia *et al*., 2009; Li *et al*., 2008). It is also important to note that TAG accumulation increases significantly during the stationary growth phase when compared to the exponential growth phase (Fuentes-Grünewald *et al.*, 2012).

1.5 Fatty Acids:

Fatty acids are aliphatic lipid compounds which are produced by eukaryotes mainly in the plastid, however the endoplasmic reticulum also plays a role in synthesis and chain elongation (Sato *et al*., 2003). The end result of plastid FA synthesis is the formation of phosphatidates, which are converted into the major chloroplast lipids (Sato *et al*., 2003). The first part of synthesis occurs in the plastid (termed the plastidial pathway), where malonyl-CoA is synthesised from acetyl-CoA via the action of acetyl-CoA carboxylase (ACCase) (Ohlrogge and Jaworski, 1997). The action of ACCase, and therefore the synthesis of malonyl-CoA, is thought to be a light dependant process. Following this, the malonyl moiety is transferred by fatty acid synthase (FAS) to an acyl carrier protein (ACP), where FAS causes acyl chain extension with malonyl-ACP. Stearoyl-ACP desaturase then inserts a *cis* double bond at the C9 position of C18:0 ACP, the saturated stearic acid. Fatty acid synthesis is terminated either by transfer of the acyl chain from the ACP or by hydrolysis, catalyzed by acyl-ACP thioesterases. Some of these synthesised FA's then leave the plastid and enter the eukaryotic lipid pathway in the endoplasmic reticulum, where they are generally esterified into glycerolipids (Ohlrogge and Jaworski, 1997).

Unsaturated FA's are distinguished by the presence of one or more double bonds between the carbon atoms (Simopoulos, 1991). This group can be split into two categories: mono and polyunsaturated FA's. Monounsaturated FA's are represented by oleic acid, which has a single double bond between the 9th and 10th carbon atom from the methyl end. Polyunsaturated FA's (PUFA's) have multiple double bonds and can be further divided into two more groups depending on the location of the first double bond: ϖ3 (eg. α-linolenic) and ϖ6 (linoleic) FA's. These two FA's can be metabolised into 20 and 22 carbon chains, for example, linolenic acid (LA) can be metabolised into arachidonic acid (AA), while α-linolenic (ALA) can be converted

into eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA). These long chain FA's are very important, as they cannot be synthesised without LA and ALA precursors in the body (Simopoulos, 1991).

It has been found that abiotic factors can cause significant effects on the FA profile of some microalgal species, such as photoperiod and light intensity. Both increasing photoperiod and increasing light intensity from 37.5 to 100 µmol photons m-2 s-1 increased the amount of saturated FA's to 28.67% while simultaneously decreasing the amount of mono and polyunsaturated FA's in *Chlorella vulgaris* (Seyfabadi *et al*., 2011). It has also been found that the addition of cadmium in combination with low nitrogen media increased saturated FA and monounsaturated FA content while decreasing PUFA's (Chia *et al*., 2013).

1.6 Abiotic Factors Affecting Lipid and Fatty Acid Compositions:

Several abiotic/ environmental factors have been shown to produce a variety of changes in lipid composition as well as FA composition in almost all classes of algae. Changes to the FA profiles and lipid composition are now accepted as being a consequence of maintaining cell structure during adverse environmental conditions (Goss and Wilhelm, 2010). For example, during sulphate starvation, the negatively charged structural membrane lipid SQDG is being consumed and not being produced; therefore, an increase of PG is observed in the membrane to balance the charges and provide structural support. A similar phenomenon occurs with phosphorus limitation, whereby, under these conditions, a decrease in phospholipids occurs with an increase in betaine lipids and glycolipids. Furthermore, the FA profile of the algae generally shifts towards more unsaturation with nutrient starvation (Goss and Wilhelm, 2010).

A study focusing on iron concentration and lipid accumulation showed that high concentrations of iron increased lipid content in *Chlorella vulgaris* to up to 56% of its total biomass (Liu *et al*., 2008). Interestingly, in another study of lipid accumulation by *Scenedesmus* sp. phosphate limitation caused an increase in lipids of 53% whereas nitrogen limitation only caused 30% (Xin *et al*., 2010). Unfortunately no FA analysis was conducted on these lipids, however this shows that results of nutrient limitation can vary widely between species.

A study by Fuentes-Grünewald *et al.* (2012) was conducted to determine the effects of aeration, temperature and nitrogen deficiency on the accumulation of TAG's in algae (Fuentes-Grünewald *et al.,* 2012). Treatment conditions of high temperature and low nitrogen causing an increase in both TAG's and free FA's. However, other than a slight increase in arachidonic acid in the treatment samples, no significant changes to the FA profile were observed (Fuentes-Grünewald *et al.,* 2012).

The combined effect of nitrogen deprivation and high media pH on lipid accumulation was investigated in *Coelastrella saipanensis* and *Scenedesmus obliquus* and though it was found that independently both conditions increased TAG accumulation significantly, in combination the effects were amplified (Gardner *et al*., 2011). This was likewise found to a certain extent by Widjaja *et al*., (2009) who subjected *Chlorella vulgaris* to low CO_2 concentrations and nitrogen deprivation. It was realised that under insufficient CO_2, the algae would use carbonate to meet carbon dioxide requirements, therefore increasing the pH of the media. Conversely, under high CO_2, pH would decrease because excess CO_2 would dissolve and become H_2CO_3 in the media. In this study, both CO_2 and nitrogen deprivation yielded an increase in TAG accumulation (Widjaja *et al*., 2009). Interestingly, Carvalho and Malcata (2005) also found that excess CO_2

concentrations caused an increase in total lipid content, however, a decrease in PUFA’s was also detected at high CO_2 concentrations (Carvalho and Malcata, 2005).

Variation in light intensity was shown to cause changes to algal lipids including FA profiles. Growth under low light tends to produce an increase in polar membrane lipids such as DGDG and MGDG as well as an increase in PUFA’s, while high light intensity tends to cause an accumulation of saturated FA's in the form of TAG’s (Goss and Wilhelm, 2010). Contrary to this trend, a different study found that exposure to high light intensities caused a large increase in TAG’s, while low light caused an increase in structural thylakoid lipids (Khotimchenko and Yakovleva, 2005). However, no significant change in the FA profile was observed (Khotimchenko and Yakovleva, 2005). Another study was conducted to determine the effects of different wavelengths on lipid accumulation, using red and blue LED lights at 1000 lux (Shu *et al*., 2012). It was found that red LED light caused an increase in biomass, while blue LED light caused an increase in lipid production (Shu *et al*., 2012).

A decrease in temperature generally leads to more FA unsaturation, whereas an increase in temperature causes a decrease in PUFA’s (Goss and Wilhelm, 2010). However, the effects of temperature can be quite variable (Goss and Wilhelm, 2010). In a study by Kalacheva *et al*. (2002), above optimal temperatures increased TAG accumulation in *Botryococcus*, and decreased the trienoic FA content (Kalacheva *et al*., 2002). Higher temperatures were also found to inhibit the synthesis of most other cellular lipids (Kalacheva *et al*., 2002). Almost the opposite results were discovered in another study, where it was found that increased temperatures caused an increase in trienoic FA's, and a decrease of unsaturated FA's (Sushchik *et al*., 2003). An earlier study showed an increase in betaine lipids and PUFA’s with a decrease in temperature (Tatsuzawa and Takizawa, 1995).

1.7 Effects of Plant Hormones on Presence of Fatty Acids:

Research into how phytohormones affect FA profiles of higher plants started very early on, following the discovery of phytohormones and adequate ways to isolate and detect them. However, this interest seems to have gone dormant and only recently resurfaced in the last decade or so. It emerged with more of a focus on algae and phytohormones, because of their wide range of FA synthesis capabilities and the ability to produce higher yields of pharmaceutically and industrially important FA's per capita than higher plants. Although this interest has re-emerged, published research on this topic remains relatively scarce.

Most of what is known about interactions of CK and FA comes from the study of higher plants. For example Kull *et al*., (1978) sought to determine whether exogenously applied CK's had an effect on both the lipid and FA profiles of *Coleus blumei* and *Impatiens sultani* leaves and shoots. In *C. blumei* leaves it was found that in the phospholipid portion linolenic and linoleic acid both decreased by 8.5 and 4% respectively, but increased in the glycolipid portion by 6.5 and 7.1% respectively with the addition of 100 µg/ml of tZ. A similar trend was seen when shoots were exposed to the same concentration of tZ; however, a decrease in palmitic acid by 9% was also identified. The results were similar when the same experiment was conducted on the shoots and leaves of *I. sultani* (Kull *et al*.,1978).

Wang and Faust (1988) investigated the effects of thidiazuron (a synthetic cytokinin) application on the FA and sterol content of golden delicious apple buds and found it caused an increase in unsaturated FA's, namely linoleic and linolenic. It produced an overall decrease in free sterol content especially sitosterol and an increase in both campesterol and stigmasterol (Wang and Faust, 1988).

In more recent studies among phytohormones, lipids, and FA's, overall lipid yield is commonly one of the first measured responses. In a study conducted on *Hibiscus sabdariffa* seeds, it was found that, the application of 100mg/L of both the gibberellin GA_3 and the aromatic CK benzyladenine (BA), caused an increase in oil yield by 22.3% above the control plant leaves (Mostafa *et al*., 2005). With this concentration, marked increases in unsaturated FA's especially oleic and linoleic were detected as well as corresponding decreases in saturated FA's, especially palmitic acid (Mostafa *et al*., 2005).

Jadhav *et al*., (2008) investigated the effect of ABA and some of its hydroxylated metabolites (7' and 9'-hydroxy ABA) and a catabolite (8'-hydroxy ABA) on the total oil yield, lipid profile, FA profile and gene induction in *Brassica napus* seeds. It was found that 8'-hydroxy ABA increased total oil content by 18% over the control, while ABA itself increased TAG content significantly. Both ABA and its metabolites doubled the amount of very long chain unsaturated FA's such as erucic (22:1) and eicosenoic (20:1) acid by inducing the expression of olein and the fatty acid elongase (*FAE1*) genes (Jadhav *et al*., 2008).

More recently, combinations and single hormones were added exogenously to the green algae *Chlamydomonas reinhartii* in order to increase biomass and lipid yield (Park *et al*., 2013). It was found that the addition of IAA, GA_3, kinetin and 1-triacontanol (TRIA) all had stimulatory effects on biomass with a maximum increase of 68% by kinetin (1 ppm). Although ABA was the only hormone which did not stimulate growth it had the most significant effect on the FA profile, causing a 13% increase in FAME content at 5 ppm (Park *et al*., 2013; Figure 11).

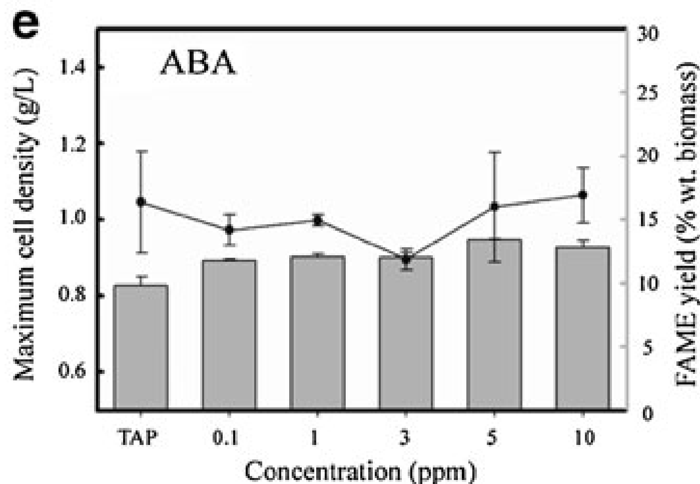

Figure 11: Differences in *C. reinhartii* cell density and FAME yield in response to ABA treatments (Park *et al*., 2013)

1.8 Thesis Rationale:

There are some minor discrepancies in the hormonal profiles of most phytohormones in algae, as their numerous forms and partially unknown pathways make the undertaking complex. However, many more clarifications and breakthroughs have been made in recent research projects which are helping to answer these questions. While there has also been an increase in published research involving exogenous hormone addition to microalgae which is helping to clarify their role in these organisms (Noble *et al*., 2014; Pitrowska *et al*., 2008; Tarakhovskaya *et al*., 2007), existing studies have not taken much care towards matching endogenous hormone profiles with exogenous application experiments. This thesis will concentrate on two groups of hormones, CK's and ABA, their endogenous profiles in *Chlorella vulgaris* and how their exogenous application affects FA presence.

The most significant gap in knowledge in published works to date on the topic of phytohormones in algae is the lack of information on MeSCK's. This group was recently

discovered and seems to be present in a diverse array of tissues, yet still has an unknown function. The forms MeSZ, MeSZR, MeSiP and MeSiPA have been positively identified in the motile algae *Euglena gracilis* in significant amounts in the media (supernatant); however no speculation on their possible function was discussed (Noble *et al*., 2014). Previously, studies on *Rhizobium* bacteria and the fungi *Ustilago maydis* also found that MeSCK's were produced and released in large quantities (Kisiala *et al*., 2013; Morrison *et al*., 2015). It should also be noted that no works on exogenous applications of MeSCK's on higher plants or algae have been attempted and published to the best knowledge of the author.

Given these challenges it is perhaps not surprising that so little has been published regarding the exogenous addition of phytohormones to microalgae to investigate the effects on the lipid and FA profiles. As it has been well documented that hormones play an integral role in all aspects of microalgae metabolism and it is also known that algae have the capacity to produce high value oils and FA's. Thus a critical scientific gap remains since so few papers have been published to ascertain if this group of phytohormones can be used as a tool to alter these oils and FA's.

A second oversight in the research to date is the lack of investigation on lipid and FA profiles of microalgae grown in resource rich conditions. As the focus recently has been the optimization of maximum lipid and FA yield from algae and as this only tends to occur under poor growth conditions, very little has been mentioned about the levels under ideal growth conditions with the exception of a few papers. However, under favourable conditions algae may provide a FA profile that is more conducive to scaffolding higher value FA for the engineering of compounds for commercial use.

1.9 Objectives, Hypotheses and Predictions:

The goal of this thesis is twofold. First, I will determine the endogenous CK and ABA content of the unicellular, non motile, freshwater green algae species *Chlorella vulgaris.* Second, I will examine the effects of their exogenous applications at three different physiologically relevant concentrations on the growth rate, total lipid production and FA profile of this microalgae.

The endogenous hormone content was determined at the beginning of the exponential growth phase of the culture using a modified version of the extraction method described by Farrow and Emery (2012) with identification of up to 25 hormones by electrospray-ionization, liquid chromatography-tandem mass spectrometry (HPLC-(ESI) MS/MS). Based on the literature and the endogenous hormone profile acquired, four prominent hormones were chosen for the exogenous additions which were added at the beginning of the exponential phase of the culture in a single dose. The cultures were harvested at the end of the exponential phase and the total lipids extracted in hexane using a Soxlhet apparatus. Total lipids were transesterified to fatty acid methyl esters (FAME's) and identified using gas chromatography flame ionization detection.

It was hypothesized that the addition of different phytohormones at different concentrations would produce changes in growth rate, lipid yield and the FA profile of the microalgae *Chlorella vulgaris.* Based on the available published literature, it was predicted that with increasing concentrations of ABA, there would be a decrease in growth rate, an increase in the total lipid yield, and an increase in the ratio of unsaturated to saturated FA's. As ABA is generally used as a stress signalling hormone, the culture was expected to react as if conditions

were unfavourable, and therefore would begin accumulating lipid stores. With respect to CK's, increasing concentrations of tZ, BA and MeSZ were expected to increase growth rate, with a decrease in overall lipid yield. The FA ratio of unsaturation to saturation was also predicted to increase. These three hormones are considered growth promoting hormones, including MeSZ by the conclusions of Morrison *et al*. (2015); therefore, the culture was expected to favour rapid growth over lipid accumulation.

2. Methods and Materials:

2.1 Culture Maintenance and Growth Conditions:

The freshwater algae species *Chlorella vulgaris* (UTEX #265) was obtained in 500ml bulk culture from the UTEX Culture Collection of Algae. The culture was maintained in autoclaved Bold 3N medium (see Table 2) containing a trace metal solution (see Table 3), and was allowed to grow until an optical density (OD) of approximately 1.5 measured at 680 nm was reached (Genesys 20 Visible Spectrophotometer, Thermo Scientific). The culture was then split and maintained as 500 ml cultures in 1L autoclaved glass bottles in a growth chamber (Conviron, Model PGR15) on an 18 hr photoperiod at 24°C/20°C in the light/dark. Full spectrum Sunblaster CFL bulbs (Sunblaster, ESP 26W, 6400K E26) and fluorescent lighting (Phillips Alto, FT2T8, 65W, C9) with a total output of 200 µmol photons m^{-2} s^{-1}, were used to simulate partial sun conditions and cultures were constantly bubbled from the base of the culture with filtered atmospheric air (Millipore, PTFF 0.22) using air pumps (Marina 200, 2x110 L/h). Each culture bottle was also covered with an air filter membrane (3M, Filtrete Microparticle and Allergen Reducer).

Table 2: Recipe for Bold 3N Medium (Table adapted from UTEX Culture Collection of Algae)

Component	Amount	Stock Solution Concentration	Final Concentration
$NaNO_3$ (Fisher BP360-500)	30 mL/L	10 g/400mL dH2O	8.82 mM
$CaCl_2 \cdot 2H_2O$ (Sigma C-3881)	10 mL/L	1 g/400mL dH2O	0.17 mM
$MgSO_4 \cdot 7H_2O$ (Sigma 230391)	10 mL/L	3 g/400mL dH2O	0.3 mM
K_2HPO_4 (Sigma P 3786)	10 mL/L	3 g/400mL dH2O	0.43 mM
KH_2PO_4 (Sigma P 0662)	10 mL/L	7 g/400mL dH2O	1.29 mM
NaCl (Fisher S271-500)	10 mL/L	1 g/400mL dH2O	0.43 mM

P-IV Metal Solution	6 mL/L

Table 3: Recipe for P-IV metal solution (Table adapted from UTEX Culture Collection of Algae)

Component	Amount	Stock Solution Concentration
$Na_2EDTA \cdot 2H_2O$ (Sigma ED255)	0.75 g/L	2 mM
$FeCl_3 \cdot 6H_2O$ (Sigma F-1513)	0.097 g/L	0.36 mM
$MnCl_2 \cdot 4H_2O$ (Baker 2540)	0.041 g/L	0.21 mM
$ZnCl_2$ (Sigma Z-0152)	0.005 g/L	0.037 mM
$CoCl_2 \cdot 6H_2O$ (Sigma C-3169)	0.002 g/L	0.0084 mM
$Na_2MoO_4 \cdot 2H_2O$ (J.T. Baker 3764)	0.004 g/L	0.017 mM

2.2 Growth Curve:

Three randomly selected 500ml healthy cultures were combined in an autoclaved 3L conical flask and mixed thoroughly. The cultures were split into 6 new 500ml cultures containing an equal ratio of algae culture to fresh autoclaved media (250 ml: 250 ml). Three of these new cultures were used to construct a growth curve using OD_{680} measured daily on a desktop spectrophotometer (see Figure 12A). Each culture had an approximate starting OD of 0.900. When an OD of 1.5 was reached, spectrophotometer samples were diluted by half so as to ensure OD values were within the linear response of the detector, then the values obtained were multiplied by two.

As the cultures required vigorous bubbling to prevent settling as well as a large headspace, the rapid evaporation of water from the media contributed to the increasing concentration of cells in the culture, thereby continuing to increase OD. This was not considered as a source of stress as the nutrient level in the media was not affected however the incresing concentration of cells made it difficult to ascertain the period of exponential growth. Therefore,

cell counts were also conducted each day giving change in number of cell per week (see Figure 12B) and correlated with OD, in order to clarify the length of the exponential phase.

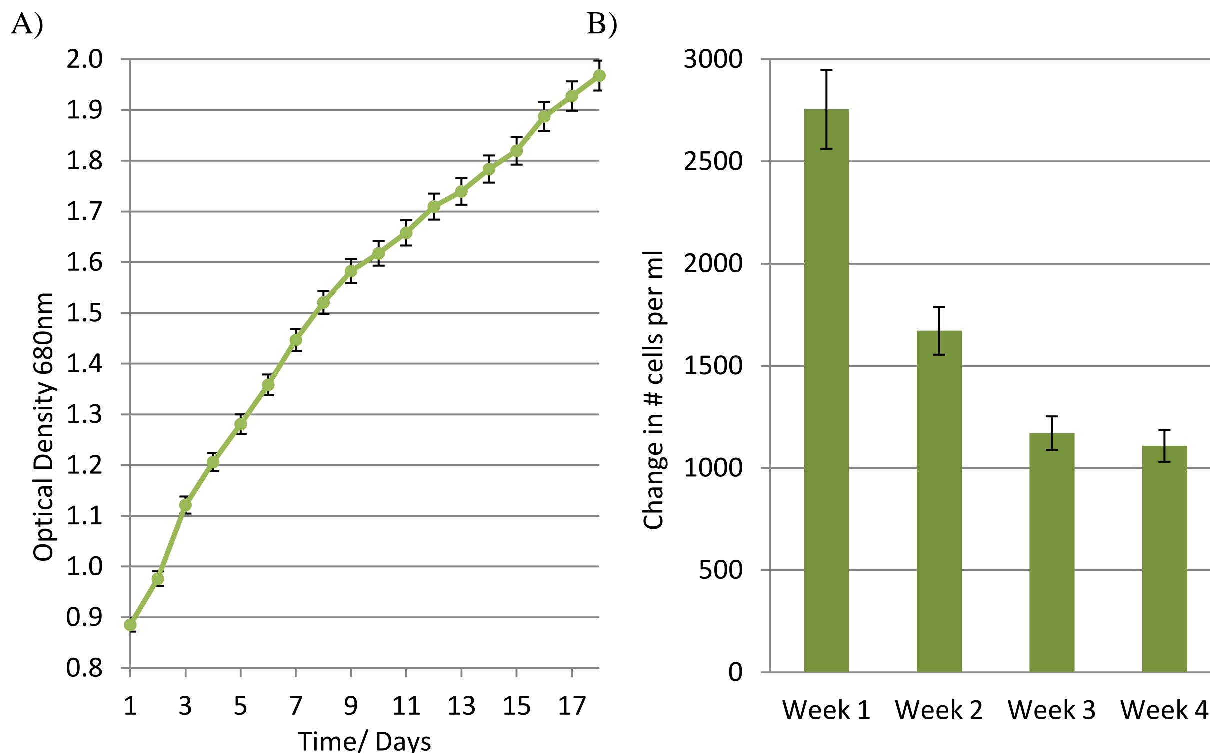

Figure 12: A) *Chlorella vulgaris* average growth curve (3 reps) measured by spectrophotometer at 680 nm, B) Change in number of cells per week of *Chlorella vulgaris* cultures during growth curve

Cell counts were conducted using a light microscope (OMAX, V434 Series Stereoscopic Microscope, NO. 00164 at x100 magnification) with 1 ml of culture on a grid slide and the average cell counts of ten grid squares was used to give a concentration value of number of cells per milliliter. Figure 12B shows the change in the number of cells per week throughout the duration of the growth curve (see Figure 12A). The largest increase in cell number occurred between day 1 and day 7 of the growth curve indicating that the growth rate was highest during this period. The second week showed a smaller increase in cell number, indicating that the growth rate was slowing down, and though the third and fourth weeks still show an increase in cell number, they are not very different, indicating the culture was in the stationary growth

phase. Both Figures 12A and B indicated that culture growth slowed after week 1; therefore, further experiments were conducted during the first week of culture to ensure they occurred during the exponential growth phase.

2.3 Endogenous Hormone Study:

Four randomly selected healthy algal cultures were selected and combined, then split into 8 new cultures, as was done for the growth curve. These cultures were allowed to grow for 4 days, then 15 ml of each culture was harvested by centrifugation at 5000rpm for 5 min (Sorvall, ST 16) into 15 ml tubes. The supernatant was removed from the pellet, filtered and transferred to a new 15 ml tube. Endogenous ABA and CK's were extracted using a modified version the methods shown in Quesnelle and Emery (2007) and Farrow and Emery (2012).

Both the supernatant and pellet were dried in a speed vacuum concentrator at 35°C (Savant SPD111V, Thermo Scientific) until their weights were constant ensuring all moisture had been removed, then stored overnight at -20°C. All samples were extracted in Bieleski's extraction buffer (CH3OH:H2O:HCOOH [15:4:1, v/v/v]) over ice, then homogenised in a ball mill (Retsch, MM300) with zirconium oxide beads at -20°C. Both the pellet and supernatant samples were spiked with the following internal standards: 149.8 ng of $^{2}H_4$ABA (PBI, Saskatchewan, Canada) and 10 ng each of the following deuterated internal standard CK's: $^{2}H_7$BA, $^{2}H_7$BAR, $^{2}H_5$ZOG, $^{2}H_7$DHZOG, $^{2}H_5$ZROG, $^{2}H_7$DHZROG, $^{2}H_6$iP7G, $^{2}H_5$Z9G, $^{2}H_5$MeSZ, $^{2}H_6$MeSiP, $^{2}H_5$MeZR, $^{2}H_6$MeSiPR, $^{2}H_6$iPR, $^{2}H_3$DHZR, $^{2}H_6$iP, $^{2}H_3$DHZ, $^{2}H_6$iPRMP, and $^{2}H_6$DHZRMP (OlchemIm Ltd., Olomouc, CZ) and vortexed. After overnight passive extraction at -20°C, supernatants were transferred and dried down at 35°C.

Separation, purification and concentration of the different CK fractions was performed by solid phase extraction on a mixed mode, reverse-phase/ cation exchange cartridge (Oasis, MCX 6 cc; Waters, Mississauga, Canada) activated with methanol and equilibrated with 1 M formic acid. ABA was eluted first using 1 ml HPLC grade methanol, followed by the nucleotide CK fraction with 1 ml 0.35 M ammonium hydroxide. The free base and riboside fractions as well as methylthiol and glucoside conjugates were eluted together last using 1 ml 0.35M ammonium hydroxide in 60% methanol. Plates were then dried down at 35°C and stored at -20°C immediately to avoid CK degradation.

Dephosphorylation of the nucleotide CK fraction is necessary for detection of the fraction using this method and was carried out using bacterial alkaline phosphatase in 1 ml 0.1 M ethanolamine-HCl overnight at 37°C, then samples were dried down at 35°C under a vacuum. Samples were rehydrated in double distilled water, and were isolated on a reversed-phase C_{18} solid phase extraction column (Oasis C_{18} 3cc; Waters, Mississauga, Canada) activated with methanol and equilibrated using double distilled water. Nucleotide samples were allowed to pass through the column by gravity and the sorbent was washed with double distilled water. Ribosides were then eluted with methanol, and both fractions were evaporated under a vacuum at 35°C, and stored at -20°C. Before LC-MS/MS analysis, all samples were reconstituted in 1.5 ml starting conditions (CH_3COOH: C_2H_3N:ddH_2O [0.08:5.0:94.92,vol/vol/vol] for CK, and CH_3COOH:CH_3OH:ddH_2O [0.08:5.0:94.92, vol/vol/vol] for ABA)) and centrifuged.

Cytokinins and ABA were identified by electrospray ionization, liquid chromatography-tandem mass spectrometry, HPLC-ESI MS/MS (Agilent 1100 HPLC connected to Sciex Applied Biosystem 5500 API Mass Spectrometer) using the method described by Noble *et al*. (2014) and Kisiala *et al*., (2013) with a Luna reverse phase C_{18} HPLC column (3 µm, 150 × 2.1 µm;

Phenomex, Torrance, California, USA). Cytokinins were eluted using an increasing gradient of 0.08% acetic acid in acetonitrile with 0.08% acetic acid in double distilled water at a flow rate of 0.2 ml per minute. ABA was eluted using an increasing gradient of 0.08% acetic acid in methanol with 0.08% acetic acid in double distilled water at a flow rate of 0.2 ml per minute.

Analysis was carried out using Analyst (v 1.5) software (AB Sciex, Framingham, Massachusetts, USA) to calculate peak area and concentration of analytes by comparison to the recovery of the deuterated internal standards which were listed earlier in this section (see Figure 14A-E). cZ isomers were identified and quantified based on the peak position of the corresponding tZ standards.

2.3.1 Rationale for Exogenous Concentrations:

The exogenous hormone additions in further experiments were based on both the endogenous hormone content of the culture and on published literature. The exogenous applications would be added to the supernatant of the culture therefore the concentrations of hormones in the supernatant were the values consulted. The endogenous ABA, tZ and MeSZ content in the supernatant were 1.15, 17.89 and 220.72 pmol/ml, which translated to 10^{-9}, 10^{-8} and 10^{-7} M, respectively. Because the exogenous applications were meant to shock the system in the hopes of creating a novel physiological response in lipid and fatty acid production, values of 10^{-7}, 10^{-6} and 10^{-5} M were chosen for exogenous application. These concentrations were equal to or higher than the endogenous production of each hormone, with the exception of BA which was not detected. All three chosen concentrations had also been added to algae previously in the literature (Noble *et al*., 2014), with the exception of MeSZ, therefore comparisons of results could be made. BA is an aromatic CK which was also chosen as it has been widely used in the

literature in exogenous applications (Noble *et al*., 2014; Piotrowska and Czerpak, 2009). Though it was not detected in this study, perhaps due to analytical differences or growth phase differences, it is known to be produced and released by *Chlorella vulgaris* (Ordog *et al*., 2004).

2.4 Exogenous Hormone Treatments:

Twenty 500ml cultures at OD_{680} ~1.5 were centrifuged into 50 ml tubes and the supernatant removed and discarded. Pellets were then re-suspended with 10 ml autoclaved media using a vortex mixer, combined into one container and mixed thoroughly. 8 ml of this stock were then used to inoculate each of 57 500 ml cultures, giving a starting OD_{680} of between 0.750-0.850 for all cultures. Four hormone treatments, BA (4 reps), MeSZ (3 reps), tZ (4 reps) and ABA (4 reps) plus a DI water control (12 reps) were added to give final concentrations in the cultures of 10^{-7}, 10^{-6} and 10^{-5} M. Cultures were then allowed to grow until they reached an OD_{680} of 1.5 (previously determined OD_{680} corresponding with the end of the exponential phase) at which point whole cultures were harvested by centrifugation for 10 minutes at 5000rpm into 50 ml tubes. The supernatant was discarded and pellets were washed 3 times with DI water before being frozen overnight at -80°C. Pellets were then individually ground for five minutes in a mortar and pestle under continuous liquid nitrogen addition, then freeze dried for two days in a bench top freeze drier (Labconco, FreeZone 4.5; Welch vacuum pump, M# 8912). Dry pellet weights were obtained using a microscale (Delta Range xP105, Mettler Toledo).

2.5 Lipid Extraction:

Total lipids were continuously extracted from the total freeze dried pellet in a soxhlet apparatus for 12 hours each in either 150 ml or 300ml reagent grade hexane at 120°C. Pellets were suspended in a thimble of filter paper (Whatman, 27cm, Grade 1). Round bottom flasks containing hexane and extracted lipids were allowed to cool to room temperature and hexane was removed using a rotary evaporator with a water bath at 40°C (Büchi, Rotovapor R-3000). Round bottom flasks were washed 5 times with 10 ml hexane to ensure all lipids were transferred into pre weighed 10 ml glass tubes. Total lipid weights were obtained by drying down 10 ml tubes in a vacuum concentrator for 12 hours.

2.6 Transesterification:

Transesterification of total lipids to FAME's was performed in the 10 ml glass tubes by first saponifying the dried lipid sample with 900µl methanolic KOH in a silicone oil bath at 120°C for 10-15 mins with the lids tightly sealed. During heating the tubes were shaken every 4-5 minutes. The tubes were removed from the oil bath and 100µl heptadecanoic acid was added as an internal standard. 1 ml methanolic BCl_3 was then added and the tubes were heated for an additional 10 minutes at 120°C and shaken every 4-5 minutes. The tubes were then allowed to cool to room temperature and 1 ml of saturated NaCl solution and 1 ml hexane were added. The tubes were tightly sealed, shaken vigorously for 5 minutes and centrifuged at 5000rpm for 10 minutes. The upper hexane layer was transferred to 5 ml tubes and 1 ml hexane was added to the 10 ml tubes once again. The tubes were shaken and centrifuged as in the previous step and the

hexane layer transferred. This was repeated 3 times. FAME samples in 5 ml tubes were then dried down in a vacuum concentrator for 2 hours.

FAME samples were redissolved in 1 ml hexane, and 1 ml DI water was added to remove any residual salts from the transesterification process. The samples were shaken vigorously in a vortex mixer for 10 minutes then centrifuged at 5000 rpm for 5 minutes. The hexane layer was transferred to new 5 ml tubes and another 1 ml hexane was added. This process was repeated for a total of 5 washes. A second wash with 0.1M HCl solution and a third with 0.5g of Na_2SO_4 anhydrous with five washes each were also carried out to remove remnants of catalyst and any moisture in the samples respectively. FAME's were then transferred to pre weighed 5 ml tubes and dried down for 48 hours in a vacuum concentrator at 35°C and dry weights were obtained using a microscale.

2.7 Fatty Acid Methyl Ester Analysis:

Fatty acid methyl ester identification was carried out using a Gas Chromatograph equipped with a Flame Ionization detector (GC-FID) (Hewlett Packard, 5890 A) and a FFAP column (Zebron Capillary GC Column, 60m, 0.25µm, 233543) with helium as the carrier gas. Both the injector and detector were set at 230°C, with an initial oven temperature of 160°C and a split ratio of 1/80. Oven temperature was held at 160 for the first 5 minutes, then temperature was set to increase to 230 at a rate of 2°C per minute with a final time of 30 minutes. Samples were dissolved and injected using volumes of 1µl n-hexane and an n-hexane column wash was conducted between each sample.

2.8 Statistical Analysis:

Statistics tests were conducted using SigmaPlot (v 12.0) software on exogenous hormone treatment affect on growth rate, lipid content, FA content and FA profile. One way analysis of variance with a Duncan's multiple range test was used to determine significant differences from the control, indicated in figures by * when significant differences were detected at the rejection level of $P = 0.05$.

3. Results

3.1 Endogenous Hormone Content of *Chlorella vulgaris*:

In all hormones identified, the largest concentrations were found to be present in the supernatant of the culture, with only small amounts present on the algae pellet. However in both the supernatant and pellet, MeSCK's were by far the most prevalent, followed by nucleotide type CK's (see Figure 13A & B). By contrast, in the pellet low amounts of ABA and riboside type CK's were found and negligible amounts of free base type CK's. However, in the supernatant the opposite was true as it contained negligible amounts of ribosides and ABA and a significant amount of free bases.

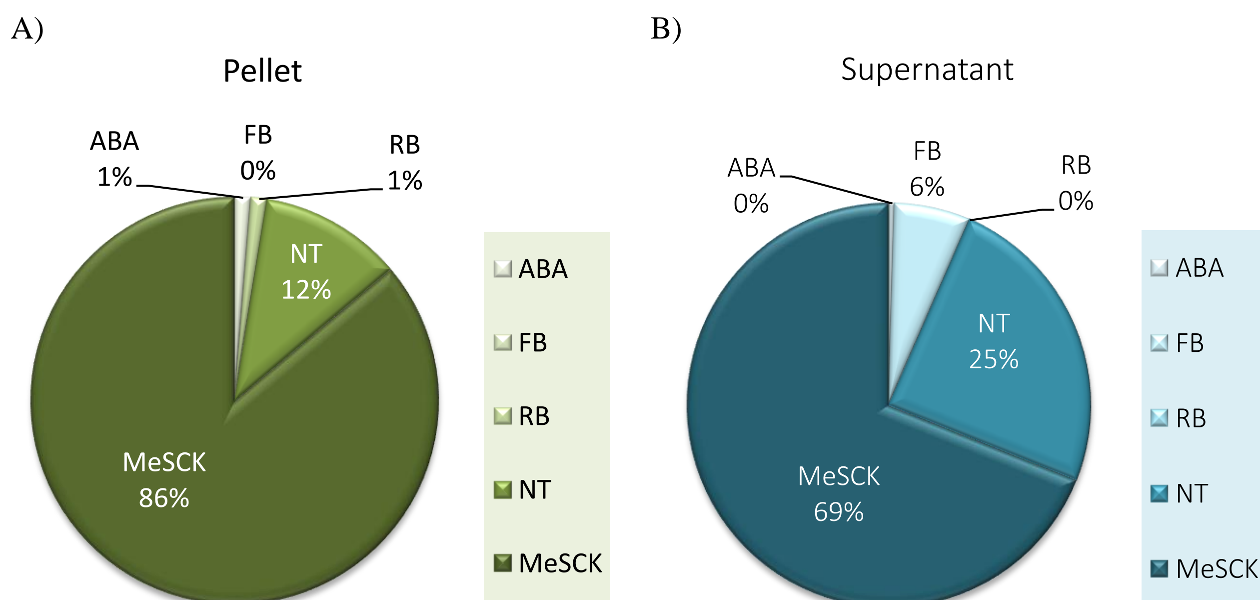

Figure 13: Hormone profiles represented as percentage of endogenous CK forms (Free base - FB, Riboside - RB, Nucleotide - NT, Methylthiol - MeSCK) and Abscisic acid (ABA) found in A) Algae pellet and B) Algae culture supernatant

Abscisic acid was found mostly in the supernatant with an average of 1.15 pmol/ml, while only a small amount of 0.09 pmol/g was found in the pellet (see Figure 14A). No free base type CK's (ie. tZ, cZ, iP, DZ) were detected in the pellet, however a significant amount was found in the supernatant of the cultures (see Figure 14B). The free base type CK tZ was the dominant form found with 17.89 pmol/ml, followed by small amounts of iP 2.20 pmol/ml and a trace amount of cZ with 0.87 pmol/ml. No DZ free bases were detected (see Figure 14B).

Riboside type CK's were detected in both the supernatant and the pellet of *Chlorella vulgaris*. In the pellet the most prevalent type was cZ riboside (cZR) (0.04 pmol/g) followed by DZR 0.03 pmol/g and tZR 0.02 pmol/g. No iPR was detected in the pellet. In the supernatant, the most prevalent riboside was iPR at 0.16 pmol/ml followed by cZR (0.05 pmol/ml), DZR (0.04 pmol/ml) and tZR (0.01 pmol/ml) (see Figure 14C).

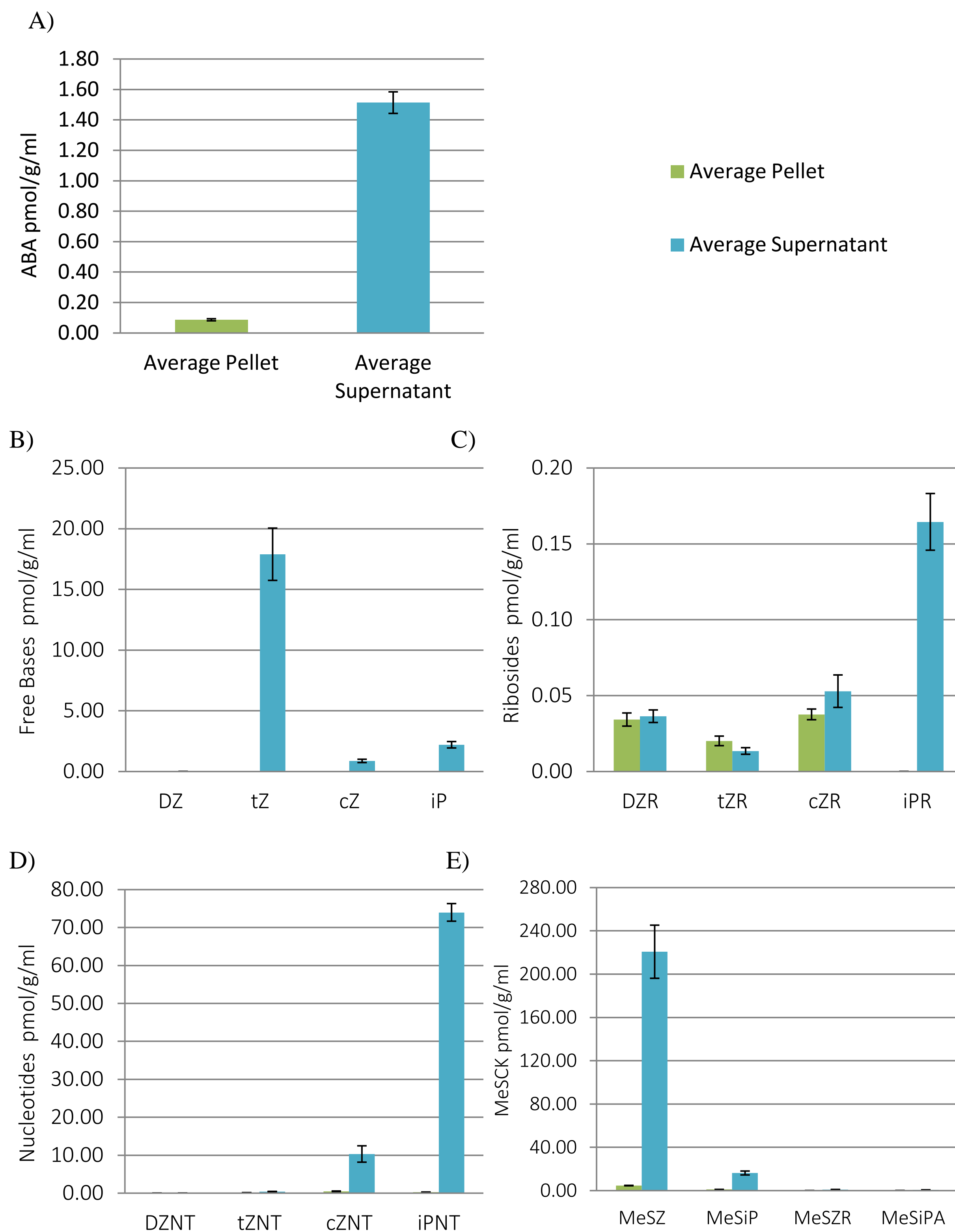

Figure 14: Average endogenous content of A) ABA and CK's B) free base (DZ, tZ, cZ, iP) C) riboside (DZR, tZR, cZR, iPR) D) nucleotide (DZNT, tZNT, cZNT, iPNT) and E) MeSCK's (MeSZ, MeSiP, MeSZR, MeSiPA) and in *Chlorella vulgaris*. Error bars represent standard error (n=7-8)

Almost all nucleotide type cytokinins were detected in the supernatant with only small amounts of cZNT (0.45 pmol/g), iPNT (0.25 pmol/g) and tZNT (0.09 pmol/g) detected in the pellet. iPNT was the most prevalent nucleotide found in the supernatant with an average concentration of 73.97 pmol/ml, followed by cZNT (10.29 pmol/ml) and a small amount of tZNT (0.38 pmol/ml) (see Figure 14D).

As mentioned previously, MeSCK's were present in the highest concentration in both the supernatant and pellet of *Chlorella vulgaris* when compared to all other detected cytokinin forms. MeSZ was the most common in the pellet (4.70 pmol/g) followed by MeSiP (1.11 pmol/g), MeSiPA (0.14 pmol/g) and MeSZR (0.12 pmol/g). Very high concentrations of MeSZ were detected in the supernatant with an average of 220.72 pmol/ml, followed by MeSiP (16.31 pmol/ml), MeSZR (0.97) and MeSiPA (0.69 pmol/ml) (see Figure 14E).

3.2 Exogenous Hormone Treatment effect on Growth Rate:

3.2.1 Hormone Effect By Concentration:

A one way analysis of variance coupled with Duncan's test (P = 0.05) was used to determine significance in all following results, and is denoted by an asterisk in all following figures. All error bars represent standard error based on Control replicates of n=12, ABA, BA and tZ replicates of n=4 each and MeSZ replicates of n=3. In this case, the slope values of each growth curve's trendlines were used to determine levels of significance for each hormone treatment on growth rate. As seen from Figure 15A, there does not appear to be any difference in growth rate between hormone treatments at 10^{-7} M, which was confirmed by statistical tests (P <

0.05). The slope values at this concentration ranged from 0.0749 (ABA) to 0.0925 (tZ). Although tZ had a higher slope value than all other treatments, indicating a higher growth rate, this difference was not significant. All cultures at this low concentration took 9-10 days to reach the harvesting OD of 1.5 (see Figure 15A).

The mid-range hormone additions of 10^{-6} M had a more obvious effect on the growth rate of the culture with slope values ranging from 0.0773 to 0.132. BA and MeSZ stimulated the greatest significant increase in growth rate ($P < 0.05$) of 0.132 and 0.126, respectively, from the control slope value of 0.0808. These treatments were also the first to reach harvesting OD on day 6. ABA caused a slight increase, while tZ caused a slight decrease in growth over the control (harvested on day 8 and 10 respectively) however these differences were not found to be significant (see Figure 15B).

The highest hormone concentration treatment of 10^{-5} M produced both increases and slight decreases in growth rate from the control, which had a slope value of 0.0808. Slope values ranged from 0.0753 (ABA) to 0.107 (tZ). *trans*-Zeatin and BA at this high concentration had significant effects on growth rate with increases in slope values to 0.107 and 0.106 respectively, and both were harvested on day 8. Treatment with MeSZ and ABA at this concentration did not cause a significant deviation from the control (see Figure 15C).

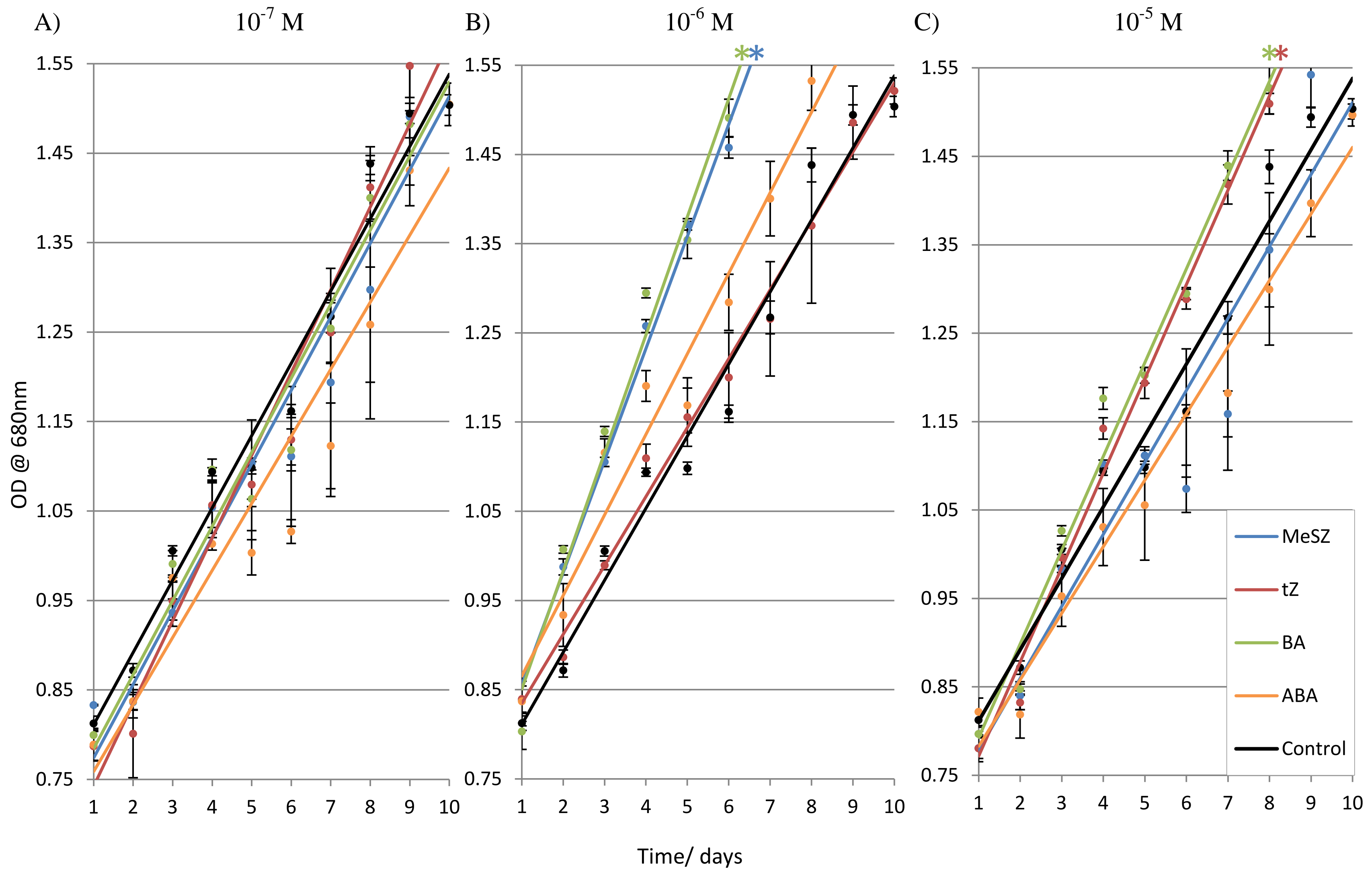

Figure 15: Growth response of *Chlorella vulgaris* measured by change in OD @680 nm to exogenous hormones treatments at A) 10^{-7} M, B) 10^{-6} M and C) 10^{-5} M. All R^2 values ≥ 0.9. * indicates statistical significance (P=0.05) from control. Control n=12, all others n=3-4

3.2.2 Hormone Effect By Timepoint:

The effect of the hormone additions on growth rate was observed mainly at medium concentration, and to a lesser extent at high concentration, and this appeared to be amplified over time, as seen from Figure 16A-C. The most obvious trend was a typical biphasic response that hormones often evoke (Davies, 2004), whereby additions at low (10^{-7} M) and high (10^{-5} M) concentrations both initially had negative effects on growth rate, while by day 6 after additions both tZ and BA at 10^{-5} M had positive effects on the growth rate increasing it by 10.9% and 11.5% respectively, above the control. Another clear trend was the addition of all hormones at 10^{-6} M had initial positive effects on growth which amplified over time, especially exemplified by the addition of BA and MeSZ which showed consistent significant increases in growth over time. The effect of each hormone on growth rate across concentrations over time will be individually discussed below.

BA caused the greatest significant ($P < 0.05$) increase in growth rate throughout the 6 days at 10^{-6} M, increasing growth from 15.5% at day 2, to 18.36% at day 4, and finally to 28.4% at day 6 above the control. Additions at 10^{-5} M also ultimately caused an increase in growth rate from -2.8% at day 2 to 11.5% at day 6. MeSZ caused significant increases in growth at 10^{-6} M, similar to that of BA, with an increase of 13.3% on day 2, to 14.97% growing to 25.5% on day 6 above the control. However, unlike BA, MeSZ decreased growth rate by 7.5% at 10^{-5} M on day 6, though this decrease was not significant.

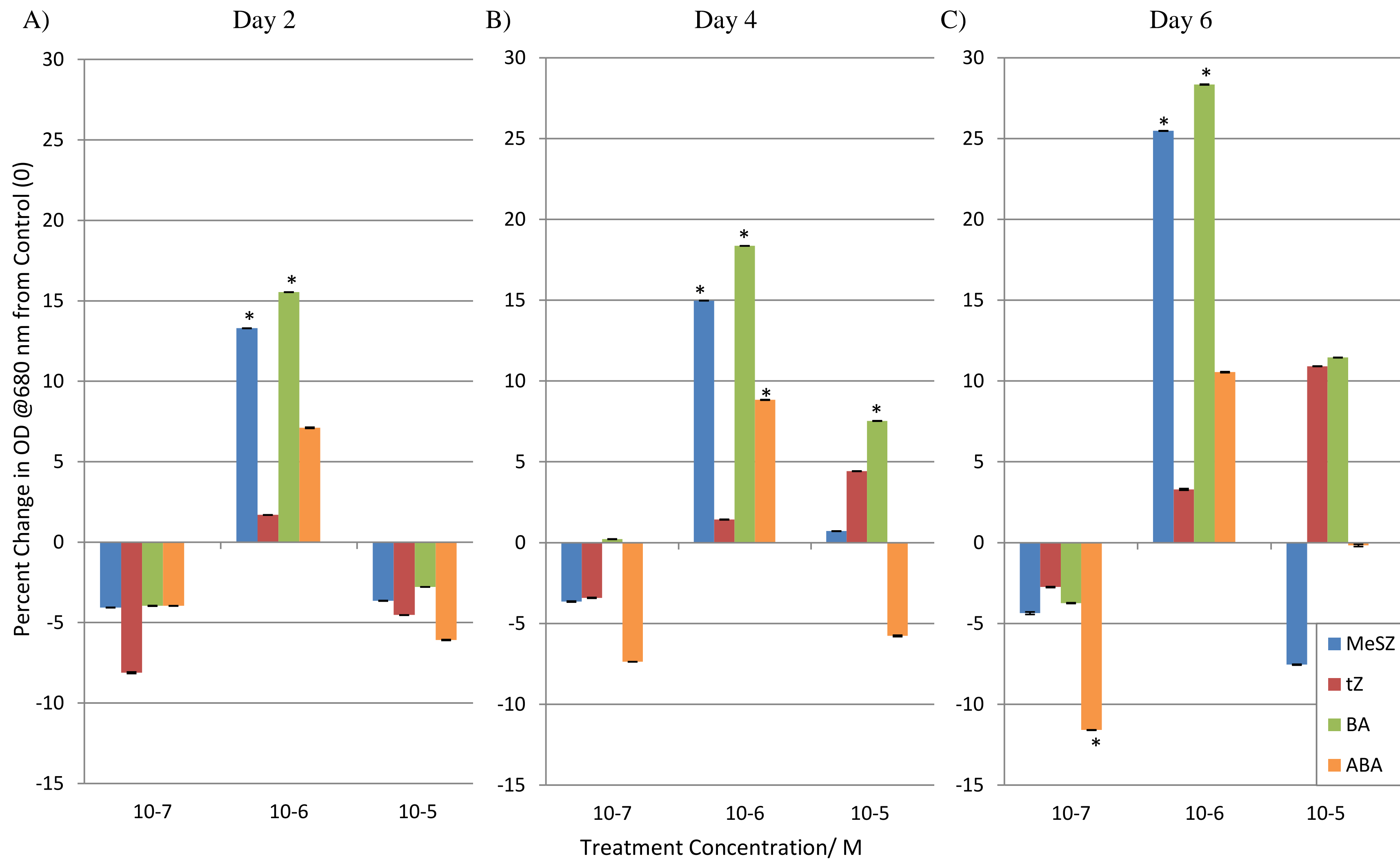

Figure 16: Percent change in OD from control of *Chlorella vulgaris* across concentrations at A) Day 2, B) Day 4 and C) Day 6 of treatment. * indicates statistical significance (*P*=0.05) from control. Error bars represent standard error (n=3-4). Data converted to percentage to show trends more clearly.

ABA also caused a significant increase in growth rate at 10^{-6} M, with an increase of 7.11% at day 2, becoming statistically significant at day 4 with an increase of 8.83% and rising to 10.55% on day 6 (not significant). At the lowest concentration ABA caused decreases in the growth rate from 4% at day 2, to 7.4% on day 4, to a significant 11.6% at day 6. This clearly shows the amplified negative effect on the growth rate over time. When added at 10^{-5} M ABA decreased growth by 6.1% on day 2 and 5.8% on day 4; however the growth rate was almost equal to the control by day 6 and no significant differences were found.

The only notable effects of tZ are seen on day 2 and day 6 when added at 10^{-7} and 10^{-5} M. At 10^{-7} M, whereby on day 2 tZ caused a decrease in growth of 8%; however this decrease became negligible by day 4 and in day 6. A small initial decrease in growth rate at 10^{-5} M on day 2 reversed to a small increase by day 4 and reached an increase of 10.9% on day 6. None of the effects of tZ were deemed significant when viewed as percentage increase/ decrease from the control. Because tZ continued to increase growth at the highest concentration of 10^{-5} M it was the only hormone which did not elicit the inhibitory phase at higher concentrations, typical of a hormonal biphasic response. Presumably this would have happened if the experiment had continued at greater concentrations.

No other statistically significant differences were identified, although trends seemed to be apparent in the data. For this reason statistical tests were conducted not only across concentrations and days but also within concentrations on individual days. These tests confirmed a lack of statistically significant differences between the control and treatments.

3.4 Exogenous Hormone Treatment effect on Lipid Content:

Although several hormone treatments altered growth rates, the effects on lipid content was only apparent in select cases. The percentage of dry weight lipid content varied with individual hormone treatments when compared to the control, which contained 1.53% dry weight (see Figure 17). The only treatment which increased lipid content significantly (*P* < 0.05) above the control was BA at 10^{-6} and 10^{-5} M. All other treatments, with the exception of tZ at 10^{-5} and MeSZ at 10^{-6} M, caused a significant decrease in lipid content (see Table 4). BA at 10^{-7} did not differ significantly from the control.

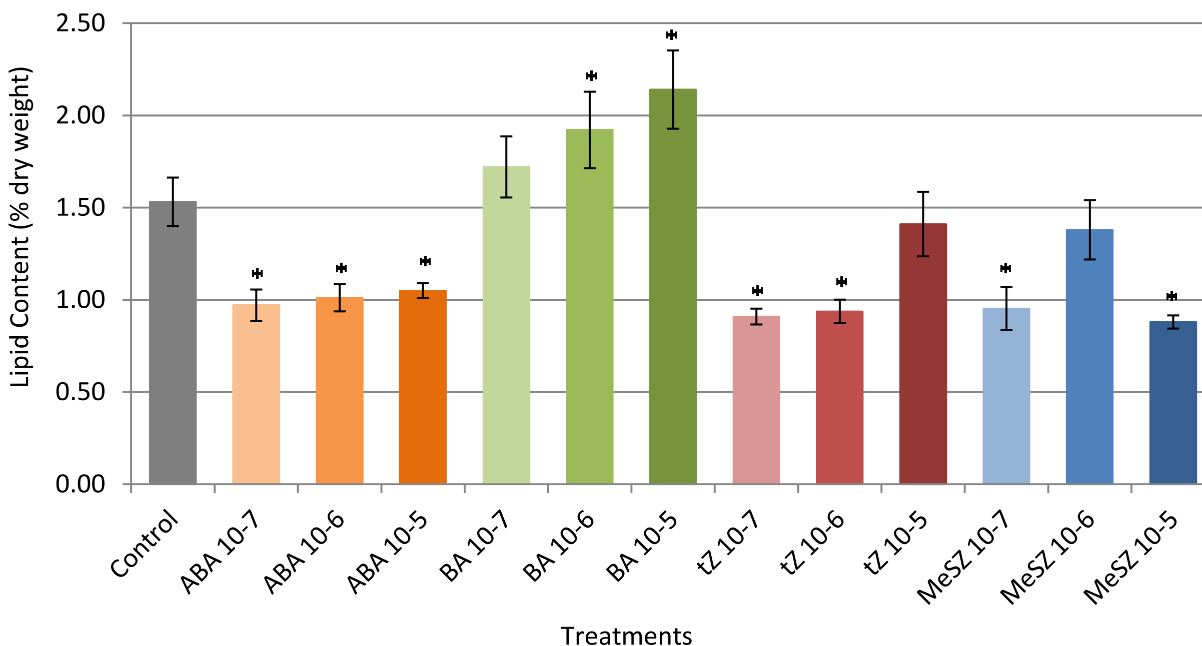

Figure 17: Lipid content shown as percentage of dry algal weight of *Chlorella vulgaris* in response to exogenous hormone treatments. * indicates statistical significance (*P*=0.05) from control. Due to expected variation within samples percent dry weight was chosen for figures to highlight trends. See Appendix for raw data. Error bars represent standard error (Control n=12, all other n=3-4)

Table 4: Summary table of percent change in lipid content from the control (n=12) with hormone additions

Treatment	Average Percent Change in Lipid Content from Control (%)	Replicates	Significant Difference ($P < 0.05$)
ABA 10^{-7}	-36.6168 ± 0.849	4	Yes
ABA 10^{-6}	-34.0059 ± 0.736	4	Yes
ABA 10^{-5}	-31.4687 ± 0.401	4	Yes
BA 10^{-7}	12.32397 ± 1.657	4	No
BA 10^{-6}	25.43085 ± 2.073	4	Yes
BA 10^{-5}	39.72111 ± 2.121	4	Yes
tZ 10^{-7}	-40.6409 ± 0.429	4	Yes
tZ 10^{-6}	-38.8285 ± 0.641	4	Yes
tZ 10^{-5}	-7.89128 ± 1.750	4	No
MeSZ 10^{-7}	-37.8099 ± 1.167	3	Yes
MeSZ 10^{-6}	-9.92413 ± 1.609	3	No
MeSZ 10^{-5}	-42.5619 ± 0.355	3	Yes

3.4 Exogenous Hormone Treatment effect on Fatty Acid Content:

Similar to the responses observed with the overall lipid content, ABA, tZ and MeSZ treatments, at all concentrations, caused a significant reduction in FAME content when compared to the control (see Figure 18). Only treatment with BA at 10^{-5} M caused a very slight increase in FAME content from 10.8 mg to 11.2 mg; however this was not significant ($P < 0.05$). BA at 10^{-7} and 10^{-6} M caused a significant decrease in FAME content. All treatments with the exception of MeSZ show a subtle dose dependant response where increasing concentrations of hormones produced increases in FAME content, however in most cases the FAME content was still below that of the control. Standard deviations ranged from 2.8-23.9% with replicates ranging from 12 to 3 (see Figure 18).

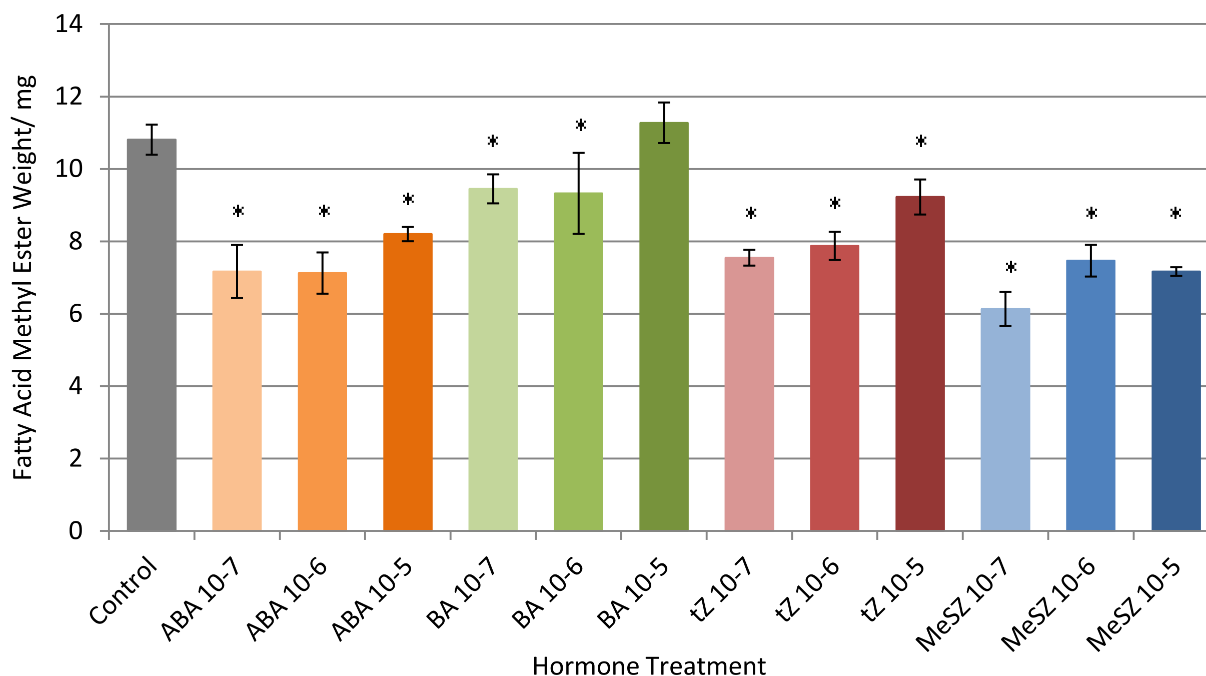

Figure 18: Total FAME content of *Chlorella vulgaris* (mg) in response to exogenous hormone treatments. * indicates statistical significance (P=0.05) from control. Reduced variation between samples allowed for trends to be visible using raw data. Error bars represent standard error (Control n=12, all other n=3-4).

The transesterification process also yielded unknown compounds which contributed to the mass of FAME's measured. This was due to both naturally found compounds within the lipids and due to excess salts and moisture from the transesterification process. As previously mentioned in the methods, the salts and moisture were removed by sample washes of dilute hydrochloric acid and anhydrous sodium sulphate. To determine the class of the unknown component of the FAME samples TLC separations followed by C-NMR analysis were conducted on two randomly selected FAME samples. These tests confirmed the presence of long chain alkane type molecules, as well as confirming successful transesterification of the FA's. The methods used and the results of these tests can be found in the Appendix. As this component was not present in the GC-FID spectra produced by the same samples, these compounds would not

affect the concentration calculations for the FAME's we were analyzing, therefore no further testing was performed.

Unfortunately, after the transesterification process, all biological replicates of the treatments ABA at 10^{-7} M and tZ at 10^{-5} M were unintentionally destroyed, and thus not available for analyses of FA profiles. Those specific dosages have therefore been omitted from any further graphs and discussion.

3.6 Exogenous Hormone Treatment effect on Fatty Acid Profile:

The control FA profile of *Chlorella vulgaris* was composed mostly of the PUFA linoleic acid (38%), the saturated palmitic acid (32%) and the monounsaturated oleic acid (23%) (see Figure 19). The saturated stearic acid and PUFA linolenic acid were small components of the profile constituting 5% and 2% respectively.

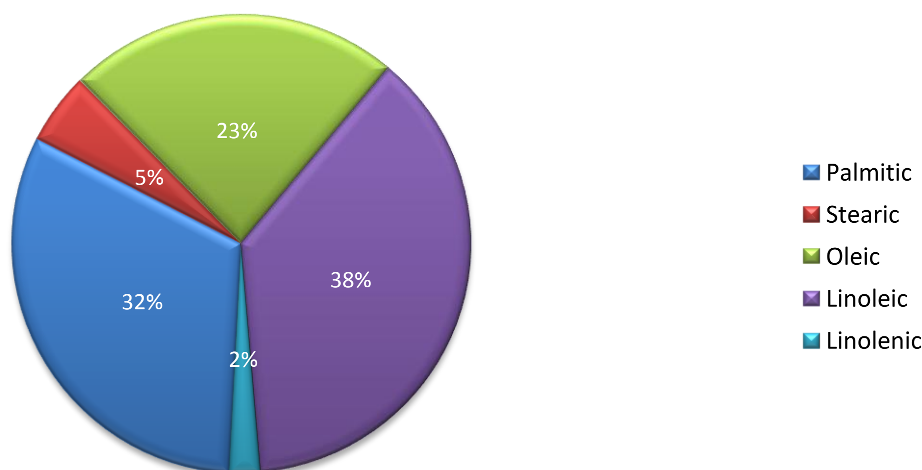

Figure 19: FAME profile of control *Chlorella vulgaris* shown as averaged percentage of total. Heptadecanoic acid standard recovery for all samples was calculated to be 68.9% +/- 15.4% with a standard error of 2.6 (n=49).

The hormone treatments produced variations in the FA profile of *Chlorella vulgaris* which included all five most common FA's found in the control cultures (see Figure 20A-E). All treatments at all concentrations caused an increase in stearic acid the highest of which was caused by the addition of ABA at 10^{-6} M with an increase of 65.56% above the control. Treatment with ABA and MeSZ produced the most consistent changes throughout the profile across all concentrations while treatment with BA and tZ produced more variable results across concentrations (see Table 5; Figure 20A-E).

Table 5: Summary of percent change in FAME content from control showing number of replicates, statistical significance and standard error values. * indicates significance from control (*P*=0.05).

		Saturated Fatty Acids				Unsaturated Fatty Acids					
Treatment	**Replicates**	**Palmitic**	**SE**	**Stearic**	**SE**	**Oleic**	**SE**	**Linoleic**	**SE**	**Linolenic**	**SE**
ABA 10^{-6}	4	16.48*	1.57	65.57*	0.62	-15.59	2.99	-14.42	2.36	25.07	0.51
ABA 10^{-5}	4	11.72*	1.10	13.48	0.85	-6.94	1.76	-5.28	0.48	-36.64	0.24
BA 10^{-7}	4	12.57*	2.64	5.47	0.45	23.06	2.48	-23.00*	3.06	-48.25	0.13
BA 10^{-6}	4	-5.33	0.97	8.51	0.53	0.27	1.50	3.96	1.47	-13.66	0.20
BA 10^{-5}	4	-6.78	0.76	14.01	0.38	-10.72	2.22	11.37	1.05	-14.89	0.55
tZ 10^{-7}	4	-11.22	1.28	42.90*	0.63	6.28	2.51	-2.99	1.71	48.63	0.29
tZ 10^{-6}	4	-0.99	1.12	31.61*	0.52	-14.76	2.57	8.17	2.65	-41.77	0.29
MeSZ 10^{-7}	3	-7.07	1.14	18.40	0.23	-13.86	2.05	0.51	1.11	204.53*	0.56
MeSZ 10^{-6}	3	-8.25	0.38	17.94	0.54	-42.10*	0.99	4.82	1.65	457.43*	1.34
MeSZ 10^{-5}	3	-5.52	0.37	41.06*	0.22	-6.37	1.11	6.07	0.88	-51.98	0.32

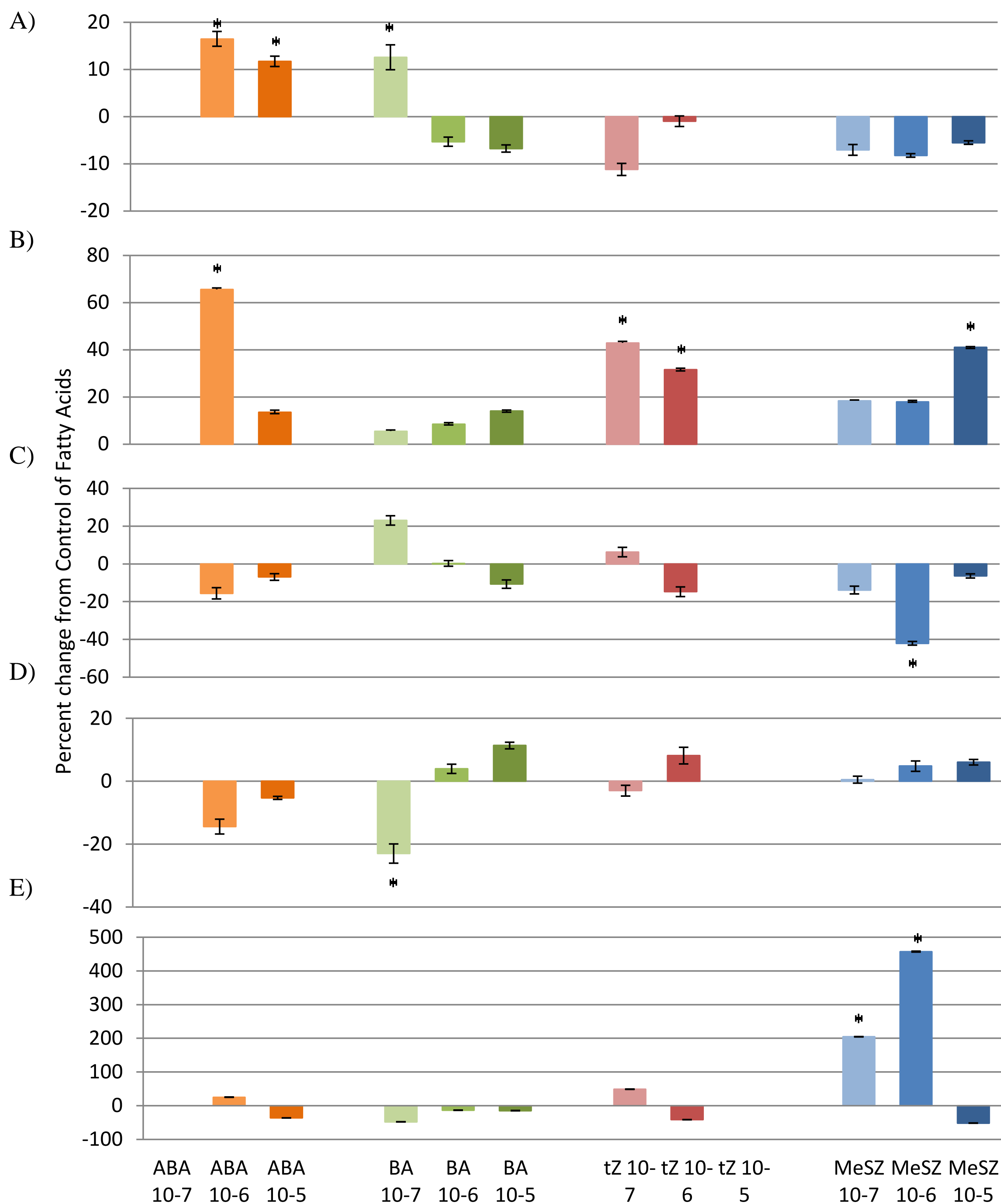

Figure 20: Percent change from control of A) palmitic acid, B) stearic acid, C) oleic acid, D) linoleic acid and E) linolenic acid in *Chlorella vulgaris* with hormone treatments. * indicates significance from control (α=0.05). Error bars represent standard error (Control n=12, all other n=3-4). Raw data was converted to percentages to reveal trends more clearly.

4. Discussion

4.1 *Chlorella vulgaris* Endogenous Hormone Content:

Phytohormones such as CK's and ABA act as signal molecules in plants, algae and bacteria (Sakakibara, 2006; Frebort *et al*., 2011). In higher plants they are generally synthesised in one part of the plant and are transported to their target organ via the phloem where receptors perceive them and react according to their concentration (Davies, 2004). In media grown unicellular algae, these molecules are synthesised in each algal cell, then released into the media where they are perceived by the rest of the cells in the culture (Spichal, 2012). Again, these cells react according to the concentration of phytohormones in the media, either accelerating or decelerating growth, mounting a pathogen response or producing different metabolites (Piotrowska and Czerpak, 2009; Tate *et al*., 2013). It is clear that this cycle is being exemplified in the results of the endogenous hormone study of *Chlorella vulgaris*. This study shows that this algal species can synthesise a mixture of CK's and ABA, as well as release them to the surrounding media. The vast majority of the detected CK's and ABA were found in the media and not in the pellet and were therefore actively signalling the rest of the cells in culture.

4.1.1 Abscisic Acid Content:

ABA is considered a “stress” hormone as it is synthesised and released in abundance when a plant is experiencing stressful or suboptimal growth conditions such as lack of nutrients (Nambara and Marion-Poll, 2005). Both the pellet and supernatant contained very low concentrations of ABA, 0.09 pmol/g and 1.15 pmol/ml respectively, when compared to the total CK content, indicating that the culture had abundant resources and was not experiencing abiotic stress. This low ABA content is representative of the exponential growth phase when the cultures

were harvested; because of the fresh input of nutrient rich media and the thinning of the cultures there was no obvious role for ABA signalling. By contrast, a study of the endogenous CK and ABA profile of *Euglena gracilis* revealed relatively high levels of ABA in both the pellet and supernatant (Noble *et al*., 2014). However, the algal cultures in that study were re-suspended in DI water and grown for 2 days prior to harvesting; therefore they were experiencing severe nutrient and osmotic stress and would be producing much more ABA than a healthy algal culture.

4.1.2 Cytokinin Content:

4.1.2.1 Methylthiols - Of all CK types, MeSCK's were the most abundant in both the pellet and supernatant of the exponentially growing cultures. Methylthiols were recently discovered and seem to be present in a diverse array of tissues, yet still have an unknown function (Sakakibara, 2006). The forms MeSZ, MeSZR, MeSiP and MeSiPA have been positively identified in the motile unicellular algae *Euglena gracilis* in significant amounts in the supernatant, however no speculation on their possible function was discussed (Noble *et al*., 2014). Previously, studies on *Rhizobium* bacteria found that MeSCK's were the predominant CK's found within the cell and were also present in the media (Kisiala *et al*., 2013).

Although the algae cultures in this study were not maintained in a flow hood, all equipment and media was thoroughly sterilized and the air supply filtered to prevent bacterial contamination. During the entirety of the culture maintenance as well as during experiments, regular microscopic examination of the cultures was also carried out in order to ensure that no contamination had occurred. It is therefore assumed that the MeSCK content is due to the algae cultures alone and not bacterial contamination.

In this study, the most abundant form of MeSCK was MeSZ, with a smaller amount of MeSiP also being produced and released into the media. In the case of the parasitic fungus, *Ustilago maydis*, it was hypothesized that an accumulation of MeSZ in infected corn tissue may be due to its release by the fungus in order to stimulate further cell proliferation and thereby the development of tumours (Morrison *et al*., 2015). This result and explanation suggests that MeSCK's may play a stimulatory role in cell growth and therefore would be abundant during the exponential growth phase of the algal cultures. The much larger quantities of the MeSCK's in comparison to the levels of CK's and ABA detected may also be due to the reduced ability of the CKX enzyme to break them down, therefore resulting in a build up in both the pellet and supernatant (Radhika *et al*., 2015; Morrison *et al*., 2015). It should also be noted that the riboside forms of MeSZ and MeSiP were low in both the supernatant and the pellet, indicating that conjugates were being readily converted into the presumably more active free base forms.

4.1.2.2 Free Bases, Ribosides and Nucleotides - Free base cytokinin forms are generally considered the most biologically active forms (Sakakibara, 2006; Spichal *et al*., 2004) and are synthesised from nucleotides and ribosides through either the direct action of the LOG gene (Sakakibara, 2006; Kurakawa *et al*., 2007) or through a two-step process starting with phosphate group cleavage from nucleotide to riboside and then, secondly, to free base form. Riboside forms are considered less active and possible short-term storage forms while nucleotides are considered inactive precursors (Sakakibara, 2006). In this study it was found that there was an abundance of iPNT as well as a small amount of cZNT, both mostly in the supernatant of the exponentially growing *Chlorella vulgaris* cultures, with very small amounts in the pellet. There was also a very small amount of tZNT found in both the pellet and supernatant but no DZNT. Since the iP and cZ types were the most abundant nucleotide forms it suggests that these are mostly inactive at

this stage of exponential growth. This is further supported by the riboside content, which also contained high amounts of iPR, cZR and DZR with low amounts of tZR indicating that tZR is readily being converted to free base form for active signalling. In this study it was found that free base forms were the second most abundant form detected in the supernatant, and were composed mostly of the free bases tZ, with very low amounts of iP and cZ. This further supports the importance of tZ as the main signal molecule, as it is the most abundant active form.

These results mirror the results found in *Euglena gracilis*, where the highest concentrations of hormones were found in the supernatant (Noble *et al*., 2014). The free base CK's most prevalent in the supernatant were also tZ and MeSZ, with the cis isomer and iP forms most abundant in the pellet as riboside and nucleotide forms. iP was also found to be at its highest concentration in nucleotide form (Noble *et al*., 2014), as it was in this study. Although GLUC storage forms and aromatic CK's such as BA are known to be produced by Chlorella sp. (Ordog *et al*., 2004), none were detected in the present study. The results of Noble *et al*., (2014) as well as the results of the current study underscore the importance of production and release of hormones in algal systems rather than their retention and use within the cells.

4.1.2.3 Active Cytokinin Synthesis Pathway - As stated previously in the literature review chapter, CK synthesis is divided into two active pathways, the MEP and MVA pathways, responsible for producing either the tZ derived CK's or the cis-isomers, respectively (Sakakibara, 2006). Previous research into the preferred CK synthesis pathways of higher plants have concluded that they tend to favour the MEP pathway and produce more tZ isomers, while lower plant organisms such as algae and other microorganisms favour the MVA pathway and produce more cis-isomers (Pertry *et al*., 2009). This was supported by an endogenous hormone study

conducted on the green algae *Euglena gracilis*, which revealed that the main CK produced was cZ, which originated from the MVA pathway (Noble *et al*., 2014).

The results of this study do not support those of previous work, as the main biologically active form being produced was tZ which originates from the MEP pathway. High free base levels of tZ were found with low amounts of cZ. As free base CK forms are believed to be the most biologically active (Sakakibara, 2006), this indicates that tZ was being synthesised at a higher rate, suggesting that it is the more active of the two isomers. High levels of cZ in the riboside and nucleotide storage forms with low levels of tZR and tZNT also support this claim. These low levels in storage indicate that tZR and tZNT is being converted to the active free base form for use in signalling, whereas the cis isomer is remaining as a storage form. Due to the presence of the cis isomer, though in low quantities, these results indicate that both the MEP and MVA pathways are active within *Chlorella vulgaris* during the exponential stage of growth. However, it is clear that the MEP pathway is the most active, due to the high levels of active tZ. For this reason, tZ was chosen for exogenous application in further experiments, as it appeared to be more active in the system than cZ.

It has been hypothesised that conversion between cis and trans isomers can occur due to the action of the cis-trans isomerase enzyme, resulting in both compounds being present (Frebort *et al*., 2011), which may also explain the presence of both isomers. It is also possible that the tZ found may be of symbiotic bacterial origin acting within the *Chlorella vulgaris* cultures, as bacteria commonly produce and release the tZ isomer in free base form (Kisiala *et al*., 2013). However, it is most likely that the MEP pathway is simply more active than the MVA pathway.

It was recently postulated that MeSZ, which was found in high quantities, is also a product of the MVA synthesis pathway (Morrison *et al*., 2015). The high levels of MeSZ therefore indicate that the MVA pathway is being activated for the production of this abundant CK during the exponential growth phase of *Chlorella vulgaris.* Recent research has also discovered that MeSCK's, though they mimic the action of the other CK's, are more stable and persistent in the system because of the lowered ability of the degradation enzyme CKX to act upon them (Radhika *et al*., 2015). This could result in elevated levels of MeSCK's, especially in closed systems such as the one examined in the current study.

4.2 Exogenous Hormone Treatments on Growth Rate:

The addition of hormones at varying concentrations to cultures of *Chlorella vulgaris* produced changes in growth rate compared to the control cultures. Therefore our hypothesis that the exogenous application of hormones would affect the growth rate is supported. Our predictions stated that the CK's BA, MeSZ and tZ would cause increases in the growth rate while the addition of the stress hormone ABA would cause decreases in growth rate compared to the control cultures. The addition of BA and MeSZ at 10^{-6} M and tZ at 10^{-5} M both caused increases in growth rate, thereby supporting the predictions. However, ABA caused no significant change in growth rate, therefore the prediction that ABA addition would decrease growth rate could not be supported.

The greatest significant increases were caused by the additions of BA and MeSZ at 10^{-6} M, which were statistically different from the control on day 2, 4 and 6. BA and MeSZ were also found to be the most consistent in terms of their effects whereas treatment with other hormones

showed more variation and therefore less predictability. Treatments with BA and tZ at 10^{-5} M also increased growth however only the increase caused by BA at 10^{-5} M was found to be statistically significant, and only on day 4. ABA also caused a statistically significant increase at 10^{-6} M on day 4 only. The only significant decrease in growth rate from the control at 10^{-7} M was observed with ABA, however this did not become statistically significant until day 6. This indicated that 10^{-7} M was generally too low of a concentration to overcome the natural metabolism of an exponentially growing culture of *Chlorella vulgaris* and only succeeded in disrupting the system enough to warrant a slight decrease in growth rate throughout the test period. It was also clear that treatment with most CK's and ABA did not have an immediate effect, as most significant changes in growth rate occurred between day 4 and 6.

All hormones tested, with the exception of tZ, showed a typical biphasic response whereby very low and very high concentrations inhibited the culture growth and the median concentration promoted growth (Noble *et al*., 2014). This allowed us to ascertain optimum concentrations for the exogenous addition of BA, ABA and MeSZ, but not tZ. As the greatest increases in growth rate were observed for treatments with BA, MeSZ and ABA at 10^{-6} M this is considered the optimum concentration for the addition of these hormones to *Chlorella vulgaris*. Although BA also caused an increase in growth above the control at 10^{-5} M, it was not as notable as that cause by additions at 10^{-6} M. Additions of tZ at 10^{-6} M caused a greater increase in growth than additions at 10^{-7} M, however 10^{-5} M caused the greatest increase in growth rate compared to the control. This indicates that the optimal concentration for tZ may be greater than 10^{-5} M.

Interestingly, 10^{-5} M was the same concentration found to be cytotoxic to *Euglena gracilis* in a previous study, where the optimum concentration for exogenous applications of tZ

was found to be 10^{-7} M (Noble *et al*., 2014). It was also revealed that ABA is cytotoxic at 10^{-5} M and its optimum concentration was determined to be far less at 10^{-9} M (Noble *et al*., 2014). Conversely, the current study showed relatively high optimum concentration and a lack of obvious cytotoxicity at higher concentrations indicating that *Chlorella vulgaris* may be more resilient to treatments with ABA and certain CK's than *Euglena gracilis*. A study on *Chlorella vulgaris* recently showed large increases in cell number with the addition of both BA and tZ at optimum concentrations of 10^{-7} and 10^{-8} M respectively, which were also lower than the optimum concentrations found in the current study (Piotrowska and Czerpak, 2009). This may be due to slight differences in growth conditions or differences in harvesting time.

BA is a well known aromatic CK which has become widely used in hormone studies in algae and higher plants (Piotrowska and Czerpak, 2009; Mostafa *et al*., 2005). Interestingly, this study has shown that MeSZ stimulates comparable increases in growth to BA at the same concentration when applied to *Chlorella vulgaris*. No studies on exogenously applied MeSCK's have been published to date, to the best knowledge of the author. As BA has become the standard CK used to increase growth rate this is a significant breakthrough. As previously stated, MeSCK's have been detected in several different organisms including algae, bacteria and fungi, however very little is known and a theory as to their precise purpose and function is mostly speculative. Morrison *et al*., (2015) hypothesised that MeSCK's were released by the parasitic fungus *Ustilago maydis* into corn cob tissue, stimulating substantial cell proliferation which facilitated the formation of cob tissue tumours (Morrison *et al*., 2015). The large increase in growth rate with the addition of MeSZ to *Chlorella vulgaris* supports the hypothesis that MeSCK introduction causes cell proliferation. This also verifies that MeSZ can be considered a growth

promoting hormone when added exogenously at 10^{-6} M, which supports our predictions that its addition would increase the growth rate of *Chlorella vulgaris*.

4.2.1 Cytokinin/ ABA Antagonism:

Many studies have shown that there appears to be an antagonistic relationship between ABA and CK content within plant cells (Emery *et al*., 1998; Cowan *et al*., 1999). This relationship was observed during lupin growth, where decreases in ABA content coincided with large increases in CK and auxin, particularly in rapidly growing tissue (Emery *et al*., 1998). This relationship was also shown in a study of ripening avocado mesocarp, where the addition of various adenine and isoprenoid CK's caused a corresponding reduction in the ABA content by increasing the deactivation of ABA into phaseic acid (Cowan *et al*., 1999). The addition of synthetic CK's such as N-(2-chloro-4-pyridyl)-N-phenylurea (CPPU) also caused a reduction in ABA content by disrupting ABA biosynthesis (Cowan *et al*., 1999). This antagonistic behaviour has also been noted in algae (Lu *et al*., 2014).

The results from the endogenous portion of this study, although not a time course study, suggest that the same antagonism may be occurring in the rapidly proliferating *Chlorella vulgaris* culture, as ABA levels were significantly lower than total CK levels. This may also indicate the favouring of rapid cell proliferation while resources are high and therefore high levels of CK's, as opposed to slow growth and the favouring of storage accumulation which occurs with high levels of ABA. This relationship is similar to that found by Lu *et al.* (2014) where endogenous ABA levels increased in response to decreased nitrogen availability while the levels of active CK's decreased in the algae *Nannochloropsis oceanica* (Lu *et al*., 2014).

The exogenous addition of ABA in our study also seemed to have the opposite effect on growth in general compared to the addition of the CK's BA and MeSZ. Although there was a slight increase in growth with the addition of ABA at 10^{-6} M, this was small in comparison to the growth caused by BA and MeSZ additions, which may also indicate the antagonistic effects of CK addition and ABA addition. This is also in agreement with the results of previous studies on the exogenous addition of ABA and CK's to algae, where ABA slowed growth and stimulated the stress response while CK's accelerated growth (Lu *et al*., 2014).

4.3 Exogenous Hormone Treatments and Effect on Lipid Content:

Lipids are synthesised by algae to be used as a form of long term energy storage and are known to accumulate during times of unfavourable growth conditions in algal cultures (Chen *et al*., 2011; Gouveia *et al*., 2009; Gardener *et al*., 2011). As the *Chlorella vulgaris* cultures in this study were grown in nutrient rich media to ensure culture growth was in exponential phase during hormone additions, maximizing lipid accumulation was not the main goal of this study. However, the addition of phytohormones was expected to cause changes in the total lipid content of the cultures, as hormones alter metabolism by signalling changes in growth conditions. For example, as ABA is considered a stress hormone which is usually synthesised more rapidly during unfavorable growth conditions (Cowan *et al*., 1997; Hartung, 2010; Tarakhovskaya *et al*., 2007), it was expected that high concentrations of ABA added to the cultures would cause them to react accordingly and therefore begin accumulating lipids.

The lipid content of the *Chlorella vulgaris* cultures in this study ranged from 0.88% to 2.14% dry weight which is within the typical range for an exponentially growing culture under

favourable growth conditions in nutrient rich media (Sostaric *et al*., 2009). Sostaric *et al*., (2009) found that the average lipid content of *Chlorella vulgaris* grown in nutrient rich media and harvested after 7 days of exponential growth was also very low at 1.69%. BA at all concentrations increased total lipid yield above the control with the highest being 39% caused by BA at 10^{-5} M. This is mirrored by the results found by Mostafa *et al*., (2005) where the addition of BA caused an increase in oil yield of 22% in *Hibiscus sabdariffa* (Mostafa *et al*., 2005).

Our hypothesis that different hormones at different concentrations would cause changes in the overall lipid content of *Chlorella vulgaris* was supported. However, our prediction that BA, MeSZ and tZ would decrease while ABA would increase lipid content can only be partially supported. All hormone additions with the exception of BA caused decreases in the lipid content from the control, therefore the prediction is supported for the growth hormones MeSZ and tZ, but not for ABA. Some studies have shown that with exogenous applications of ABA, growth is in fact increased (Kobayashi *et al*., 1997; Noble *et al*., 2014). Kobayashi *et al*., (1997) found that the addition of ABA to *Haematococcus pluvialis* increased cell growth rate as well as causing an increase in carotenoid production, while Noble *et al*., (2014) found that ABA addition to *Euglena gracilis* increased cell growth rate the most out of all single hormone treatments. Interestingly, BA showed a dose dependant response producing a positive correlation between hormone concentration and lipid content, therefore the prediction was unsupported.

It is possible that the addition of the growth hormones MeSZ and tZ stimulated the cells to remain in exponential growth phase; therefore no accumulation of lipids occurred. This is supported by the increase in growth rates observed with the addition of MeSZ and tZ at certain concentrations which would cause the cell to metabolize more lipids when compared to the control. Research into lipid and FA production in pigs and rats has shown that growth hormones

antagonise fatty acid synthase (*FAS*) transcription, therefore reducing the production of FA's and the structural lipids which are made from them (Sul and Wang, 1998). Although this relationship has not been proven in plants, it is possible that this may be occurring with the addition of the growth hormones MeSZ and tZ, as plants also contain *FAS* and fatty acid elongase (*FAE*) (Jadhav *et al*., 2008). However, it is unusual that BA, which caused the greatest increase in growth rate, produced lipid yields above that of the control. As BA was the only aromatic CK exogenously applied, perhaps this has to do with the difference in structure compared to the other two CK's added. The addition of ABA was predicted to increase lipid yield, however the opposite occurred. This result when compared to the published literature may be due to differences in the test organism (Jadhav *et al*., 2008), or differences in growth conditions and growth phase.

4.4 Exogenous Hormone Treatments and Effect on Fatty Acid Profile:

The FA profile of the control *Chlorella vulgaris* cultures tested proved to be typical for the species when grown in favourable conditions, marked by a ratio of ~1:3 saturated to unsaturated FA's (Stephenson *et al*., 2010; Tsuzuki *et al*., 1990). Treatment with phytohormones induced changes in the FA profile, therefore our hypothesis was supported.

Based on the literature available, it was predicted that all four hormone additions would increase unsaturated FA's at the expense of saturated FA's. This however was not the case, as the hormone treatments produced much more diverse effects both among hormone treatments and among concentrations. ABA was the only hormone which produced a clear trend in the results but this went against the predictions. No other hormones produced the changes that were

predicted. The only consistent change was an increase in stearic acid with the addition of all hormones at all concentrations, whereby the most dramatic was caused by ABA at 10^{-6} M.

ABA produced a definite trend of increasing saturated palmitic and stearic acid while decreasing the unsaturated FA's with the exception of linolenic acid at 10^{-6} M. This was a clear indication that treatment with ABA had the opposite effect than was predicted. This was also opposite to the findings of Jadhav *et al*., (2008), where ABA additions caused a twofold increase in the very long chain monounsaturated FA's erucic (22:1) and eicosenoic (20:1) acid in *Brassica napus* seeds (Jadhav *et al*., 2008). ABA also decreased total FA content at all three concentrations in this study whereas Park *et al*., (2013) found it caused an increase of 13% from the control in *Chlamydomonas reinhartii* (Park *et al*., 2013) and an 18% increase in *Brassica napus* (Jadhav *et al*., 2008).

Treatment with BA specifically had almost the same pattern at 10^{-6} and 10^{-5} M where saturated palmitic acid and oleic acid were decreased slightly while linoleic increased. These results are mostly in agreement with the findings of a previous study conducted on hibiscus seeds (*Hibiscus sabdariffa*) where palmitic decreased while oleic and linoleic increased (Mostafa *et al*., 2005). However, in this study, BA addition at 10^{-7} M increased palmitic and oleic acid while decreasing linoleic acid, which conflicts with the findings of Mostafa *et al*., (2005).

MeSZ and tZ increased overall unsaturation the most across treatments, with tZ increasing oleic acid and MeSZ increasing linoleic acid across concentrations and causing very large increases in linolenic acid, especially at 10^{-6} M. A study conducted on *Coleus blumei* and *Impatiens sultani* shoots and leaves found that the addition of tZ caused a decrease in palmitic acid of approximately 9% (Kull et al., 1978), which was comparable to the findings of this study

where tZ at 10^{-7} M caused a decrease in palmitic acid of 11.2% from the control. As stated previously, growth hormones in mammalian systems have been known to suppress the production of FA's (Sul and Wang, 1998), which may be why the growth hormones MeSZ and tZ had negative effects on total FA content. Again, as BA is an aromatic CK, perhaps its structural differences caused it to have a more positive effect on total FA content.

As was stated previously, no study on exogenous MeSZ application to *Chlorella vulgaris* has been published to date, therefore the effects of MeSZ on the FA profile of this species are completely unknown. The most interesting point to note is the very large increases in linolenic acid caused by this hormone at both 10^{-7} and 10^{-6} M. This effect was not seen with any other hormone at any other concentration to this extent. Linolenic acid is composed of an 18-carbon chain with three double bonds (18:3) which makes it incredibly useful and versatile as a feedstock for industrial applications. Through ozonolysis, one 1,3-propanediol molecule and a propanol molecule can be made from this long chain FA (Narine *et al*., 2007). 1,3-propandiol is a ubiquitous chemical usually synthesised from crude oil, and has applications in everything from adhesives to carpeting (Kraus, 2008). Due to the unique spiralling shape of 1,3-propanediol, products produced with it come with advantages such as greater stretching and recovery, stain resistance and better colour fastness including UV fade resistance, lower dyeing temperatures and the option of a more extensive colour range (Kraus, 2008). If optimized, MeSZ treatments may make *Chlorella vulgaris* a viable candidate for a sustainable source of this valuable chemical.

4.5 Future Research:

The study design used in this experiment was built around the addition of hormones during a certain phase of culture growth, so as to ensure the hormones were fully responsible for any changes observed. An exponentially growing culture is actively utilizing the media for nutrition and signalling during this phase therefore the hormones would have been readily perceived by the cells in culture. It would be very interesting to see if these hormones have the same effect when the culture is in the stationary phase of growth, which coincides with the lipid accumulation phase. This way perhaps hormone additions could be fine tuned to maximize lipid yield and potentially FA yield. It would also be useful to explore the relationship between hormone addition and the transcription rates of *FAS* and *FAE*. Given the relationship in animals (Sul and Wang, 1998), perhaps one may exist in plants which can be exploited for the maximization of lipid yield or specific FA's of value.

The addition of MeSCK's to the cultures had a significant effect on growth, comparable to the increases in growth caused by BA at the same concentrations. This is further evidence to suggest that MeSZ plays an important role in causing rapid cell proliferation in an algal culture, and may be applied to other organisms to see if the same effect occurs. This was also the only hormone which caused a very significant increase in linolenic acid, a FA which has multiple applications in the pharmaceutical and chemical industries. Perhaps this hormone can be used to produce other sustainable sources of this FA for example another algal species or canola in order to maximize yield.

5. Conclusions

In summary, our endogenous study showed that *Chlorella vulgaris* is able to synthesise and release a mixture of CK's including cZ and tZ, iP and DZ, as well their corresponding riboside, nucleotide and methylthiolated counterparts. Our hypothesis that the exogenous application of the growth hormones BA, MeSZ and tZ and the stress hormone ABA would cause changes in the growth rate was supported. MeSZ was the most abundant CK detected endogenously, and was very successful at stimulating growth with exogenous application, which was comparable to that of BA application. The optimal concentrations for growth stimulation were discovered for BA, MeSZ and ABA at 10^{-6} M but the optimal concentration for tZ is hypothesised to be 10^{-5} M or more.

Hormone additions were successful at altering both the total lipid and total FA content which supported our hypothesis, however only BA was successful at increasing lipid content significantly above the control. All hormones at all concentrations caused changes in the FA profile from the control which also supported our hypothesis, the most significant of which was the large increase in linolenic acid by MeSZ. As research on the exogenous applications of BA, tZ, MeSZ and ABA and their effect on FA profile in *Chlorella vulgaris* has not been published to date, these results contribute a new piece of information to the existing pool of knowledge in both FA's and hormones in algal systems.

6. References

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7. Appendices

7.1 Endogenous Hormone Study Raw Data:

The following includes all raw data produced from the endogenous hormone study of Chlorella vulgaris. Data tables for ABA and all cytokinin types found (Free Bases, Ribosides, Nucleotides and Methylthiols) have been included as well as all supplementary numbers used for calculations such as molecular weight and tissue weight. All sample names proceeded by "P" represent pellet samples, while all samples labelled "S" are supernatant samples.

7.1.1 ABA:

Table 6: Endogenous ABA content of *Chlorella vulgaris*

#	Name	MW ABA	2HABA	ABA	Tisue Weight (g fwt.)	Internal Stnd (ng)	ABA
ABA 1	P 2	264.32	1.62E+07	4.52E+04	0.2170	2.70E+01	1.3134
ABA 2	P 3	264.32	1.95E+07	4.74E+04	0.2380	2.70E+01	1.0433
ABA 3	P 4	264.32	1.58E+07	4.52E+04	0.2250	2.70E+01	1.2988
ABA 4	P 5	264.32	2.10E+07	4.75E+04	0.2490	2.70E+01	0.9279
ABA 5	P 6	264.32	1.78E+07	3.58E+04	0.1150	2.70E+01	1.7865
ABA 6	P 7	264.32	1.70E+07	3.66E+04	0.1810	2.70E+01	1.2150
ABA 7	P 8	264.32	1.83E+07	3.88E+04	0.1400	2.70E+01	1.5470
ABA 8	S 1	264.32	7.92E+06	3.35E+05	0.1650	2.70E+01	26.1860
ABA 9	S 2	264.32	9.39E+06	5.58E+05	0.2170	2.70E+01	27.9732
ABA 10	S 3	264.32	9.46E+06	4.22E+05	0.2380	2.70E+01	19.1460
ABA 11	S 4	264.32	7.62E+06	4.03E+05	0.2250	2.70E+01	24.0105
ABA 12	S 5	264.32	9.08E+06	4.21E+05	0.2490	2.70E+01	19.0209
ABA 13	S 6	264.32	1.11E+07	2.59E+05	0.1150	2.70E+01	20.7259
ABA 14	S 7	264.32	1.05E+07	4.19E+05	0.1810	2.70E+01	22.5206
ABA 15	S 8	264.32	1.20E+07	3.63E+05	0.1400	2.70E+01	22.0715

7.1.2 Free Bases:

Table 7: Endogenous cytokinin free base content of *Chlorella vulgaris*

#	Name	MW Z	2HDZ	tZ	cisZ	MW DZ	2HDZ	DZ	MW iP	2HiP	iP	Tissue Weight (g fwt.)	Internal Stnd (ng)	TransZ	CisZ	DZ	iP
FB 1	P 2	219.25	9.14E+05	0.00E+00	0.00E+00	221.259	9.14E+05	0.00E+00	203.25	4.59E+06	0.00E+00	0.2170	10	0	0	0	0
FB 2	P 3	219.25	9.83E+05	0.00E+00	0.00E+00	221.259	9.83E+05	0.00E+00	203.25	4.25E+06	0.00E+00	0.2380	10	0	0	0	0

FB 3	P 4	219.25	9.04E+05	0.00E+00	0.00E+00	221.259	9.04E+05	0.00E+00	203.25	3.55E+06	0.00E+00	0.2250	10	0	0	0	0
FB 4	P 5	219.25	8.77E+05	0.00E+00	0.00E+00	221.259	8.77E+05	0.00E+00	203.25	4.58E+06	0.00E+00	0.2490	10	0	0	0	0
FB 5	P 6	219.25	9.91E+05	0.00E+00	0.00E+00	221.259	9.91E+05	0.00E+00	203.25	4.30E+06	0.00E+00	0.1150	10	0	0	0	0
FB 6	P 7	219.25	9.95E+05	0.00E+00	0.00E+00	221.259	9.95E+05	0.00E+00	203.25	4.23E+06	0.00E+00	0.1810	10	0	0	0	0
FB 7	P 8	219.25	6.93E+05	0.00E+00	0.00E+00	221.259	6.93E+05	0.00E+00	203.25	3.21E+06	0.00E+00	0.1400	10	0	0	0	0
FB 8	S 1	219.25	5.54E+05	6.85E+05	4.74E+04	221.259	5.54E+05	0.00E+00	203.25	3.10E+06	1.84E+05	0.1650	10	341.788	23.651	0	17.6987
FB 9	S 2	219.25	9.90E+05	1.56E+06	2.98E+04	221.259	9.90E+05	0.00E+00	203.25	4.59E+06	4.99E+05	0.2170	10	331.2	6.3268	0	24.6489
FB 10	S 3	219.25	8.47E+05	1.26E+06	5.16E+04	221.259	8.47E+05	0.00E+00	203.25	5.03E+06	6.40E+05	0.2380	10	285.083	11.675	0	26.303
FB 11	S 4	219.25	8.64E+05	9.96E+05	3.37E+04	221.259	8.64E+05	0.00E+00	203.25	4.12E+06	6.51E+05	0.2250	10	233.681	7.9067	0	34.5518
FB 12	S 5	219.25	4.72E+05	7.73E+05	2.17E+04	221.259	4.72E+05	0.00E+00	203.25	2.19E+06	6.34E+05	0.2490	10	299.984	8.4213	0	57.2025
FB 13	S 6	219.25	6.99E+05	2.30E+05	3.64E+04	221.259	6.99E+05	0.00E+00	203.25	3.10E+06	2.08E+05	0.1150	10	130.501	20.653	0	28.706
FB 14	S 7	219.25	6.25E+05	9.82E+05	2.81E+04	221.259	6.25E+05	0.00E+00	203.25	3.21E+06	4.58E+05	0.1810	10	395.925	11.329	0	38.7839
FB 15	S 8	219.25	8.92E+05	3.51E+05	3.80E+04	221.259	8.92E+05	0.00E+00	203.25	4.36E+06	4.42E+05	0.1400	10	128.196	13.879	0	35.6268

7.1.3 Ribosides:

Table 8: Endogenous cytokinin riboside content of *Chlorella vulgaris*

#	Name	MW ZR	2HZR	tZR	CisZR	MW DZR	2HDZ	DZR	MW iPR	2HiPR	iPR	Weight (g fwt.)	Internal Stnd (ng)	TransZR	CisZR	DZR	iPR
R 1	P 2	351.37	3.13 E+06	4.94 E+03	1.59 E+04	353.38	2.91E+06	1.24E+04	335.37	1.89E+06	0.00E+00	0.2170	10	0.207	0.666	0.556	0.000
R 2	P 3	351.37	3.19 E+06	5.07 E+03	1.58 E+04	353.38	2.98E+06	9.31E+03	335.37	1.81E+06	0.00E+00	0.2380	10	0.190	0.592	0.371	0.000
R 3	P 4	351.37	2.81 E+06	4.62 E+03	1.09 E+04	353.38	2.22E+06	1.02E+04	335.37	1.49E+06	0.00E+00	0.2250	10	0.208	0.491	0.578	0.000
R 4	P 5	351.37	3.56 E+06	5.45 E+03	1.95 E+04	353.38	3.17E+06	1.01E+04	335.37	1.19E+04	0.00E+00	0.2490	10	0.175	0.626	0.362	0.000
R 5	P 6	351.37	4.06 E+06	7.97 E+03	4.56 E+03	353.38	3.24E+06	1.15E+04	335.37	1.09E+04	0.00E+00	0.1150	10	0.486	0.278	0.873	0.000
R 6	P 7	351.37	3.20 E+06	8.55 E+03	1.52 E+04	353.38	3.25E+06	7.18E+03	335.37	2.02E+06	0.00E+00	0.1810	10	0.420	0.747	0.345	0.000
R 7	P 8	351.37	2.78 E+06	5.83 E+03	7.47 E+03	353.38	2.38E+06	5.93E+03	335.37	1.52E+06	0.00E+00	0.1400	10	0.426	0.546	0.504	0.000
R 8	S 1	351.37	1.90 E+06	3.83 E+03	1.92 E+04	353.38	2.10E+06	5.39E+03	335.37	1.93E+06	4.00E+04	0.1650	10	0.348	1.743	0.440	3.745
R 9	S 2	351.37	3.34 E+06	2.39 E+03	1.23 E+04	353.38	3.59E+06	7.60E+03	335.37	3.85E+06	4.50E+04	0.2170	10	0.094	0.483	0.276	1.606
R 10	S 3	351.37	4.10 E+06	1.69 E+03	1.22 E+04	353.38	3.75E+06	1.27E+04	335.37	5.76E+06	7.76E+04	0.2380	10	0.049	0.356	0.403	1.688
R 11	S 4	351.37	2.81 E+06	3.78 E+03	1.80 E+04	353.38	2.23E+06	9.91E+03	335.37	2.82E+06	4.32E+04	0.2250	10	0.170	0.810	0.559	2.030
R 12	S 5	351.37	1.60 E+06	4.21 E+03	6.41 E+03	353.38	1.68E+06	7.67E+03	335.37	1.60E+06	3.41E+04	0.2490	10	0.301	0.458	0.519	2.552
R	S 6	351.37	2.88	2.67	5.38	353.38	3.29E+06	8.77E+03	335.37	2.94E+06	2.52E+04	0.1150	10	0.229	0.462	0.656	2.222

13			E+06	E+03	E+03												
R 14	S 7	351.37	2.13E+06	2.99E+03	1.73E+04	353.38	2.11E+06	8.29E+03	335.37	2.24E+06	5.11E+04	0.1810	10	0.221	1.277	0.614	3.758
R 15	S 8	351.37	3.29E+06	3.29E+03	1.22E+04	353.38	3.13E+06	1.39E+04	335.37	3.27E+06	3.27E+04	0.1400	10	0.203	0.754	0.898	2.130

7.1.4 Nucleotides:

Table 9: Endogenous cytokinin nucleotide content of *Chlorella vulgaris*

#	Name	MW ZNT	2H ZNT	Trans ZNT	CisZNT	MW DZNT	2H DZNT	DZNT	MW iPNT	2HiPNT	iPNT	Weight (g fwt.)	Internal Stnd (ng)	TransZNT	CisZNT	DZNT	iPNT
NT 1	P 6	351.37	2.07E+05	1.66E+03	4.69E+03	353.38	9.63E+02	0.00E+00	335.37	5.48E+05	8.31E+03	0.115	10	1.98	5.61	0.00	3.93
NT 2	P 7	351.37	1.57E+05	9.27E+02	9.85E+03	353.38	7.78E+02	0.00E+00	335.37	4.81E+05	1.02E+04	0.181	10	0.93	9.86	0.00	3.49
NT 3	P 8	351.37	2.08E+05	1.39E+03	5.10E+03	353.38	6.64E+03	0.00E+00	335.37	6.11E+05	1.05E+04	0.14	10	1.36	4.98	0.00	3.66
NT 4	S 5	351.37	1.47E+04	9.47E+02	2.63E+04	353.38	7.68E+02	0.00E+00	335.37	9.33E+03	9.29E+04	0.249	10	7.36	204.49	0.00	1192.37
NT 5	S 6	351.37	2.51E+04	3.96E+02	7.74E+03	353.38	1.10E+03	0.00E+00	335.37	1.34E+04	5.61E+04	0.115	10	3.90	76.31	0.00	1085.52
NT 6	S 7	351.37	2.56E+04	9.44E+02	2.97E+04	353.38	1.39E+03	0.00E+00	335.37	1.85E+04	1.18E+05	0.181	10	5.80	182.42	0.00	1050.77

7.1.5 Methylthiols:

Table 10: Endogenous cytokinin methylthiol content of *Chlorella vulgaris*

#	Name	MW MeS Z	2H MeSZ	MeSZ	MW MeS iP	2H MeS iP	MeSiP	MW MeS ZR	2H MeS ZR	MeSZR	MW MeS iPA	2H MeS iPA	MeS iPA	Tissue Weight (g fwt.)	Internal Stnd (ng)	MeSZ	MeSiP	MeSZR	MeS iPA
M 1	P 2	265	1.67E+05	7.46E+04	249	1.25E+06	9.65E+04	397	1.47E+06	2.45E+04	3.81E+02	2.81E+06	3.66E+04	0.2170	10	77.68	14.29	1.93	1.58
M 2	P 3	265	1.52E+05	5.69E+04	249	9.31E+05	8.99E+04	397	1.44E+06	2.49E+04	3.81E+02	2.30E+06	3.02E+04	0.2380	10	59.35	16.29	1.83	1.45
M 3	P 4	265	1.08E+05	4.48E+04	249	6.33E+05	5.57E+04	397	1.00E+06	1.56E+04	3.81E+02	1.53E+06	2.48E+04	0.2250	10	69.57	15.71	1.75	1.89
M 4	P 5	265	1.44E+05	6.14E+04	249	8.94E+05	8.19E+04	397	1.59E+06	2.64E+04	3.81E+02	2.55E+06	4.32E+04	0.2490	10	64.62	14.78	1.68	1.79
M 5	P 6	265	1.36E+05	2.81E+04	249	9.11E+05	3.53E+04	397	1.41E+06	1.49E+04	3.81E+02	2.06E+06	2.55E+04	0.1150	10	67.80	13.53	2.31	2.83
M 6	P 7	265	1.62E+05	6.58E+04	249	9.67E+05	1.01E+05	397	1.56E+06	1.78E+04	3.81E+02	2.25E+06	2.72E+04	0.1810	10	84.68	23.17	1.59	1.75

M 7	P 8	265	9.60 E+04	2.49 E+04	249	4.30 E+05	2.74 E+04	397	1.08 E+06	8.99 E+03	3.81 E+02	1.48 E+06	2.58 E+04	0.1400	10	69.91	18.28	1.50	3.27
M 8	S 1	265	1.16 E+05	1.75 E+06	249	5.62 E+05	5.60 E+05	397	7.22 E+05	1.25 E+05	3.81 E+02	8.91 E+05	8.66 E+04	0.1650	10	3450.25	242.53	26.43	15.46
M 9	S 2	265	1.49 E+05	3.59 E+06	249	9.20 E+05	9.81 E+05	397	1.05 E+06	1.06 E+05	3.81 E+02	1.60 E+06	8.56 E+04	0.2170	10	4189.89	197.34	11.72	6.47
M 10	S 3	265	1.82 E+05	3.84 E+06	249	9.55 E+05	8.34 E+05	397	1.18 E+06	1.47 E+05	3.81 E+02	1.82 E+06	1.08 E+05	0.2380	10	3345.31	147.36	13.18	6.54
M 11	S 4	265	1.27 E+05	2.97 E+06	249	6.15 E+05	6.56 E+05	397	9.20 E+05	1.22 E+05	3.81 E+02	1.13 E+06	8.91 E+04	0.2250	10	3922.15	190.39	14.85	9.20
M 12	S 5	265	7.23 E+04	1.40 E+06	249	3.23 E+05	4.13 E+05	397	5.65 E+05	3.59 E+04	3.81 E+02	5.89 E+05	3.67 E+04	0.2490	10	2934.57	206.23	6.43	6.57
M 13	S 6	265	9.87 E+04	7.13 E+05	249	5.62 E+05	6.64 E+05	397	6.41 E+05	2.37 E+04	3.81 E+02	6.00 E+05	2.79 E+04	0.1150	10	2370.44	412.61	8.10	10.61
M 14	S 7	265	9.39 E+04	2.22 E+06	249	5.90 E+05	7.24 E+05	397	6.68 E+05	1.05 E+05	3.81 E+02	9.93 E+05	9.58 E+04	0.1810	10	4929.05	272.28	21.87	13.99
M 15	S 8	265	1.96 E+05	9.78 E+05	249	8.01 E+05	8.04 E+05	397	1.13 E+06	8.61 E+04	3.81 E+02	1.62 E+06	1.18 E+05	0.1400	10	1344.96	287.94	13.71	13.66

7.2 Exogenous Hormone Study Raw Data:

7.2.1 Hormone Addition OD Values:

Table 11: Optical densities of MeSZ, tZ, BA, ABA and Control cultures across all concentrations

	10-7 M					10-6 M					10-5 M				
	MeSZ	tZ	BA	ABA	Control	MeSZ	tZ	BA	ABA	Control	MeSZ	tZ	BA	ABA	Control
day 1	0.831	0.758	0.816	0.769	0.815	0.856	0.825	0.776	0.871	0.874	0.77	0.779	0.793	0.869	0.82
	0.834	0.802	0.786	0.752	0.809	0.77	0.826	0.754	0.762	0.803	0.809	0.735	0.805	0.802	0.813
	0.834	0.834	0.783	0.789	0.82	0.89	0.817	0.827	0.873	0.875	0.763	0.82	0.8	0.787	0.817
		0.754	0.813	0.845	0.8		0.89	0.856	0.842	0.726		0.788	0.789	0.829	0.778
day 2	0.817	0.635	0.79	0.813	0.811	0.97	0.87	0.997	0.822	0.927	0.831	0.849	0.853	0.911	0.754
	0.84	0.863	0.891	0.861	0.845	1.008	0.903	1.019	0.925	0.999	0.877	0.817	0.861	0.793	0.842
	0.852	0.822	0.848	0.852	0.907	0.985	0.871	1.002	0.993	1.004	0.812	0.815	0.846	0.781	0.772
		0.884	0.82	0.823	0.85		0.902	1.011	0.995	0.961		0.848	0.83	0.79	0.789
day 3	0.957	0.899	0.992	0.978	0.996	1.093	0.975	1.159	1.111	1.121	0.978	1.015	1.028	1.069	0.926
	0.899	0.947	1.008	0.984	0.946	1.114	0.986	1.134	1.067	1.123	0.993	0.975	1.044	0.93	0.996
	0.953	0.935	0.942	0.966	1.042	1.109	1.001	1.133	1.145	1.103	0.979	0.976	1.012	0.901	0.896
		1.016	1.021	0.972	0.952		0.996	1.132	1.14	1.07		1.019	1.022	0.91	0.894
day 4	1.09	1	1.117	1.031	1.093	1.24	1.094	1.31	1.189	1.202	1.09	1.18	1.198	1.18	1.037
	0.983	1.055	1.091	1.017	0.983	1.268	1.069	1.286	1.137	1.219	1.106	1.117	1.195	0.965	1.105
	1.089	1.035	1.06	0.992	1.08	1.265	1.154	1.3	1.232	1.244	1.109	1.126	1.136	1.002	0.957
		1.136	1.117	1.013	1.006		1.121	1.283	1.204	1.152		1.146	1.176	0.976	1.048
day 5	1.17	1.043	1.182	1.068	1.206	1.356	1.128	1.421	1.175	1.036	1.091	1.248	1.182	1.25	1.036
	0.985	1.132	1.117	1.026	0.953	1.38	1.066	1.33	1.213	1.258	1.133	1.193	1.224	0.948	1.052
	1.155	1.066	1.006	0.934	1.069	1.379	1.187	1.311	1.221	1.294	1.111	1.184	1.188	1.078	0.861
		1.077	0.949	0.985	1.076		1.241	1.355	1.066	1.287		1.15	1.215	0.946	1.051
day 6	1.196	1.089	1.192	1.025	1.182	1.439	1.181	1.552	1.268	1.363	1.094	1.305	1.285	1.408	1.066
	0.92	1.203	1.117	1.071	1.035	1.486	1.045	1.498	1.289	1.338	1.01	1.316	1.281	1.104	1.106
	1.217	1.062	1.101	1.005	1.129	1.448	1.267	1.479	1.378	1.332	1.118	1.269	1.297	1.085	0.971
		1.165	1.063	1.007	1.126		1.307	1.435	1.202	1.219		1.264	1.316	1.042	1.073
day 7	1.384	1.233	1.352	1.269	1.417		1.217		1.445	1.489	1.16	1.483	1.455	1.469	1.178
	0.884	1.317	1.307	1.133	1.032		1.078		1.424	1.484	1.103	1.412	1.406	1.095	1.299
	1.313	1.148	1.198	1.006	1.308		1.375		1.474	1.458	1.213	1.358	1.41	1.16	0.995
		1.3	1.159	1.083	1.085		1.393		1.259	1.208		1.42	1.487	1.005	1.255
day 8	1.572	1.429	1.451	1.454	1.593		1.297		1.562	1.588	1.391	1.483	1.455	1.469	1.438
	0.967	1.499	1.453	1.277	1.058		1.122		1.55	1.614	1.19	1.512	1.599	1.132	1.525
	1.353	1.301	1.354	1.103	1.494		1.497		1.596	1.631	1.452	1.497	1.568	1.356	1.221
		1.418	1.342	1.199	1.244		1.565		1.422	1.442		1.546	1.487	1.241	1.41
day 9	1.572	1.612	1.451	1.454	1.593		1.531		1.562		1.601			1.469	1.438
	1.251	1.499	1.453	1.47	1.472		1.35		1.55		1.574			1.335	1.525
	1.649	1.458	1.512	1.387	1.494		1.497		1.596		1.452			1.475	1.366
		1.621	1.513	1.412	1.525		1.565		1.599					1.309	1.542
day 10	1.572			1.454			1.531							1.469	1.438
	1.525			1.47			1.492							1.519	1.525
	1.649			1.518			1.497							1.475	1.51
				1.576			1.565							1.524	1.542

7.2.2 Lipid Sample Weights:

Table 12: Algae pellet dry weight/g and lipid sample weight/g calculations

	Algae Pellet Dry Weight/ g	Lipid Tube /g	Tubes + Lipid	Lipid Sample/ g
C 51	0.542	11.4764	11.5057	0.0293
52	0.562	11.3442	11.3542	0.01
53	0.613	11.3781	11.3865	0.0084
54	0.487	11.431	11.4378	0.0068
61	0.697	11.4872	11.4953	0.0081
62	0.689	11.455	11.4657	0.0107
63	0.685	11.4503	11.4575	0.0072
64	0.63	11.3448	11.3581	0.0133
71	0.648	11.5408	11.547	0.0062
72	0.61	11.5749	11.5866	0.0117
73	0.612	11.4766	11.4839	0.0073
74	0.624	11.3903	11.405	0.0147
BA 51	0.577	11.6392	11.6512	0.012
52	0.646	11.6507	11.6623	0.0116
53	0.638	11.5488	11.5605	0.0117
54	0.614	11.677	11.6945	0.0175
61	0.443	11.5808	11.5912	0.0104
62	0.61	11.7023	11.7143	0.012
63	0.597	11.4069	11.4196	0.0127
64	0.612	11.5398	11.5474	0.0076
71	0.55	11.59	11.601	0.011
72	0.578	11.394	11.4034	0.0094
73	0.58	11.6209	11.6298	0.0089
74	0.578	11.4991	11.506	0.0069
ABA 51	0.593	11.4579	11.4647	0.0068
52	0.678	11.4935	11.5039	0.0104
53	0.628	11.4899	11.4961	0.0062
54	0.611	11.3279	11.3341	0.0062
61	0.68	11.3953	11.3984	0.0031
62	0.64	11.5555	11.561	0.0055
63	0.683	11.4363	11.4443	0.008
64	0.689	11.5385	11.5454	0.0069
71	0.595	11.5503	11.5552	0.0049
72	0.62	11.511	11.5146	0.0036
73	0.615	11.4416	11.463	0.0214
74	0.653	11.3764	11.3837	0.0073
tZ 51	0.574	11.4534	11.4613	0.0079
52	0.588	11.4865	11.4981	0.0116
53	0.611	11.5129	11.5207	0.0078
54	0.649	11.4369	11.4435	0.0066
61	0.646	11.4724	11.4778	0.0054
62	0.663	11.7141	11.7196	0.0055
63	0.654	11.71	11.7175	0.0075
64	0.663	11.3966	11.4028	0.0062
71	0.662	11.5222	11.5275	0.0053
72	0.614	11.5349	11.5403	0.0054
73	0.632	11.4574	11.4632	0.0058
74	0.674	11.5234	11.5304	0.007
MeSZ 51	0.641	11.5254	11.5314	0.006
52	0.655	11.6245	11.6297	0.0052
53	0.638	11.5351	11.5409	0.0058
61	0.589	11.4932	11.5035	0.0103
62	0.614	11.5042	11.5108	0.0066
63	0.616	11.4459	11.454	0.0081
71	0.634	11.6364	11.6433	0.0069
72	0.635	11.6229	11.6299	0.007
73	0.69	11.4876	11.4922	0.0046

7.2.3 FAME Sample Weights:

Table 13: FAME sample weight/g calculations

	Tubes	Tubes + FAME/ g	FAME/ g
C 51	2.4835	2.5104	0.0269
C 52	2.4814	2.4935	0.0121
C 53	2.5237	2.5355	0.0118
C 54	2.4737	2.4843	0.0106
C 61	2.4896	2.4993	0.0097
C 62	2.4834	2.4949	0.0115
C 63	2.4899	2.5013	0.0114
64	2.4924	2.5037	0.0113
71	2.5005	2.508	0.0075
72	2.4831	2.495	0.0119
73	2.4827	2.4919	0.0092
74	2.5009	2.5128	0.0119
BA 51	2.4849	2.4953	0.0104
52	2.4835	2.4942	0.0107
53	2.4854	2.4962	0.0108
54	2.49	2.5032	0.0132
61	2.4901	2.4969	0.0068
62	2.489	2.5004	0.0114
63	2.4845	2.4962	0.0117
64	2.4744	2.4818	0.0074
71	2.493	2.5032	0.0102
72	2.4737	2.4838	0.0101
73	2.5233	2.5326	0.0093
74	2.473	2.4812	0.0082
ABA 51	2.4819	2.4901	0.0082
52	2.4819	2.4907	0.0088
53	2.4848	2.4925	0.0077
54	2.482	2.4901	0.0081
61	2.4809	2.4863	0.0054
62	2.5017	2.5089	0.0072
63	2.4832	2.4918	0.0086
64	2.5013	2.5086	0.0073
71	2.524	2.531	0.007
72	2.4896	2.4953	0.0057
73	2.5236	2.5419	0.0183
74	2.5008	2.5096	0.0088
tZ 51	2.523	2.5317	0.0087
52	2.4828	2.4937	0.0109
53	2.4845	2.4931	0.0086
54	2.4852	2.4939	0.0087
61	2.4744	2.4816	0.0072
62	2.5246	2.5322	0.0076
63	2.4835	2.4927	0.0092
64	2.5236	2.5311	0.0075
71	2.4892	2.4964	0.0072
72	2.4823	2.4896	0.0073
73	2.4824	2.4898	0.0074
74	2.4884	2.4967	0.0083
MeSZ 51	2.4918	2.4992	0.0074
52	2.4814	2.4886	0.0072
53	2.4817	2.4886	0.0069
61	2.4835	2.492	0.0085
62	2.4847	2.4914	0.0067
63	2.5232	2.5304	0.0072
71	2.5241	2.531	0.0069
72	2.5021	2.5086	0.0065
73	2.4843	2.4893	0.005

7.2.4 FAME Profile:

Table 14: Peak to mass conversion of FAME's (samples in 500µl, injection volume 1µl)

	Palmitic		Stearic		Oleic		Linoleic		Linolenic		Heptadecanoic	
	Peak Area	m (mg)	Peak Area	m (mg)	Peak Area	m (mg)	Peak Area	m (mg)	Peak Area	m (mg)	Peak Area	m (mg)
C 51	50238	0.628504	8965	0.1097171	13762	0.089802	11924	0.325365	2228	0.035197	128819	1.78
52	43605	0.548957	8212	0.1007774	37936	0.428117	37916	0.649901	5606	0.10704	92313	1.27
53	41421	0.522765	6000	0.0745164	27764	0.28576	27892	0.524741	1045	0.010036	76180	1.05
54	55032	0.685997	6930	0.0855574	48037	0.56948	53851	0.848866	1774	0.025541	111444	1.54
61	52583	0.656627	8143	0.0999582	41556	0.478779	45181	0.740612	1765	0.025349	105671	1.46
62	60407	0.750457	10809	0.1316092	46010	0.541112	54983	0.863	3036	0.052381	125642	1.73
63	36769	0.466976	6549	0.0810341	36461	0.407474	29919	0.55005	1332	0.01614	82256	1.13
64	31594	0.404914	6194	0.0768195	23033	0.219549	30395	0.555994	4194	0.07701	71299	0.98
72	58330	0.725549	10085	0.1230138	55761	0.677578	62011	0.950751	2156	0.033665	106718	1.47
73	25809	0.335536	3680	0.0469731	20388	0.182533	15569	0.370876	1020	0.009505	58714	0.80
74	46821	0.587525	7179	0.0885136	49052	0.583685	44708	0.734706	1522	0.020181	99290	1.37
BA 71	58605	0.728846	10085	0.1230138	51564	0.618841	50763	0.810309	1853	0.027221	147046	2.03
72	64040	0.794026	9973	0.1216842	36975	0.414668	21166	0.44076	1822	0.026562	180489	2.49
73	59943	0.744893	8560	0.1049089	57245	0.698346	53037	0.838702	1482	0.019331	163179	2.25
74	64364	0.797912	8795	0.1076988	65162	0.809145	23595	0.471089	1575	0.021309	174666	2.41
61	80697	0.993788	12553	0.1523142	68753	0.859401	99206	1.415168	4117	0.075372	180601	2.50
62	90021	1.105607	13969	0.169125	80072	1.01781	89227	1.29057	3258	0.057103	199824	2.76
63	45183	0.567881	9795	0.1195709	36546	0.408664	55256	0.866408	2760	0.046511	138949	1.92
64	31185	0.400009	6854	0.0846551	25736	0.257378	22024	0.451473	1338	0.016268	100610	1.39
51	29944	0.385126	6482	0.0802387	19570	0.171085	29626	0.546392	1742	0.02486	100792	1.39
52	46737	0.586518	8006	0.0983318	46241	0.544345	51418	0.818487	1302	0.015502	157032	2.17
53	30822	0.395655	7251	0.0893683	30336	0.321755	33375	0.593202	1212	0.013588	97944	1.35
54	31350	0.401988	5744	0.0714771	27004	0.275124	29502	0.544844	2784	0.047022	89903	1.24
ABA 61	17653	0.237725	4681	0.0588571	12071	0.066136	2980	0.21369	1262	0.014652	126062	1.74
62	30248	0.388772	6834	0.0844177	27679	0.28457	21501	0.444943	1483	0.019352	124329	1.71
63	35025	0.44606	8928	0.1092778	22496	0.212034	15884	0.374809	3016	0.051956	166967	2.31
64	56480	0.703362	11254	0.1368923	42700	0.494789	22191	0.453559	2538	0.04179	215558	2.98
51	38359	0.486044	4268	0.0539539	27939	0.288209	24620	0.483887	1634	0.022563	165797	2.29
52	46814	0.587441	10432	0.1271335	25256	0.25066	29153	0.540486	1988	0.030092	179503	2.48
53	50717	0.634249	9370	0.1145253	38124	0.430748	35845	0.624042	1406	0.017714	172370	2.38
54	47934	0.600873	6545	0.0809866	41700	0.480794	40678	0.684387	1270	0.014822	130112	1.79
tZ 71	24234	0.316648	7054	0.0870295	21019	0.191364	17383	0.393526	1858	0.027327	129297	1.78
72	26924	0.348908	8099	0.0994359	26328	0.265663	20929	0.437801	2064	0.031709	113180	1.56
73	53130	0.663187	11469	0.1394448	62717	0.774927	44760	0.735355	4218	0.07752	191162	2.64
74	20191	0.268162	5408	0.0674881	27952	0.288391	20755	0.435629	2703	0.045299	82859	1.14
61	15084	0.206915	2878	0.0374517	12587	0.073358	11482	0.319846	798	0.004783	44747	0.61
62	25543	0.332346	4742	0.0595813	22164	0.207388	14024	0.351585	886	0.006655	75373	1.04
63	30766	0.394984	6723	0.0830999	31585	0.339235	27446	0.519173	1497	0.01965	71874	0.99
64	23554	0.308493	7139	0.0880387	23694	0.2288	17517	0.395199	1598	0.021798	101723	1.40
MeSZ 71	17314	0.233659	3837	0.048837	22947	0.218346	11897	0.325028	2815	0.047681	79452	1.09
72	20398	0.270644	3970	0.050416	19190	0.165767	10610	0.308958	3732	0.067184	81031	1.11
73	15789	0.21537	3630	0.0463795	15697	0.116882	8826	0.286683	2673	0.044661	103008	1.42
61	25750	0.334829	4852	0.0608872	17988	0.148945	25230	0.491504	5805	0.111273	112614	1.55
62	25048	0.32641	4886	0.0612909	20478	0.183792	22445	0.45673	6505	0.12616	109651	1.51
63	25400	0.330631	6511	0.080583	16740	0.131479	17647	0.396822	8448	0.167484	106265	1.46
51	25359	0.33014	6996	0.086341	24198	0.235854	23059	0.464396	1406	0.017714	99969	1.38
52	26651	0.345634	6054	0.0751575	23845	0.230913	22910	0.462536	1232	0.014014	137774	1.90
53	18515	0.248062	4660	0.0586078	21842	0.202881	10700	0.310082	680	0.002274	90113	1.24

7.3 Nuclear Magnetic Resonance (NMR):

^{13}C-NMR was conducted on a Varian spectrometer (Varian Inc., USA) using labelled chloroform as the solvent and data was collected on a Biosystem Mariner high resolution electronspray spectrometer (PerSeptive Biosystems Inc., USA). TMS was used as an internal standard (0.0 PPM). Figure 21 shows a spectrum collected from a random lipid sample before transesterification, showing the intact bonds of mono-, di-, and triacylglycerols (3.6-5.6 PPM). Other peaks are most likely pigment lipids such as carotenoid. Figure 22 shows the spectrum of a random FAME sample after transesterification, showing the absence of glycerol meaning transesterification was successful, fatty acid methyl esters (0.8-1.0 PPM) and a peak suspected to be a long chain alkane (1.2-1.4 PPM). This was confirmed by TLC (see Figure 23).

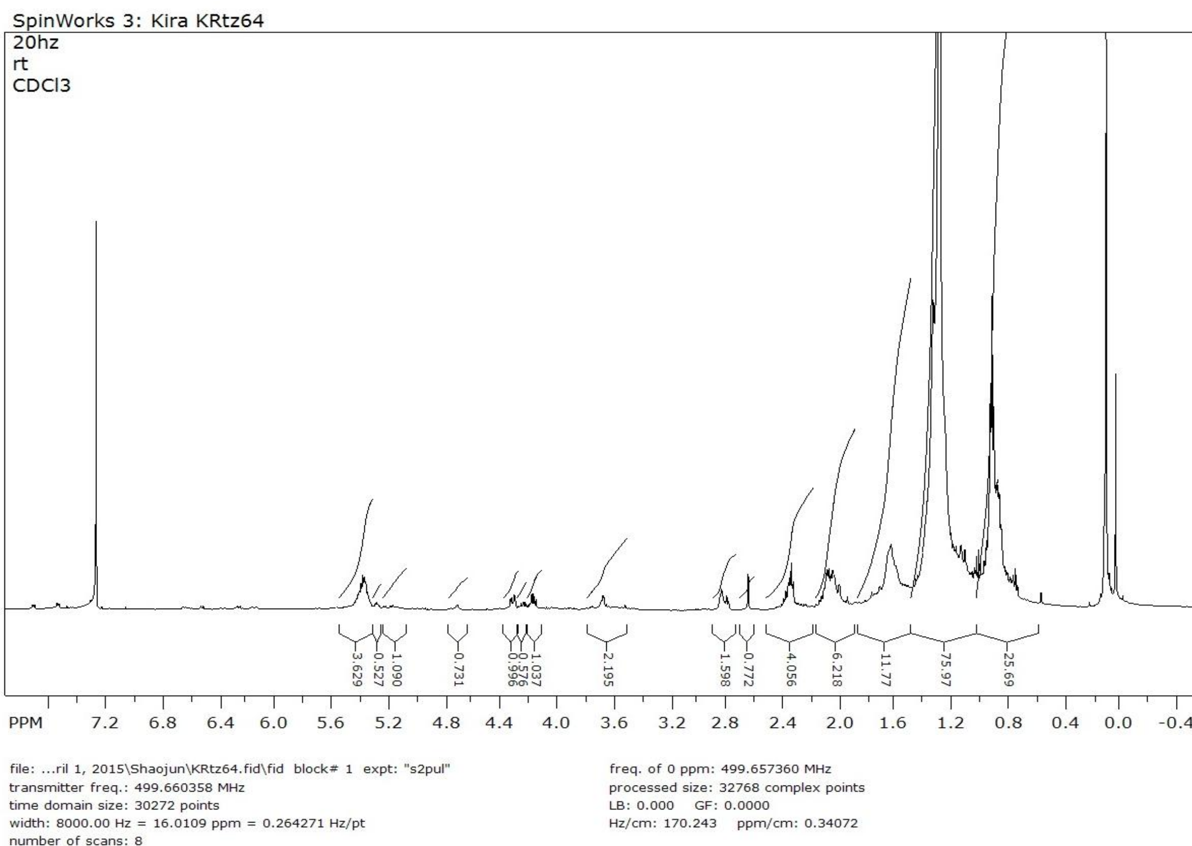

Figure 21: Lipid NMR spectrum

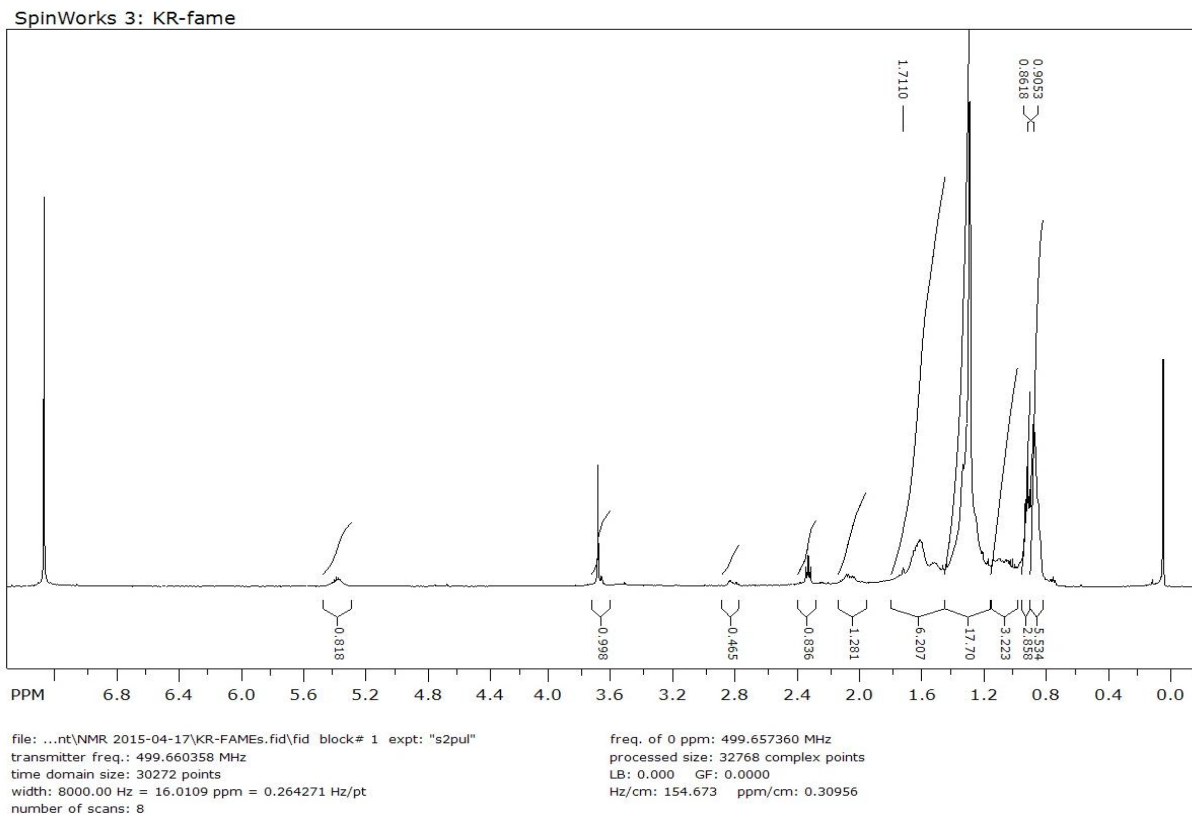

Figure 22: FAME NMR spectrum

7.4 Thin Layer Chromatography (TLC):

A TLC was conducted on the FAME NMR sample using hexane and ethyl acetate as the solvents to confirm the presence of a long chain alkane. Four ratios of hexane to ethyl acetate were used from left to right in Figure 23: 5:1, 1:0, 20:1 and 40:1. Plate 3 indicated the presence of free fatty acids and fatty acid methyl esters, while plate 1, 3 and 4 revealed the presence of a long chain alkane compound.

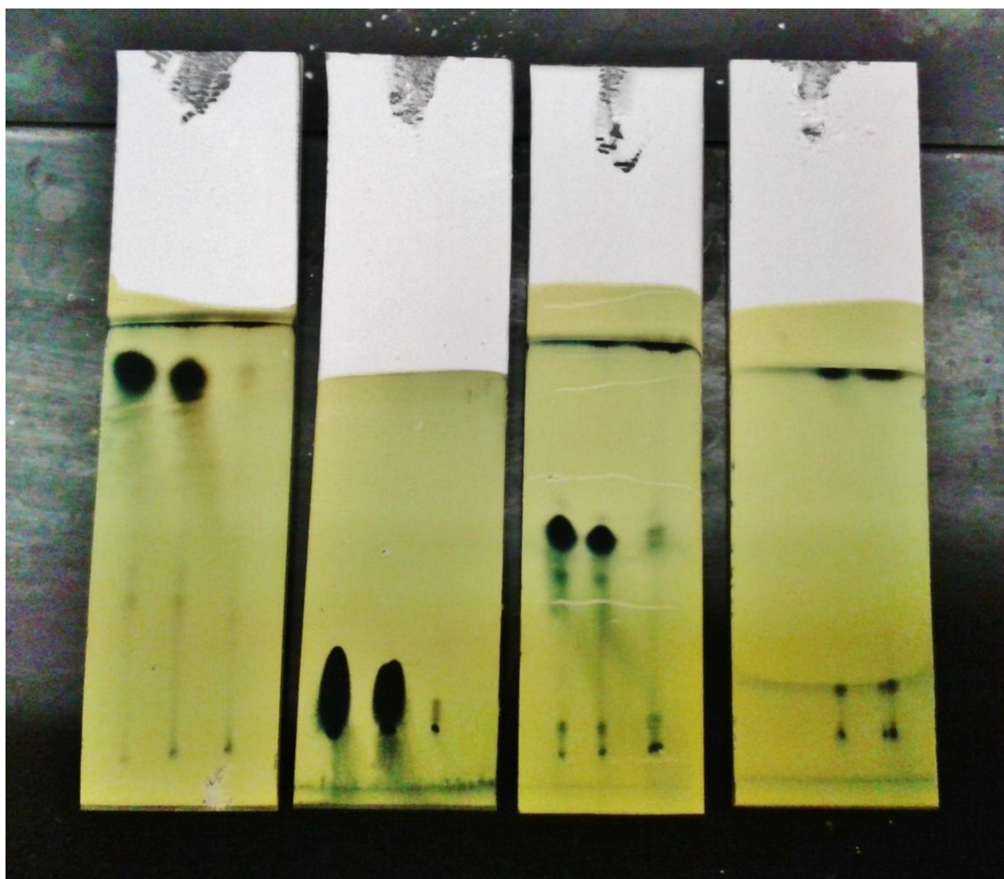

Figure 23: TLC of FAME sample

7.5 ANOVA Statistical Reports:

For simplicity only ANOVA Reports which indicated significance from the control have been included.

One Way Analysis of Variance Tuesday, September 15, 2015, 1:01:37 PM

Data source: Slopes Hormones 10-6

Group Name	N	Missing	Mean	Std Dev	SEM
Control	12	0	0.0808	0.0163	0.00470
BA 10-6	4	0	0.132	0.0196	0.00980
ABA 10-6	4	0	0.0903	0.0120	0.00600
tZ 10-6	4	0	0.0773	0.00560	0.00280
MeSZ 10-6	3	0	0.126	0.00883	0.00510

Source of Variation	DF	SS	MS	F	P
Between Groups	4	0.0122	0.00304	14.092	<0.001
Residual	22	0.00475	0.000216		
Total	26	0.0169			

The differences in the mean values among the treatment groups are greater than would be expected by chance; there is a statistically significant difference (P = <0.001).

Power of performed test with alpha = 0.050: 1.000

All Pairwise Multiple Comparison Procedures (Duncan's Method) :

Comparisons for factor:

Comparison	Diff of Means	p	q	P	P<0.050
Row 2 vs. Row 4	0.0551	5	7.499	<0.001	Yes
Row 2 vs. Row 1	0.0516	4	8.601	<0.001	Yes
Row 2 vs. Row 3	0.0421	3	5.730	<0.001	Yes
Row 2 vs. Row 5	0.00670	2	0.844	0.557	No
Row 5 vs. Row 4	0.0484	4	6.099	<0.001	Yes
Row 5 vs. Row 1	0.0449	3	6.694	<0.001	Yes
Row 5 vs. Row 3	0.0354	2	4.461	0.005	Yes
Row 3 vs. Row 4	0.0130	3	1.769	0.250	No
Row 3 vs. Row 1	0.00950	2	1.584	0.275	Do Not Test
Row 1 vs. Row 4	0.00350	2	0.583	0.684	Do Not Test

A result of "Do Not Test" occurs for a comparison when no significant difference is found between two means that enclose that comparison. For example, if you had four means sorted in order, and found no difference between means 4 vs. 2, then you would not test 4 vs. 3 and 3 vs. 2, but still test 4 vs. 1 and 3 vs. 1 (4 vs. 3 and 3 vs. 2 are enclosed by 4 vs. 2: 4 3 2 1). Note that not testing the enclosed means is a procedural rule, and a result of Do Not Test should be treated as if there is no significant difference between the means, even though one may appear to exist.

One Way Analysis of Variance Tuesday, September 15, 2015, 1:14:10 PM

Data source: Slopes Hormones 10-5

Group Name	N	Missing	Mean	Std Dev	SEM
Control	12	0	0.0808	0.0163	0.00470
BA 10-5	4	0	0.106	0.0116	0.00580
ABA 10-5	4	0	0.0753	0.00740	0.00370
tZ 10-5	4	0	0.107	0.00920	0.00460
MeSZ 10-5	3	0	0.0814	0.0180	0.0104

Source of Variation	DF	SS	MS	F	P
Between Groups	4	0.00409	0.00102	5.127	0.005
Residual	22	0.00439	0.000199		
Total	26	0.00848			

The differences in the mean values among the treatment groups are greater than would be expected by chance; there is a statistically significant difference (P = 0.005).

Power of performed test with alpha = 0.050: 0.852

All Pairwise Multiple Comparison Procedures (Duncan's Method) :

Comparisons for factor:

Comparison	Diff of Means	p	q	P	P<0.050
Row 4 vs. Row 3	0.0314	5	4.447	0.009	Yes
Row 4 vs. Row 1	0.0259	4	4.493	0.007	Yes
Row 4 vs. Row 5	0.0253	3	3.318	0.036	Yes
Row 4 vs. Row 2	0.000700	2	0.0991	0.945	No
Row 2 vs. Row 3	0.0307	4	4.348	0.009	Yes
Row 2 vs. Row 1	0.0252	3	4.371	0.007	Yes
Row 2 vs. Row 5	0.0246	2	3.226	0.033	Yes
Row 5 vs. Row 3	0.00610	3	0.800	0.600	No
Row 5 vs. Row 1	0.000600	2	0.0931	0.948	Do Not Test
Row 1 vs. Row 3	0.00550	2	0.954	0.507	Do Not Test

A result of "Do Not Test" occurs for a comparison when no significant difference is found between two means that enclose that comparison. For example, if you had four means sorted in order, and found no difference between means 4 vs. 2, then you would not test 4 vs. 3 and 3 vs. 2, but still test 4 vs. 1 and 3 vs. 1 (4 vs. 3 and 3 vs. 2 are enclosed by 4 vs. 2: 4 3 2 1). Note that not testing the enclosed means is a

One Way Analysis of Variance Friday, August 28, 2015, 1:18:53 PM

Data source: Hormone Treatment Day 2

Group Name	N	Missing	Mean	Std Dev	SEM
Control	12	0	0.872	0.0829	0.0239
BA 10-7	4	0	0.837	0.0372	0.0186
Ba 10-6	4	0	1.007	0.00844	0.00422
BA 10-5	4	0	0.848	0.0114	0.00571
ABA 10-7	4	0	0.837	0.0198	0.00991
ABA 10-6	4	0	0.934	0.0704	0.0352
ABA 10-5	4	0	0.819	0.0534	0.0267
tZ 10-7	4	0	0.801	0.0984	0.0492
tZ 10-6	4	0	0.886	0.0160	0.00800
tZ 10-5	4	0	0.832	0.0163	0.00813
MeSZ 10-7	3	0	0.836	0.0145	0.00838
MeSZ 10-6	3	0	0.988	0.0156	0.00902
MeSZ 10-5	3	0	0.840	0.0273	0.0158

Source of Variation	DF	SS	MS	F	P
Between Groups	12	0.186	0.0155	4.950	<0.001
Residual	44	0.138	0.00313		
Total	56	0.324			

The differences in the mean values among the treatment groups are greater than would be expected by chance; there is a statistically significant difference (P = <0.001).

Power of performed test with alpha = 0.050: 0.996

All Pairwise Multiple Comparison Procedures (Duncan's Method) :

Comparisons for factor:

Comparison	Diff of Means	p	q	P	P<0.050
Row 3 vs. Row 8	0.206	13	7.369	<0.001	Yes
Row 3 vs. Row 7	0.189	12	6.734	<0.001	Yes
Row 3 vs. Row 10	0.175	11	6.252	<0.001	Yes
Row 3 vs. Row 11	0.171	10	5.653	<0.001	Yes
Row 3 vs. Row 2	0.170	9	6.074	<0.001	Yes
Row 3 vs. Row 5	0.170	8	6.074	<0.001	Yes
Row 3 vs. Row 13	0.167	7	5.532	<0.001	Yes
Row 3 vs. Row 4	0.160	6	5.707	<0.001	Yes
Row 3 vs. Row 1	0.135	5	5.929	<0.001	Yes
Row 3 vs. Row 9	0.121	4	4.314	0.007	Yes
Row 3 vs. Row 6	0.0735	3	2.626	0.085	No
Row 3 vs. Row 12	0.0196	2	0.648	0.649	Do Not Test
Row 12 vs. Row 8	0.187	12	6.174	<0.001	Yes
Row 12 vs. Row 7	0.169	11	5.587	0.001	Yes
Row 12 vs. Row 10	0.155	10	5.141	0.003	Yes
Row 12 vs. Row 11	0.151	9	4.682	0.006	Yes
Row 12 vs. Row 2	0.150	8	4.975	0.003	Yes
Row 12 vs. Row 5	0.150	7	4.975	0.003	Yes
Row 12 vs. Row 13	0.148	6	4.569	0.005	Yes
Row 12 vs. Row 4	0.140	5	4.636	0.004	Yes
Row 12 vs. Row 1	0.116	4	4.537	0.004	Yes

Row 12 vs. Row 9	0.101	3	3.346	0.029	Yes
Row 12 vs. Row 6	0.0539	2	1.783	0.214	Do Not Test
Row 6 vs. Row 8	0.133	11	4.743	0.006	Yes
Row 6 vs. Row 7	0.115	10	4.109	0.016	Yes
Row 6 vs. Row 10	0.101	9	3.626	0.032	Yes
Row 6 vs. Row 11	0.0974	8	3.222	0.055	No
Row 6 vs. Row 2	0.0965	7	3.448	0.038	Do Not Test
Row 6 vs. Row 5	0.0965	6	3.448	0.036	Do Not Test
Row 6 vs. Row 13	0.0938	5	3.101	0.055	Do Not Test
Row 6 vs. Row 4	0.0862	4	3.081	0.051	Do Not Test
Row 6 vs. Row 1	0.0620	3	2.713	0.076	Do Not Test
Row 6 vs. Row 9	0.0473	2	1.688	0.239	Do Not Test
Row 9 vs. Row 8	0.0855	10	3.055	0.074	No
Row 9 vs. Row 7	0.0677	9	2.420	0.154	Do Not Test
Row 9 vs. Row 10	0.0542	8	1.938	0.249	Do Not Test
Row 9 vs. Row 11	0.0502	7	1.659	0.319	Do Not Test
Row 9 vs. Row 2	0.0492	6	1.760	0.285	Do Not Test
Row 9 vs. Row 5	0.0492	5	1.760	0.276	Do Not Test
Row 9 vs. Row 13	0.0465	4	1.538	0.330	Do Not Test
Row 9 vs. Row 4	0.0390	3	1.393	0.360	Do Not Test
Row 9 vs. Row 1	0.0147	2	0.645	0.650	Do Not Test
Row 1 vs. Row 8	0.0707	9	3.096	0.068	Do Not Test
Row 1 vs. Row 7	0.0530	8	2.319	0.168	Do Not Test
Row 1 vs. Row 10	0.0395	7	1.728	0.299	Do Not Test
Row 1 vs. Row 11	0.0354	6	1.386	0.399	Do Not Test
Row 1 vs. Row 2	0.0345	5	1.510	0.351	Do Not Test
Row 1 vs. Row 5	0.0345	4	1.510	0.339	Do Not Test
Row 1 vs. Row 13	0.0318	3	1.243	0.414	Do Not Test
Row 1 vs. Row 4	0.0242	2	1.061	0.457	Do Not Test
Row 4 vs. Row 8	0.0465	8	1.661	0.323	Do Not Test
Row 4 vs. Row 7	0.0288	7	1.027	0.538	Do Not Test
Row 4 vs. Row 10	0.0152	6	0.545	0.742	Do Not Test
Row 4 vs. Row 11	0.0112	5	0.369	0.822	Do Not Test
Row 4 vs. Row 2	0.0102	4	0.366	0.817	Do Not Test
Row 4 vs. Row 5	0.0102	3	0.366	0.810	Do Not Test
Row 4 vs. Row 13	0.00750	2	0.248	0.862	Do Not Test
Row 13 vs. Row 8	0.0390	7	1.290	0.439	Do Not Test
Row 13 vs. Row 7	0.0212	6	0.703	0.670	Do Not Test
Row 13 vs. Row 10	0.00775	5	0.256	0.877	Do Not Test
Row 13 vs. Row 11	0.00367	4	0.113	0.945	Do Not Test
Row 13 vs. Row 2	0.00275	3	0.0910	0.953	Do Not Test
Row 13 vs. Row 5	0.00275	2	0.0910	0.949	Do Not Test
Row 5 vs. Row 8	0.0363	6	1.295	0.431	Do Not Test
Row 5 vs. Row 7	0.0185	5	0.661	0.683	Do Not Test
Row 5 vs. Row 10	0.00500	4	0.179	0.913	Do Not Test
Row 5 vs. Row 11	0.000917	3	0.0303	0.985	Do Not Test
Row 5 vs. Row 2	0.000	2	0.000	1.000	Do Not Test
Row 2 vs. Row 8	0.0363	5	1.295	0.423	Do Not Test
Row 2 vs. Row 7	0.0185	4	0.661	0.676	Do Not Test
Row 2 vs. Row 10	0.00500	3	0.179	0.907	Do Not Test
Row 2 vs. Row 11	0.000917	2	0.0303	0.983	Do Not Test
Row 11 vs. Row 8	0.0353	4	1.169	0.459	Do Not Test
Row 11 vs. Row 7	0.0176	3	0.582	0.702	Do Not Test
Row 11 vs. Row 10	0.00408	2	0.135	0.924	Do Not Test
Row 10 vs. Row 8	0.0313	3	1.116	0.463	Do Not Test
Row 10 vs. Row 7	0.0135	2	0.482	0.735	Do Not Test

Row 7 vs. Row 8	0.0177	2	0.634	0.656	Do Not Test

A result of "Do Not Test" occurs for a comparison when no significant difference is found between two means that enclose that comparison. For example, if you had four means sorted in order, and found no difference between means 4 vs. 2, then you would not test 4 vs. 3 and 3 vs. 2, but still test 4 vs. 1 and 3 vs. 1 (4 vs. 3 and 3 vs. 2 are enclosed by 4 vs. 2: 4 3 2 1). Note that not testing the enclosed means is a procedural rule, and a result of Do Not Test should be treated as if there is no significant difference between the means, even though one may appear to exist.

One Way Analysis of Variance Friday, August 28, 2015, 1:33:21 PI

Data source: Hormone Treatment Day 4

Group Name	N	Missing	Mean	Std Dev	SEM
Control	12	0	1.094	0.0965	0.0279
BA 10-7	4	0	1.096	0.0235	0.0117
BA 10-6	4	0	1.295	0.0109	0.00545
BA 10-5	4	0	1.176	0.0247	0.0124
ABA 10-7	4	0	1.013	0.0140	0.00699
ABA 10-6	4	0	1.190	0.0345	0.0173
ABA 10-5	4	0	1.031	0.0872	0.0436
tZ 10-7	4	0	1.057	0.0499	0.0250
tZ 10-6	4	0	1.109	0.0316	0.0158
tZ 10-5	4	0	1.142	0.0242	0.0121
MeSZ 10-7	3	0	1.054	0.0502	0.0290
MeSZ 10-6	3	0	1.258	0.0126	0.00725
MeSZ 10-5	3	0	1.102	0.00834	0.00482

Source of Variation	DF	SS	MS	F	P
Between Groups	12	0.332	0.0277	8.062	<0.001
Residual	44	0.151	0.00343		
Total	56	0.483			

The differences in the mean values among the treatment groups are greater than would be expected by chance; there is a statistically significant difference (P = <0.001).

Power of performed test with alpha = 0.050: 1.000

All Pairwise Multiple Comparison Procedures (Duncan's Method) :

Comparisons for factor:

Comparison	Diff of Means	p	q	P	P<0.050
Row 3 vs. Row 5	0.282	13	9.610	<0.001	Yes
Row 3 vs. Row 7	0.264	12	9.013	<0.001	Yes
Row 3 vs. Row 11	0.241	11	7.609	<0.001	Yes
Row 3 vs. Row 8	0.238	10	8.133	<0.001	Yes
Row 3 vs. Row 1	0.201	9	8.400	<0.001	Yes
Row 3 vs. Row 2	0.199	8	6.776	<0.001	Yes
Row 3 vs. Row 13	0.193	7	6.103	<0.001	Yes
Row 3 vs. Row 9	0.185	6	6.324	<0.001	Yes
Row 3 vs. Row 10	0.153	5	5.206	0.001	Yes
Row 3 vs. Row 4	0.119	4	4.045	0.011	Yes
Row 3 vs. Row 6	0.104	3	3.559	0.020	Yes
Row 3 vs. Row 12	0.0371	2	1.172	0.412	No
Row 12 vs. Row 5	0.244	12	7.725	<0.001	Yes
Row 12 vs. Row 7	0.227	11	7.172	<0.001	Yes
Row 12 vs. Row 11	0.204	10	6.021	<0.001	Yes
Row 12 vs. Row 8	0.201	9	6.358	<0.001	Yes
Row 12 vs. Row 1	0.164	8	6.127	<0.001	Yes
Row 12 vs. Row 2	0.161	7	5.102	0.002	Yes
Row 12 vs. Row 13	0.156	6	4.612	0.005	Yes
Row 12 vs. Row 9	0.148	5	4.683	0.004	Yes
Row 12 vs. Row 10	0.115	4	3.648	0.021	Yes

Row 12 vs. Row 4	0.0814	3	2.573	0.092	No
Row 12 vs. Row 6	0.0672	2	2.123	0.141	Do Not Test
Row 6 vs. Row 5	0.177	11	6.051	<0.001	Yes
Row 6 vs. Row 7	0.160	10	5.454	0.001	Yes
Row 6 vs. Row 11	0.136	9	4.314	0.011	Yes
Row 6 vs. Row 8	0.134	8	4.575	0.006	Yes
Row 6 vs. Row 1	0.0967	7	4.042	0.015	Yes
Row 6 vs. Row 2	0.0942	6	3.218	0.050	Yes
Row 6 vs. Row 13	0.0888	5	2.808	0.082	No
Row 6 vs. Row 9	0.0810	4	2.765	0.080	Do Not Test
Row 6 vs. Row 10	0.0482	3	1.647	0.279	Do Not Test
Row 6 vs. Row 4	0.0142	2	0.486	0.733	Do Not Test
Row 4 vs. Row 5	0.163	10	5.565	0.001	Yes
Row 4 vs. Row 7	0.145	9	4.967	0.003	Yes
Row 4 vs. Row 11	0.122	8	3.864	0.021	Yes
Row 4 vs. Row 8	0.120	7	4.088	0.014	Yes
Row 4 vs. Row 1	0.0824	6	3.446	0.036	Yes
Row 4 vs. Row 2	0.0800	5	2.731	0.091	No
Row 4 vs. Row 13	0.0746	4	2.357	0.135	Do Not Test
Row 4 vs. Row 9	0.0668	3	2.279	0.135	Do Not Test
Row 4 vs. Row 10	0.0340	2	1.161	0.416	Do Not Test
Row 10 vs. Row 5	0.129	9	4.404	0.009	Yes
Row 10 vs. Row 7	0.111	8	3.806	0.023	Yes
Row 10 vs. Row 11	0.0882	7	2.789	0.094	No
Row 10 vs. Row 8	0.0857	6	2.927	0.075	Do Not Test
Row 10 vs. Row 1	0.0484	5	2.024	0.210	Do Not Test
Row 10 vs. Row 2	0.0460	4	1.570	0.320	Do Not Test
Row 10 vs. Row 13	0.0406	3	1.283	0.399	Do Not Test
Row 10 vs. Row 9	0.0328	2	1.118	0.434	Do Not Test
Row 9 vs. Row 5	0.0962	8	3.286	0.051	No
Row 9 vs. Row 7	0.0787	7	2.688	0.106	Do Not Test
Row 9 vs. Row 11	0.0555	6	1.754	0.286	Do Not Test
Row 9 vs. Row 8	0.0530	5	1.809	0.263	Do Not Test
Row 9 vs. Row 1	0.0157	4	0.655	0.678	Do Not Test
Row 9 vs. Row 2	0.0132	3	0.452	0.766	Do Not Test
Row 9 vs. Row 13	0.00783	2	0.248	0.862	Do Not Test
Row 13 vs. Row 5	0.0884	7	2.794	0.093	Do Not Test
Row 13 vs. Row 7	0.0709	6	2.241	0.173	Do Not Test
Row 13 vs. Row 11	0.0477	5	1.409	0.384	Do Not Test
Row 13 vs. Row 8	0.0452	4	1.428	0.366	Do Not Test
Row 13 vs. Row 1	0.00783	3	0.293	0.847	Do Not Test
Row 13 vs. Row 2	0.00542	2	0.171	0.904	Do Not Test
Row 2 vs. Row 5	0.0830	6	2.833	0.084	Do Not Test
Row 2 vs. Row 7	0.0655	5	2.236	0.166	Do Not Test
Row 2 vs. Row 11	0.0422	4	1.335	0.398	Do Not Test
Row 2 vs. Row 8	0.0397	3	1.357	0.372	Do Not Test
Row 2 vs. Row 1	0.00242	2	0.101	0.943	Do Not Test
Row 1 vs. Row 5	0.0806	5	3.369	0.037	Do Not Test
Row 1 vs. Row 7	0.0631	4	2.638	0.095	Do Not Test
Row 1 vs. Row 11	0.0398	3	1.490	0.328	Do Not Test
Row 1 vs. Row 8	0.0373	2	1.561	0.276	Do Not Test
Row 8 vs. Row 5	0.0433	4	1.476	0.350	Do Not Test
Row 8 vs. Row 7	0.0257	3	0.879	0.563	Do Not Test
Row 8 vs. Row 11	0.00250	2	0.0790	0.956	Do Not Test
Row 11 vs. Row 5	0.0408	3	1.288	0.397	Do Not Test
Row 11 vs. Row 7	0.0232	2	0.735	0.606	Do Not Test

Row 7 vs. Row 5	0.0175	2	0.597	0.675	Do Not Test

A result of "Do Not Test" occurs for a comparison when no significant difference is found between two means that enclose that comparison. For example, if you had four means sorted in order, and found no difference between means 4 vs. 2, then you would not test 4 vs. 3 and 3 vs. 2, but still test 4 vs. 1 and 3 vs. 1 (4 vs. 3 and 3 vs. 2 are enclosed by 4 vs. 2: 4 3 2 1). Note that not testing the enclosed means is a procedural rule, and a result of Do Not Test should be treated as if there is no significant difference between the means, even though one may appear to exist.

One Way Analysis of Variance Friday, August 28, 2015, 1:36:36 PM

Data source: Hormone Treatment Day 6

Group Name	N	Missing	Mean	Std Dev	SEM
Control	12	0	1.162	0.122	0.0353
BA 10-7	4	0	1.118	0.0469	0.0234
BA 10-6	4	0	1.491	0.0420	0.0210
BA 10-5	4	0	1.295	0.0136	0.00680
ABA 10-7	4	0	1.027	0.0266	0.0133
ABA 10-6	4	0	1.284	0.0629	0.0315
ABA 10-5	4	0	1.160	0.145	0.0725
tZ 10-7	4	0	1.130	0.0567	0.0283
tZ 10-6	4	0	1.200	0.100	0.0502
tZ 10-5	4	0	1.289	0.0224	0.0112
MeSZ 10-7	3	0	1.111	0.135	0.0781
MeSZ 10-6	3	0	1.458	0.0204	0.0118
MeSZ 10-5	3	0	1.074	0.0463	0.0267

Source of Variation	DF	SS	MS	F	P
Between Groups	12	0.892	0.0743	9.704	<0.001
Residual	44	0.337	0.00766		
Total	56	1.229			

The differences in the mean values among the treatment groups are greater than would be expected by chance; there is a statistically significant difference (P = <0.001).

Power of performed test with alpha = 0.050: 1.000

All Pairwise Multiple Comparison Procedures (Duncan's Method) :

Comparisons for factor:

Comparison	Diff of Means	p	q	P	P<0.050
Row 3 vs. Row 5	0.464	13	10.604	<0.001	Yes
Row 3 vs. Row 13	0.417	12	8.823	<0.001	Yes
Row 3 vs. Row 11	0.380	11	8.040	<0.001	Yes
Row 3 vs. Row 2	0.373	10	8.519	<0.001	Yes
Row 3 vs. Row 8	0.361	9	8.256	<0.001	Yes
Row 3 vs. Row 7	0.331	8	7.570	<0.001	Yes
Row 3 vs. Row 1	0.329	7	9.218	<0.001	Yes
Row 3 vs. Row 9	0.291	6	6.650	<0.001	Yes
Row 3 vs. Row 6	0.207	5	4.725	0.004	Yes
Row 3 vs. Row 10	0.203	4	4.628	0.004	Yes
Row 3 vs. Row 4	0.196	3	4.485	0.004	Yes
Row 3 vs. Row 12	0.0333	2	0.705	0.621	No
Row 12 vs. Row 5	0.431	12	9.112	<0.001	Yes
Row 12 vs. Row 13	0.384	11	7.593	<0.001	Yes
Row 12 vs. Row 11	0.347	10	6.861	<0.001	Yes
Row 12 vs. Row 2	0.339	9	7.182	<0.001	Yes
Row 12 vs. Row 8	0.328	8	6.938	<0.001	Yes
Row 12 vs. Row 7	0.298	7	6.303	<0.001	Yes
Row 12 vs. Row 1	0.296	6	7.410	<0.001	Yes
Row 12 vs. Row 9	0.258	5	5.452	<0.001	Yes
Row 12 vs. Row 6	0.173	4	3.669	0.020	Yes

Row 12 vs. Row 10	0.169	3	3.579	0.020	Yes
Row 12 vs. Row 4	0.163	2	3.447	0.019	Yes
Row 4 vs. Row 5	0.268	11	6.119	<0.001	Yes
Row 4 vs. Row 13	0.221	10	4.671	0.006	Yes
Row 4 vs. Row 11	0.184	9	3.888	0.022	Yes
Row 4 vs. Row 2	0.177	8	4.034	0.016	Yes
Row 4 vs. Row 8	0.165	7	3.771	0.023	Yes
Row 4 vs. Row 7	0.135	6	3.085	0.060	No
Row 4 vs. Row 1	0.133	5	3.725	0.021	Do Not Test
Row 4 vs. Row 9	0.0948	4	2.165	0.170	Do Not Test
Row 4 vs. Row 6	0.0105	3	0.240	0.875	Do Not Test
Row 4 vs. Row 10	0.00625	2	0.143	0.920	Do Not Test
Row 10 vs. Row 5	0.262	10	5.976	<0.001	Yes
Row 10 vs. Row 13	0.214	9	4.538	0.007	Yes
Row 10 vs. Row 11	0.177	8	3.756	0.025	Yes
Row 10 vs. Row 2	0.170	7	3.891	0.019	Yes
Row 10 vs. Row 8	0.159	6	3.628	0.027	Yes
Row 10 vs. Row 7	0.129	5	2.942	0.068	Do Not Test
Row 10 vs. Row 1	0.127	4	3.550	0.025	Do Not Test
Row 10 vs. Row 9	0.0885	3	2.023	0.184	Do Not Test
Row 10 vs. Row 6	0.00425	2	0.0971	0.946	Do Not Test
Row 6 vs. Row 5	0.257	9	5.879	<0.001	Yes
Row 6 vs. Row 13	0.210	8	4.449	0.008	Yes
Row 6 vs. Row 11	0.173	7	3.666	0.027	Yes
Row 6 vs. Row 2	0.166	6	3.794	0.021	Yes
Row 6 vs. Row 8	0.154	5	3.531	0.029	Yes
Row 6 vs. Row 7	0.124	4	2.845	0.071	Do Not Test
Row 6 vs. Row 1	0.123	3	3.431	0.025	Do Not Test
Row 6 vs. Row 9	0.0842	2	1.925	0.180	Do Not Test
Row 9 vs. Row 5	0.173	8	3.954	0.018	Yes
Row 9 vs. Row 13	0.126	7	2.666	0.109	No
Row 9 vs. Row 11	0.0890	6	1.883	0.252	Do Not Test
Row 9 vs. Row 2	0.0817	5	1.868	0.248	Do Not Test
Row 9 vs. Row 8	0.0702	4	1.605	0.309	Do Not Test
Row 9 vs. Row 7	0.0402	3	0.920	0.545	Do Not Test
Row 9 vs. Row 1	0.0383	2	1.073	0.452	Do Not Test
Row 1 vs. Row 5	0.135	7	3.769	0.023	Yes
Row 1 vs. Row 13	0.0877	6	2.195	0.182	Do Not Test
Row 1 vs. Row 11	0.0507	5	1.268	0.433	Do Not Test
Row 1 vs. Row 2	0.0434	4	1.215	0.441	Do Not Test
Row 1 vs. Row 8	0.0319	3	0.893	0.557	Do Not Test
Row 1 vs. Row 7	0.00192	2	0.0536	0.970	Do Not Test
Row 7 vs. Row 5	0.133	6	3.034	0.065	No
Row 7 vs. Row 13	0.0857	5	1.814	0.262	Do Not Test
Row 7 vs. Row 11	0.0488	4	1.031	0.514	Do Not Test
Row 7 vs. Row 2	0.0415	3	0.948	0.533	Do Not Test
Row 7 vs. Row 8	0.0300	2	0.686	0.630	Do Not Test
Row 8 vs. Row 5	0.103	5	2.348	0.146	Do Not Test
Row 8 vs. Row 13	0.0557	4	1.180	0.455	Do Not Test
Row 8 vs. Row 11	0.0188	3	0.397	0.794	Do Not Test
Row 8 vs. Row 2	0.0115	2	0.263	0.854	Do Not Test
Row 2 vs. Row 5	0.0913	4	2.085	0.186	Do Not Test
Row 2 vs. Row 13	0.0442	3	0.936	0.538	Do Not Test
Row 2 vs. Row 11	0.00725	2	0.153	0.914	Do Not Test
Row 11 vs. Row 5	0.0840	3	1.777	0.243	Do Not Test
Row 11 vs. Row 13	0.0370	2	0.732	0.607	Do Not Test

Row 13 vs. Row 5	0.0470	2	0.994	0.486	Do Not Test

A result of "Do Not Test" occurs for a comparison when no significant difference is found between two means that enclose that comparison. For example, if you had four means sorted in order, and found no difference between means 4 vs. 2, then you would not test 4 vs. 3 and 3 vs. 2, but still test 4 vs. 1 and 3 vs. 1 (4 vs. 3 and 3 vs. 2 are enclosed by 4 vs. 2: 4 3 2 1). Note that not testing the enclosed means is a procedural rule, and a result of Do Not Test should be treated as if there is no significant difference between the means, even though one may appear to exist.

One Way Analysis of Variance Tuesday, September 15, 2015, 1:21:57 PM

Data source: Lipid Percent Dry Weight

Group Name	N	Missing	Mean	Std Dev	SEM
Control	12	0	1.532	0.435	0.126
BA 10-7	4	0	1.720	0.287	0.144
BA 10-6	4	0	1.921	0.415	0.207
BA 10-5	4	0	2.140	0.424	0.212
ABA 10-7	4	0	0.971	0.147	0.0736
ABA 10-6	4	0	1.011	0.128	0.0638
ABA 10-5	4	0	1.050	0.0696	0.0348
tZ 10-7	4	0	0.909	0.0859	0.0429
tZ 10-6	4	0	0.937	0.128	0.0641
tZ 10-5	4	0	1.411	0.350	0.175
MeSZ 10-7	3	0	0.952	0.202	0.117
MeSZ 10-6	3	0	1.380	0.279	0.161
MeSZ 10-5	3	0	0.880	0.0616	0.0356

Source of Variation	DF	SS	MS	F	P
Between Groups	12	8.748	0.729	7.640	<0.001
Residual	44	4.199	0.0954		
Total	56	12.947			

The differences in the mean values among the treatment groups are greater than would be expected by chance; there is a statistically significant difference (P = <0.001).

Power of performed test with alpha = 0.050: 1.000

All Pairwise Multiple Comparison Procedures (Duncan's Method) :

Comparisons for factor:

Comparison	Diff of Means	p	q	P	P<0.050
Row 4 vs. Row 13	1.260	13	7.554	<0.001	Yes
Row 4 vs. Row 8	1.231	12	7.968	<0.001	Yes
Row 4 vs. Row 9	1.203	11	7.789	<0.001	Yes
Row 4 vs. Row 11	1.187	10	7.117	<0.001	Yes
Row 4 vs. Row 5	1.169	9	7.569	<0.001	Yes
Row 4 vs. Row 6	1.129	8	7.311	<0.001	Yes
Row 4 vs. Row 7	1.090	7	7.059	<0.001	Yes
Row 4 vs. Row 12	0.760	6	4.557	0.006	Yes
Row 4 vs. Row 10	0.729	5	4.721	0.004	Yes
Row 4 vs. Row 1	0.608	4	4.824	0.002	Yes
Row 4 vs. Row 2	0.420	3	2.717	0.075	No
Row 4 vs. Row 3	0.219	2	1.417	0.322	Do Not Test
Row 3 vs. Row 13	1.041	12	6.242	<0.001	Yes
Row 3 vs. Row 8	1.012	11	6.551	<0.001	Yes
Row 3 vs. Row 9	0.984	10	6.372	<0.001	Yes
Row 3 vs. Row 11	0.969	9	5.806	<0.001	Yes
Row 3 vs. Row 5	0.950	8	6.152	<0.001	Yes
Row 3 vs. Row 6	0.910	7	5.894	<0.001	Yes
Row 3 vs. Row 7	0.871	6	5.642	<0.001	Yes
Row 3 vs. Row 12	0.541	5	3.246	0.044	Yes
Row 3 vs. Row 10	0.510	4	3.304	0.036	Yes

Row 3 vs. Row 1	0.389	3	3.088	0.044	Yes
Row 3 vs. Row 2	0.201	2	1.300	0.363	Do Not Test
Row 2 vs. Row 13	0.841	11	5.039	0.003	Yes
Row 2 vs. Row 8	0.811	10	5.252	0.002	Yes
Row 2 vs. Row 9	0.783	9	5.072	0.003	Yes
Row 2 vs. Row 11	0.768	8	4.602	0.006	Yes
Row 2 vs. Row 5	0.750	7	4.853	0.004	Yes
Row 2 vs. Row 6	0.710	6	4.594	0.005	Yes
Row 2 vs. Row 7	0.671	5	4.342	0.007	Yes
Row 2 vs. Row 12	0.341	4	2.042	0.196	No
Row 2 vs. Row 10	0.310	3	2.004	0.188	Do Not Test
Row 2 vs. Row 1	0.189	2	1.497	0.296	Do Not Test
Row 1 vs. Row 13	0.652	10	4.623	0.007	Yes
Row 1 vs. Row 8	0.622	9	4.935	0.004	Yes
Row 1 vs. Row 9	0.595	8	4.715	0.005	Yes
Row 1 vs. Row 11	0.579	7	4.107	0.013	Yes
Row 1 vs. Row 5	0.561	6	4.447	0.007	Yes
Row 1 vs. Row 6	0.521	5	4.130	0.011	Yes
Row 1 vs. Row 7	0.482	4	3.822	0.016	Yes
Row 1 vs. Row 12	0.152	3	1.078	0.478	Do Not Test
Row 1 vs. Row 10	0.121	2	0.958	0.502	Do Not Test
Row 10 vs. Row 13	0.531	9	3.183	0.060	No
Row 10 vs. Row 8	0.502	8	3.247	0.053	Do Not Test
Row 10 vs. Row 9	0.474	7	3.068	0.065	Do Not Test
Row 10 vs. Row 11	0.458	6	2.747	0.095	Do Not Test
Row 10 vs. Row 5	0.440	5	2.848	0.078	Do Not Test
Row 10 vs. Row 6	0.400	4	2.589	0.101	Do Not Test
Row 10 vs. Row 7	0.361	3	2.338	0.125	Do Not Test
Row 10 vs. Row 12	0.0311	2	0.187	0.896	Do Not Test
Row 12 vs. Row 13	0.500	8	2.803	0.096	Do Not Test
Row 12 vs. Row 8	0.470	7	2.820	0.090	Do Not Test
Row 12 vs. Row 9	0.443	6	2.653	0.106	Do Not Test
Row 12 vs. Row 11	0.427	5	2.395	0.138	Do Not Test
Row 12 vs. Row 5	0.409	4	2.450	0.120	Do Not Test
Row 12 vs. Row 6	0.369	3	2.211	0.147	Do Not Test
Row 12 vs. Row 7	0.330	2	1.978	0.169	Do Not Test
Row 7 vs. Row 13	0.170	7	1.018	0.541	Do Not Test
Row 7 vs. Row 8	0.140	6	0.909	0.581	Do Not Test
Row 7 vs. Row 9	0.113	5	0.730	0.652	Do Not Test
Row 7 vs. Row 11	0.0971	4	0.582	0.713	Do Not Test
Row 7 vs. Row 5	0.0788	3	0.510	0.737	Do Not Test
Row 7 vs. Row 6	0.0389	2	0.252	0.860	Do Not Test
Row 6 vs. Row 13	0.131	6	0.785	0.634	Do Not Test
Row 6 vs. Row 8	0.102	5	0.658	0.685	Do Not Test
Row 6 vs. Row 9	0.0739	4	0.478	0.762	Do Not Test
Row 6 vs. Row 11	0.0583	3	0.349	0.819	Do Not Test
Row 6 vs. Row 5	0.0400	2	0.259	0.856	Do Not Test
Row 5 vs. Row 13	0.0910	5	0.546	0.737	Do Not Test
Row 5 vs. Row 8	0.0616	4	0.399	0.801	Do Not Test
Row 5 vs. Row 9	0.0339	3	0.219	0.886	Do Not Test
Row 5 vs. Row 11	0.0183	2	0.110	0.939	Do Not Test
Row 11 vs. Row 13	0.0728	4	0.408	0.797	Do Not Test
Row 11 vs. Row 8	0.0434	3	0.260	0.865	Do Not Test
Row 11 vs. Row 9	0.0156	2	0.0935	0.948	Do Not Test
Row 9 vs. Row 13	0.0572	3	0.343	0.822	Do Not Test
Row 9 vs. Row 8	0.0278	2	0.180	0.900	Do Not Test

Row 8 vs. Row 13	0.0294	2	0.176	0.901	Do Not Test

A result of "Do Not Test" occurs for a comparison when no significant difference is found between two means that enclose that comparison. For example, if you had four means sorted in order, and found no difference between means 4 vs. 2, then you would not test 4 vs. 3 and 3 vs. 2, but still test 4 vs. 1 and 3 vs. 1 (4 vs. 3 and 3 vs. 2 are enclosed by 4 vs. 2: 4 3 2 1). Note that not testing the enclosed means is a procedural rule, and a result of Do Not Test should be treated as if there is no significant difference between the means, even though one may appear to exist.

One Way Analysis of Variance Tuesday, September 15, 2015, 1:31:07 PM

Data source: Fatty Acid Weights mg

Group Name	N	Missing	Mean	Std Dev	SEM
Control	12	0	10.809	1.379	0.398
BA 10-7	4	0	9.450	0.802	0.401
BA 10-6	4	0	9.325	2.238	1.119
BA 10-5	4	0	11.275	1.121	0.561
ABA 10-7	4	0	7.167	1.271	0.636
ABA 10-6	4	0	7.125	1.139	0.569
ABA 10-5	4	0	8.200	0.394	0.197
tZ 10-7	4	0	7.550	0.439	0.219
tZ 10-6	4	0	7.875	0.779	0.390
tZ 10-5	4	0	9.225	0.968	0.484
MeSZ 10-7	3	0	6.133	0.818	0.472
MeSZ 10-6	3	0	7.467	0.759	0.438
MeSZ 10-5	3	0	7.167	0.205	0.119

Source of Variation	DF	SS	MS	F	P
Between Groups	12	144.119	12.010	9.016	<0.001
Residual	44	58.612	1.332		
Total	56	202.731			

The differences in the mean values among the treatment groups are greater than would be expected by chance; there is a statistically significant difference (P = <0.001).

Power of performed test with alpha = 0.050: 1.000

All Pairwise Multiple Comparison Procedures (Duncan's Method) :

Comparisons for factor:

Comparison	Diff of Means	p	q	P	P<0.050
Row 4 vs. Row 11	5.142	13	8.249	<0.001	Yes
Row 4 vs. Row 6	4.150	12	7.191	<0.001	Yes
Row 4 vs. Row 13	4.108	11	6.591	<0.001	Yes
Row 4 vs. Row 5	4.108	10	7.119	<0.001	Yes
Row 4 vs. Row 12	3.808	9	6.110	<0.001	Yes
Row 4 vs. Row 8	3.725	8	6.455	<0.001	Yes
Row 4 vs. Row 9	3.400	7	5.892	<0.001	Yes
Row 4 vs. Row 7	3.075	6	5.329	0.001	Yes
Row 4 vs. Row 10	2.050	5	3.552	0.028	Yes
Row 4 vs. Row 3	1.950	4	3.379	0.032	Yes
Row 4 vs. Row 2	1.825	3	3.162	0.039	Yes
Row 4 vs. Row 1	0.466	2	0.989	0.488	No
Row 1 vs. Row 11	4.676	12	8.876	<0.001	Yes
Row 1 vs. Row 6	3.684	11	7.819	<0.001	Yes
Row 1 vs. Row 13	3.642	10	6.914	<0.001	Yes
Row 1 vs. Row 5	3.642	9	7.730	<0.001	Yes
Row 1 vs. Row 12	3.342	8	6.345	<0.001	Yes
Row 1 vs. Row 8	3.259	7	6.917	<0.001	Yes
Row 1 vs. Row 9	2.934	6	6.227	<0.001	Yes
Row 1 vs. Row 7	2.609	5	5.537	<0.001	Yes
Row 1 vs. Row 10	1.584	4	3.362	0.033	Yes

Row 1 vs. Row 3	1.484	3	3.150	0.040	Yes
Row 1 vs. Row 2	1.359	2	2.884	0.048	Yes
Row 2 vs. Row 11	3.317	11	5.321	0.002	Yes
Row 2 vs. Row 6	2.325	10	4.029	0.018	Yes
Row 2 vs. Row 13	2.283	9	3.663	0.031	Yes
Row 2 vs. Row 5	2.283	8	3.957	0.018	Yes
Row 2 vs. Row 12	1.983	7	3.182	0.056	No
Row 2 vs. Row 8	1.900	6	3.292	0.045	Do Not Test
Row 2 vs. Row 9	1.575	5	2.729	0.091	Do Not Test
Row 2 vs. Row 7	1.250	4	2.166	0.170	Do Not Test
Row 2 vs. Row 10	0.225	3	0.390	0.798	Do Not Test
Row 2 vs. Row 3	0.125	2	0.217	0.879	Do Not Test
Row 3 vs. Row 11	3.192	10	5.120	0.003	Yes
Row 3 vs. Row 6	2.200	9	3.812	0.024	Yes
Row 3 vs. Row 13	2.158	8	3.463	0.039	Yes
Row 3 vs. Row 5	2.158	7	3.740	0.024	Yes
Row 3 vs. Row 12	1.858	6	2.981	0.069	Do Not Test
Row 3 vs. Row 8	1.775	5	3.076	0.057	Do Not Test
Row 3 vs. Row 9	1.450	4	2.513	0.111	Do Not Test
Row 3 vs. Row 7	1.125	3	1.949	0.201	Do Not Test
Row 3 vs. Row 10	0.1000	2	0.173	0.903	Do Not Test
Row 10 vs. Row 11	3.092	9	4.960	0.003	Yes
Row 10 vs. Row 6	2.100	8	3.639	0.030	Yes
Row 10 vs. Row 13	2.058	7	3.302	0.047	Yes
Row 10 vs. Row 5	2.058	6	3.567	0.030	Yes
Row 10 vs. Row 12	1.758	5	2.821	0.081	Do Not Test
Row 10 vs. Row 8	1.675	4	2.903	0.066	Do Not Test
Row 10 vs. Row 9	1.350	3	2.339	0.125	Do Not Test
Row 10 vs. Row 7	1.025	2	1.776	0.216	Do Not Test
Row 7 vs. Row 11	2.067	8	3.316	0.048	Yes
Row 7 vs. Row 6	1.075	7	1.863	0.263	No
Row 7 vs. Row 13	1.033	6	1.658	0.314	Do Not Test
Row 7 vs. Row 5	1.033	5	1.791	0.268	Do Not Test
Row 7 vs. Row 12	0.733	4	1.176	0.456	Do Not Test
Row 7 vs. Row 8	0.650	3	1.126	0.459	Do Not Test
Row 7 vs. Row 9	0.325	2	0.563	0.693	Do Not Test
Row 9 vs. Row 11	1.742	7	2.794	0.093	No
Row 9 vs. Row 6	0.750	6	1.300	0.430	Do Not Test
Row 9 vs. Row 13	0.708	5	1.136	0.482	Do Not Test
Row 9 vs. Row 5	0.708	4	1.227	0.437	Do Not Test
Row 9 vs. Row 12	0.408	3	0.655	0.667	Do Not Test
Row 9 vs. Row 8	0.325	2	0.563	0.693	Do Not Test
Row 8 vs. Row 11	1.417	6	2.273	0.167	Do Not Test
Row 8 vs. Row 6	0.425	5	0.736	0.649	Do Not Test
Row 8 vs. Row 13	0.383	4	0.615	0.697	Do Not Test
Row 8 vs. Row 5	0.383	3	0.664	0.662	Do Not Test
Row 8 vs. Row 12	0.0833	2	0.134	0.925	Do Not Test
Row 12 vs. Row 11	1.333	5	2.001	0.216	Do Not Test
Row 12 vs. Row 6	0.342	4	0.548	0.729	Do Not Test
Row 12 vs. Row 13	0.300	3	0.450	0.767	Do Not Test
Row 12 vs. Row 5	0.300	2	0.481	0.735	Do Not Test
Row 5 vs. Row 11	1.033	4	1.658	0.294	Do Not Test
Row 5 vs. Row 6	0.0417	3	0.0722	0.963	Do Not Test
Row 5 vs. Row 13	0.000	2	0.000	1.000	Do Not Test
Row 13 vs. Row 11	1.033	3	1.551	0.308	Do Not Test
Row 13 vs. Row 6	0.0417	2	0.0668	0.963	Do Not Test

Row 6 vs. Row 11	0.992	2	1.591	0.267	Do Not Test

A result of "Do Not Test" occurs for a comparison when no significant difference is found between two means that enclose that comparison. For example, if you had four means sorted in order, and found no difference between means 4 vs. 2, then you would not test 4 vs. 3 and 3 vs. 2, but still test 4 vs. 1 and 3 vs. 1 (4 vs. 3 and 3 vs. 2 are enclosed by 4 vs. 2: 4 3 2 1). Note that not testing the enclosed means is a procedural rule, and a result of Do Not Test should be treated as if there is no significant difference between the means, even though one may appear to exist.

One Way Analysis of Variance Friday, August 28, 2015, 12:10:37 PM

Data source: Palmitic Acid

Group Name	N	Missing	Mean	Std Dev	SEM
Control	10	0	31.724	2.517	0.796
BA 10-7	4	0	35.713	5.278	2.639
BA 10-6	4	0	30.032	1.937	0.969
BA 10-5	4	0	29.574	1.529	0.765
ABA 10-6	4	0	36.954	3.140	1.570
ABA 10-5	4	0	35.441	2.194	1.097
tZ 10-7	4	0	28.166	2.555	1.278
tZ 10-6	4	0	31.410	2.238	1.119
MeSZ 10-7	3	0	29.482	1.978	1.142
MeSZ 10-6	3	0	29.108	0.652	0.376
MeSZ 10-5	3	0	29.972	0.643	0.371

Source of Variation	DF	SS	MS	F	P
Between Groups	10	354.096	35.410	5.162	<0.001
Residual	36	246.960	6.860		
Total	46	601.056			

The differences in the mean values among the treatment groups are greater than would be expected by chance; there is a statistically significant difference (P = <0.001).

Power of performed test with alpha = 0.050: 0.992

All Pairwise Multiple Comparison Procedures (Duncan's Method) :

Comparisons for factor:

Comparison	Diff of Means	p	q	P	P<0.050
Row 5 vs. Row 7	8.788	11	6.711	<0.001	Yes
Row 5 vs. Row 10	7.846	10	5.547	0.001	Yes
Row 5 vs. Row 9	7.472	9	5.282	0.002	Yes
Row 5 vs. Row 4	7.380	8	5.635	<0.001	Yes
Row 5 vs. Row 11	6.981	7	4.935	0.003	Yes
Row 5 vs. Row 3	6.921	6	5.285	0.002	Yes
Row 5 vs. Row 8	5.543	5	4.233	0.010	Yes
Row 5 vs. Row 1	5.229	4	4.773	0.003	Yes
Row 5 vs. Row 6	1.513	3	1.155	0.448	No
Row 5 vs. Row 2	1.240	2	0.947	0.508	Do Not Test
Row 2 vs. Row 7	7.548	10	5.764	<0.001	Yes
Row 2 vs. Row 10	6.606	9	4.670	0.006	Yes
Row 2 vs. Row 9	6.232	8	4.406	0.009	Yes
Row 2 vs. Row 4	6.139	7	4.688	0.005	Yes
Row 2 vs. Row 11	5.741	6	4.059	0.014	Yes
Row 2 vs. Row 3	5.681	5	4.338	0.008	Yes
Row 2 vs. Row 8	4.303	4	3.286	0.039	Yes
Row 2 vs. Row 1	3.989	3	3.641	0.019	Yes
Row 2 vs. Row 6	0.272	2	0.208	0.884	Do Not Test
Row 6 vs. Row 7	7.275	9	5.556	0.001	Yes
Row 6 vs. Row 10	6.333	8	4.477	0.008	Yes
Row 6 vs. Row 9	5.959	7	4.213	0.012	Yes
Row 6 vs. Row 4	5.867	6	4.480	0.007	Yes

Row 6 vs. Row 11	5.469	5	3.866	0.018	Yes
Row 6 vs. Row 3	5.409	4	4.130	0.010	Yes
Row 6 vs. Row 8	4.030	3	3.078	0.046	Yes
Row 6 vs. Row 1	3.717	2	3.392	0.022	Yes
Row 1 vs. Row 7	3.559	8	3.248	0.054	No
Row 1 vs. Row 10	2.616	7	2.146	0.197	Do Not Test
Row 1 vs. Row 9	2.243	6	1.840	0.263	Do Not Test
Row 1 vs. Row 4	2.150	5	1.963	0.225	Do Not Test
Row 1 vs. Row 11	1.752	4	1.437	0.363	Do Not Test
Row 1 vs. Row 3	1.692	3	1.544	0.311	Do Not Test
Row 1 vs. Row 8	0.314	2	0.286	0.841	Do Not Test
Row 8 vs. Row 7	3.245	7	2.478	0.137	Do Not Test
Row 8 vs. Row 10	2.303	6	1.628	0.322	Do Not Test
Row 8 vs. Row 9	1.929	5	1.364	0.399	Do Not Test
Row 8 vs. Row 4	1.837	4	1.402	0.374	Do Not Test
Row 8 vs. Row 11	1.438	3	1.017	0.504	Do Not Test
Row 8 vs. Row 3	1.378	2	1.052	0.462	Do Not Test
Row 3 vs. Row 7	1.867	6	1.425	0.385	Do Not Test
Row 3 vs. Row 10	0.924	5	0.653	0.686	Do Not Test
Row 3 vs. Row 9	0.551	4	0.389	0.806	Do Not Test
Row 3 vs. Row 4	0.458	3	0.350	0.818	Do Not Test
Row 3 vs. Row 11	0.0599	2	0.0423	0.976	Do Not Test
Row 11 vs. Row 7	1.807	5	1.277	0.429	Do Not Test
Row 11 vs. Row 10	0.865	4	0.572	0.717	Do Not Test
Row 11 vs. Row 9	0.491	3	0.324	0.831	Do Not Test
Row 11 vs. Row 4	0.398	2	0.282	0.843	Do Not Test
Row 4 vs. Row 7	1.408	4	1.075	0.496	Do Not Test
Row 4 vs. Row 10	0.466	3	0.329	0.829	Do Not Test
Row 4 vs. Row 9	0.0922	2	0.0652	0.964	Do Not Test
Row 9 vs. Row 7	1.316	3	0.930	0.541	Do Not Test
Row 9 vs. Row 10	0.374	2	0.247	0.862	Do Not Test
Row 10 vs. Row 7	0.942	2	0.666	0.641	Do Not Test

A result of "Do Not Test" occurs for a comparison when no significant difference is found between two means that enclose that comparison. For example, if you had four means sorted in order, and found no difference between means 4 vs. 2, then you would not test 4 vs. 3 and 3 vs. 2, but still test 4 vs. 1 and 3 vs. 1 (4 vs. 3 and 3 vs. 2 are enclosed by 4 vs. 2: 4 3 2 1). Note that not testing the enclosed means is a procedural rule, and a result of Do Not Test should be treated as if there is no significant difference between the means, even though one may appear to exist.

One Way Analysis of Variance Friday, August 28, 2015, 12:21:04 PM

Data source: Stearic Acid

Group Name	N	Missing	Mean	Std Dev	SEM
Control	10	0	5.058	0.550	0.174
BA 10-7	4	0	5.334	0.896	0.448
BA 10-6	4	0	5.488	1.059	0.529
BA 10-5	4	0	5.766	0.753	0.377
ABA 10-6	4	0	8.374	1.231	0.616
ABA 10-5	4	0	5.740	1.700	0.850
tZ 10-7	4	0	7.227	1.262	0.631
tZ 10-6	4	0	6.657	1.043	0.522
MeSZ 10-7	3	0	5.988	0.398	0.230
MeSZ 10-6	3	0	5.965	0.929	0.537
MeSZ 10-5	3	0	7.134	0.388	0.224

Source of Variation	DF	SS	MS	F	P
Between Groups	10	45.974	4.597	4.897	<0.001
Residual	36	33.796	0.939		
Total	46	79.771			

The differences in the mean values among the treatment groups are greater than would be expected by chance; there is a statistically significant difference (P = <0.001).

Power of performed test with alpha = 0.050: 0.987

All Pairwise Multiple Comparison Procedures (Duncan's Method) :

Comparisons for factor:

Comparison	Diff of Means	p	q	P	P<0.050
Row 5 vs. Row 1	3.316	11	8.182	<0.001	Yes
Row 5 vs. Row 2	3.040	10	6.274	<0.001	Yes
Row 5 vs. Row 3	2.886	9	5.957	<0.001	Yes
Row 5 vs. Row 6	2.634	8	5.438	0.001	Yes
Row 5 vs. Row 4	2.608	7	5.383	0.001	Yes
Row 5 vs. Row 10	2.409	6	4.604	0.006	Yes
Row 5 vs. Row 9	2.385	5	4.559	0.005	Yes
Row 5 vs. Row 8	1.717	4	3.545	0.026	Yes
Row 5 vs. Row 11	1.240	3	2.369	0.122	No
Row 5 vs. Row 7	1.147	2	2.367	0.103	Do Not Test
Row 7 vs. Row 1	2.170	10	5.353	0.002	Yes
Row 7 vs. Row 2	1.893	9	3.907	0.022	Yes
Row 7 vs. Row 3	1.739	8	3.590	0.034	Yes
Row 7 vs. Row 6	1.488	7	3.071	0.066	No
Row 7 vs. Row 4	1.461	6	3.016	0.067	Do Not Test
Row 7 vs. Row 10	1.262	5	2.412	0.136	Do Not Test
Row 7 vs. Row 9	1.239	4	2.368	0.135	Do Not Test
Row 7 vs. Row 8	0.571	3	1.178	0.439	Do Not Test
Row 7 vs. Row 11	0.0931	2	0.178	0.901	Do Not Test
Row 11 vs. Row 1	2.077	9	4.604	0.007	Yes
Row 11 vs. Row 2	1.800	8	3.440	0.042	Yes
Row 11 vs. Row 3	1.646	7	3.146	0.060	No
Row 11 vs. Row 6	1.395	6	2.665	0.106	Do Not Test

Row 11 vs. Row 4	1.368	5	2.615	0.107	Do Not Test
Row 11 vs. Row 10	1.169	4	2.090	0.186	Do Not Test
Row 11 vs. Row 9	1.146	3	2.048	0.180	Do Not Test
Row 11 vs. Row 8	0.478	2	0.913	0.523	Do Not Test
Row 8 vs. Row 1	1.599	8	3.945	0.020	Yes
Row 8 vs. Row 2	1.322	7	2.729	0.102	No
Row 8 vs. Row 3	1.169	6	2.412	0.143	Do Not Test
Row 8 vs. Row 6	0.917	5	1.893	0.242	Do Not Test
Row 8 vs. Row 4	0.891	4	1.838	0.245	Do Not Test
Row 8 vs. Row 10	0.692	3	1.322	0.385	Do Not Test
Row 8 vs. Row 9	0.668	2	1.277	0.373	Do Not Test
Row 9 vs. Row 1	0.931	7	2.064	0.215	No
Row 9 vs. Row 2	0.654	6	1.250	0.446	Do Not Test
Row 9 vs. Row 3	0.500	5	0.956	0.554	Do Not Test
Row 9 vs. Row 6	0.249	4	0.476	0.763	Do Not Test
Row 9 vs. Row 4	0.222	3	0.425	0.780	Do Not Test
Row 9 vs. Row 10	0.0234	2	0.0418	0.977	Do Not Test
Row 10 vs. Row 1	0.907	6	2.012	0.221	Do Not Test
Row 10 vs. Row 2	0.631	5	1.205	0.456	Do Not Test
Row 10 vs. Row 3	0.477	4	0.912	0.563	Do Not Test
Row 10 vs. Row 6	0.225	3	0.431	0.777	Do Not Test
Row 10 vs. Row 4	0.199	2	0.380	0.790	Do Not Test
Row 4 vs. Row 1	0.708	5	1.748	0.280	Do Not Test
Row 4 vs. Row 2	0.432	4	0.891	0.572	Do Not Test
Row 4 vs. Row 3	0.278	3	0.574	0.706	Do Not Test
Row 4 vs. Row 6	0.0265	2	0.0547	0.969	Do Not Test
Row 6 vs. Row 1	0.682	4	1.682	0.287	Do Not Test
Row 6 vs. Row 2	0.405	3	0.836	0.582	Do Not Test
Row 6 vs. Row 3	0.252	2	0.519	0.716	Do Not Test
Row 3 vs. Row 1	0.430	3	1.062	0.485	Do Not Test
Row 3 vs. Row 2	0.154	2	0.317	0.824	Do Not Test
Row 2 vs. Row 1	0.277	2	0.683	0.632	Do Not Test

A result of "Do Not Test" occurs for a comparison when no significant difference is found between two means that enclose that comparison. For example, if you had four means sorted in order, and found no difference between means 4 vs. 2, then you would not test 4 vs. 3 and 3 vs. 2, but still test 4 vs. 1 and 3 vs. 1 (4 vs. 3 and 3 vs. 2 are enclosed by 4 vs. 2: 4 3 2 1). Note that not testing the enclosed means is a procedural rule, and a result of Do Not Test should be treated as if there is no significant difference between the means, even though one may appear to exist.

One Way Analysis of Variance Friday, August 28, 2015, 12:26:15 PM

Data source: Oleic Acid

Group Name	**N**	**Missing**	**Mean**	**Std Dev**	**SEM**
Control	10	0	23.476	3.694	1.168
BA 10-7	4	0	28.890	4.966	2.483
BA 10-6	4	0	23.540	2.999	1.500
BA 10-5	4	0	20.959	4.444	2.222
ABA 10-6	4	0	19.816	5.974	2.987
ABA 10-5	4	0	21.847	3.524	1.762
tZ 10-7	4	0	24.950	5.018	2.509
tZ 10-6	4	0	20.011	5.131	2.566
MeSZ 10-7	3	0	20.222	3.556	2.053
MeSZ 10-6	3	0	13.593	1.707	0.985
MeSZ 10-5	3	0	21.980	1.917	1.107

Source of Variation	**DF**	**SS**	**MS**	**F**	**P**
Between Groups	10	515.378	51.538	2.991	0.008
Residual	36	620.371	17.233		
Total	46	1135.749			

The differences in the mean values among the treatment groups are greater than would be expected by chance; there is a statistically significant difference (P = 0.008).

Power of performed test with alpha = 0.050: 0.784

All Pairwise Multiple Comparison Procedures (Duncan's Method) :

Comparisons for factor:

Comparison	**Diff of Means**	**p**	**q**	**P**	**P<0.050**
Row 2 vs. Row 10	15.297	11	6.823	<0.001	Yes
Row 2 vs. Row 5	9.074	10	4.372	0.011	Yes
Row 2 vs. Row 8	8.879	9	4.278	0.012	Yes
Row 2 vs. Row 9	8.668	8	3.866	0.022	Yes
Row 2 vs. Row 4	7.931	7	3.821	0.022	Yes
Row 2 vs. Row 6	7.043	6	3.393	0.040	Yes
Row 2 vs. Row 11	6.910	5	3.082	0.057	No
Row 2 vs. Row 1	5.414	4	3.118	0.050	Do Not Test
Row 2 vs. Row 3	5.350	3	2.578	0.093	Do Not Test
Row 2 vs. Row 7	3.940	2	1.898	0.188	Do Not Test
Row 7 vs. Row 10	11.357	10	5.066	0.003	Yes
Row 7 vs. Row 5	5.134	9	2.474	0.145	No
Row 7 vs. Row 8	4.939	8	2.380	0.157	Do Not Test
Row 7 vs. Row 9	4.728	7	2.109	0.205	Do Not Test
Row 7 vs. Row 4	3.991	6	1.923	0.242	Do Not Test
Row 7 vs. Row 6	3.103	5	1.495	0.355	Do Not Test
Row 7 vs. Row 11	2.970	4	1.325	0.401	Do Not Test
Row 7 vs. Row 1	1.475	3	0.849	0.577	Do Not Test
Row 7 vs. Row 3	1.411	2	0.680	0.634	Do Not Test
Row 3 vs. Row 10	9.947	9	4.437	0.009	Yes
Row 3 vs. Row 5	3.724	8	1.794	0.285	Do Not Test
Row 3 vs. Row 8	3.529	7	1.700	0.307	Do Not Test
Row 3 vs. Row 9	3.317	6	1.480	0.368	Do Not Test

Row 3 vs. Row 4	2.580	5	1.243	0.442	Do Not Test
Row 3 vs. Row 6	1.692	4	0.815	0.605	Do Not Test
Row 3 vs. Row 11	1.559	3	0.696	0.647	Do Not Test
Row 3 vs. Row 1	0.0639	2	0.0368	0.979	Do Not Test
Row 1 vs. Row 10	9.883	8	5.114	0.003	Yes
Row 1 vs. Row 5	3.660	7	2.107	0.206	Do Not Test
Row 1 vs. Row 8	3.465	6	1.995	0.225	Do Not Test
Row 1 vs. Row 9	3.253	5	1.684	0.298	Do Not Test
Row 1 vs. Row 4	2.517	4	1.449	0.359	Do Not Test
Row 1 vs. Row 6	1.628	3	0.938	0.538	Do Not Test
Row 1 vs. Row 11	1.495	2	0.774	0.588	Do Not Test
Row 11 vs. Row 10	8.387	7	3.500	0.036	Yes
Row 11 vs. Row 5	2.164	6	0.965	0.556	Do Not Test
Row 11 vs. Row 8	1.969	5	0.878	0.587	Do Not Test
Row 11 vs. Row 9	1.758	4	0.734	0.642	Do Not Test
Row 11 vs. Row 4	1.021	3	0.455	0.765	Do Not Test
Row 11 vs. Row 6	0.133	2	0.0593	0.967	Do Not Test
Row 6 vs. Row 10	8.254	6	3.682	0.026	Yes
Row 6 vs. Row 5	2.031	5	0.979	0.545	Do Not Test
Row 6 vs. Row 8	1.836	4	0.885	0.575	Do Not Test
Row 6 vs. Row 9	1.625	3	0.725	0.634	Do Not Test
Row 6 vs. Row 4	0.888	2	0.428	0.764	Do Not Test
Row 4 vs. Row 10	7.366	5	3.286	0.043	Yes
Row 4 vs. Row 5	1.143	4	0.551	0.727	Do Not Test
Row 4 vs. Row 8	0.948	3	0.457	0.764	Do Not Test
Row 4 vs. Row 9	0.737	2	0.329	0.818	Do Not Test
Row 9 vs. Row 10	6.629	4	2.766	0.081	No
Row 9 vs. Row 5	0.406	3	0.181	0.905	Do Not Test
Row 9 vs. Row 8	0.211	2	0.0943	0.947	Do Not Test
Row 8 vs. Row 10	6.418	3	2.863	0.062	Do Not Test
Row 8 vs. Row 5	0.195	2	0.0939	0.947	Do Not Test
Row 5 vs. Row 10	6.223	2	2.776	0.058	Do Not Test

A result of "Do Not Test" occurs for a comparison when no significant difference is found between two means that enclose that comparison. For example, if you had four means sorted in order, and found no difference between means 4 vs. 2, then you would not test 4 vs. 3 and 3 vs. 2, but still test 4 vs. 1 and 3 vs. 1 (4 vs. 3 and 3 vs. 2 are enclosed by 4 vs. 2: 4 3 2 1). Note that not testing the enclosed means is a procedural rule, and a result of Do Not Test should be treated as if there is no significant difference between the means, even though one may appear to exist.

One Way Analysis of Variance Friday, August 28, 2015, 12:31:09 PM

Data source: Linoleic Acid

Group Name	N	Missing	Mean	Std Dev	SEM
Control	10	0	37.604	1.707	0.540
BA 10-7	4	0	28.956	6.129	3.065
BA 10-6	4	0	39.094	2.940	1.470
BA 10-5	4	0	41.881	2.104	1.052
ABA 10-6	4	0	32.182	4.719	2.360
ABA 10-5	4	0	35.617	0.965	0.482
tZ 10-7	4	0	36.479	3.417	1.709
tZ 10-6	4	0	40.677	5.295	2.647
MeSZ 10-7	3	0	37.796	1.915	1.105
MeSZ 10-6	3	0	39.415	2.855	1.648
MeSZ 10-5	3	0	39.886	1.527	0.882

Source of Variation	DF	SS	MS	F	P
Between Groups	10	574.051	57.405	5.230	<0.001
Residual	36	395.166	10.977		
Total	46	969.218			

The differences in the mean values among the treatment groups are greater than would be expected by chance; there is a statistically significant difference (P = <0.001).

Power of performed test with alpha = 0.050: 0.993

All Pairwise Multiple Comparison Procedures (Duncan's Method) :

Comparisons for factor:

Comparison	Diff of Means	p	q	P	P<0.050
Row 4 vs. Row 2	12.925	11	7.802	<0.001	Yes
Row 4 vs. Row 5	9.699	10	5.855	<0.001	Yes
Row 4 vs. Row 6	6.264	9	3.781	0.026	Yes
Row 4 vs. Row 7	5.402	8	3.261	0.053	No
Row 4 vs. Row 1	4.277	7	3.086	0.065	Do Not Test
Row 4 vs. Row 9	4.085	6	2.283	0.165	Do Not Test
Row 4 vs. Row 3	2.787	5	1.682	0.298	Do Not Test
Row 4 vs. Row 10	2.466	4	1.378	0.383	Do Not Test
Row 4 vs. Row 11	1.995	3	1.115	0.464	Do Not Test
Row 4 vs. Row 8	1.204	2	0.727	0.611	Do Not Test
Row 8 vs. Row 2	11.721	10	7.076	<0.001	Yes
Row 8 vs. Row 5	8.494	9	5.128	0.003	Yes
Row 8 vs. Row 6	5.059	8	3.054	0.070	No
Row 8 vs. Row 7	4.198	7	2.534	0.128	Do Not Test
Row 8 vs. Row 1	3.073	6	2.217	0.178	Do Not Test
Row 8 vs. Row 9	2.881	5	1.610	0.319	Do Not Test
Row 8 vs. Row 3	1.583	4	0.956	0.545	Do Not Test
Row 8 vs. Row 10	1.261	3	0.705	0.643	Do Not Test
Row 8 vs. Row 11	0.791	2	0.442	0.757	Do Not Test
Row 11 vs. Row 2	10.931	9	6.109	<0.001	Yes
Row 11 vs. Row 5	7.704	8	4.305	0.011	Yes
Row 11 vs. Row 6	4.269	7	2.386	0.152	Do Not Test
Row 11 vs. Row 7	3.407	6	1.904	0.247	Do Not Test

Row 11 vs. Row 1	2.282	5	1.480	0.360	Do Not Test
Row 11 vs. Row 9	2.090	4	1.093	0.489	Do Not Test
Row 11 vs. Row 3	0.792	3	0.443	0.771	Do Not Test
Row 11 vs. Row 10	0.471	2	0.246	0.863	Do Not Test
Row 10 vs. Row 2	10.460	8	5.846	<0.001	Yes
Row 10 vs. Row 5	7.233	7	4.042	0.016	Yes
Row 10 vs. Row 6	3.798	6	2.123	0.197	Do Not Test
Row 10 vs. Row 7	2.937	5	1.641	0.310	Do Not Test
Row 10 vs. Row 1	1.811	4	1.175	0.457	Do Not Test
Row 10 vs. Row 9	1.619	3	0.847	0.578	Do Not Test
Row 10 vs. Row 3	0.321	2	0.180	0.900	Do Not Test
Row 3 vs. Row 2	10.138	7	6.120	<0.001	Yes
Row 3 vs. Row 5	6.912	6	4.172	0.012	Yes
Row 3 vs. Row 6	3.477	5	2.099	0.195	Do Not Test
Row 3 vs. Row 7	2.615	4	1.579	0.317	Do Not Test
Row 3 vs. Row 1	1.490	3	1.075	0.480	Do Not Test
Row 3 vs. Row 9	1.298	2	0.725	0.611	Do Not Test
Row 9 vs. Row 2	8.840	6	4.941	0.003	Yes
Row 9 vs. Row 5	5.614	5	3.137	0.053	No
Row 9 vs. Row 6	2.179	4	1.218	0.440	Do Not Test
Row 9 vs. Row 7	1.317	3	0.736	0.628	Do Not Test
Row 9 vs. Row 1	0.192	2	0.125	0.930	Do Not Test
Row 1 vs. Row 2	8.648	5	6.240	<0.001	Yes
Row 1 vs. Row 5	5.422	4	3.912	0.014	Do Not Test
Row 1 vs. Row 6	1.987	3	1.433	0.347	Do Not Test
Row 1 vs. Row 7	1.125	2	0.812	0.570	Do Not Test
Row 7 vs. Row 2	7.523	4	4.541	0.005	Yes
Row 7 vs. Row 5	4.296	3	2.594	0.091	Do Not Test
Row 7 vs. Row 6	0.861	2	0.520	0.715	Do Not Test
Row 6 vs. Row 2	6.662	3	4.021	0.010	Yes
Row 6 vs. Row 5	3.435	2	2.074	0.151	Do Not Test
Row 5 vs. Row 2	3.227	2	1.948	0.177	No

A result of "Do Not Test" occurs for a comparison when no significant difference is found between two means that enclose that comparison. For example, if you had four means sorted in order, and found no difference between means 4 vs. 2, then you would not test 4 vs. 3 and 3 vs. 2, but still test 4 vs. 1 and 3 vs. 1 (4 vs. 3 and 3 vs. 2 are enclosed by 4 vs. 2: 4 3 2 1). Note that not testing the enclosed means is a procedural rule, and a result of Do Not Test should be treated as if there is no significant difference between the means, even though one may appear to exist.

One Way Analysis of Variance Friday, August 28, 2015, 12:35:15 PM

Data source: Linolenic Acid

Group Name	**N**	**Missing**	**Mean**	**Std Dev**	**SEM**
Control	10	0	2.138	1.871	0.592
BA 10-7	4	0	1.106	0.252	0.126
BA 10-6	4	0	1.846	0.401	0.201
BA 10-5	4	0	1.820	1.094	0.547
ABA 10-6	4	0	2.674	1.024	0.512
ABA 10-5	4	0	1.355	0.484	0.242
tZ 10-7	4	0	3.178	0.579	0.289
tZ 10-6	4	0	1.245	0.572	0.286
MeSZ 10-7	3	0	6.511	0.963	0.556
MeSZ 10-6	3	0	11.919	2.325	1.343
MeSZ 10-5	3	0	1.027	0.546	0.315

Source of Variation	**DF**	**SS**	**MS**	**F**	**P**
Between Groups	10	342.179	34.218	22.446	<0.001
Residual	36	54.880	1.524		
Total	46	397.059			

The differences in the mean values among the treatment groups are greater than would be expected by chance; there is a statistically significant difference (P = <0.001).

Power of performed test with alpha = 0.050: 1.000

All Pairwise Multiple Comparison Procedures (Duncan's Method) :

Comparisons for factor:

Comparison	**Diff of Means**	**p**	**q**	**P**	**P<0.050**
Row 10 vs. Row 11	10.892	11	15.279	<0.001	Yes
Row 10 vs. Row 2	10.812	10	16.215	<0.001	Yes
Row 10 vs. Row 8	10.674	9	16.007	<0.001	Yes
Row 10 vs. Row 6	10.564	8	15.843	<0.001	Yes
Row 10 vs. Row 4	10.099	7	15.145	<0.001	Yes
Row 10 vs. Row 3	10.073	6	15.106	<0.001	Yes
Row 10 vs. Row 1	9.780	5	17.018	<0.001	Yes
Row 10 vs. Row 5	9.245	4	13.864	<0.001	Yes
Row 10 vs. Row 7	8.741	3	13.108	<0.001	Yes
Row 10 vs. Row 9	5.407	2	7.586	<0.001	Yes
Row 9 vs. Row 11	5.485	10	7.694	<0.001	Yes
Row 9 vs. Row 2	5.405	9	8.106	<0.001	Yes
Row 9 vs. Row 8	5.266	8	7.898	<0.001	Yes
Row 9 vs. Row 6	5.157	7	7.733	<0.001	Yes
Row 9 vs. Row 4	4.692	6	7.036	<0.001	Yes
Row 9 vs. Row 3	4.665	5	6.996	<0.001	Yes
Row 9 vs. Row 1	4.373	4	7.609	<0.001	Yes
Row 9 vs. Row 5	3.837	3	5.755	<0.001	Yes
Row 9 vs. Row 7	3.333	2	4.999	0.001	Yes
Row 7 vs. Row 11	2.151	9	3.226	0.058	No
Row 7 vs. Row 2	2.072	8	3.356	0.047	Do Not Test
Row 7 vs. Row 8	1.933	7	3.131	0.061	Do Not Test
Row 7 vs. Row 6	1.823	6	2.953	0.073	Do Not Test

Row 7 vs. Row 4	1.358	5	2.200	0.174	Do Not Test
Row 7 vs. Row 3	1.332	4	2.157	0.173	Do Not Test
Row 7 vs. Row 1	1.040	3	2.013	0.188	Do Not Test
Row 7 vs. Row 5	0.504	2	0.816	0.568	Do Not Test
Row 5 vs. Row 11	1.647	8	2.471	0.142	Do Not Test
Row 5 vs. Row 2	1.568	7	2.539	0.128	Do Not Test
Row 5 vs. Row 8	1.429	6	2.315	0.159	Do Not Test
Row 5 vs. Row 6	1.319	5	2.137	0.187	Do Not Test
Row 5 vs. Row 4	0.854	4	1.384	0.381	Do Not Test
Row 5 vs. Row 3	0.828	3	1.341	0.379	Do Not Test
Row 5 vs. Row 1	0.536	2	1.038	0.468	Do Not Test
Row 1 vs. Row 11	1.111	7	1.934	0.245	Do Not Test
Row 1 vs. Row 2	1.032	6	1.998	0.224	Do Not Test
Row 1 vs. Row 8	0.893	5	1.729	0.285	Do Not Test
Row 1 vs. Row 6	0.783	4	1.517	0.337	Do Not Test
Row 1 vs. Row 4	0.318	3	0.616	0.685	Do Not Test
Row 1 vs. Row 3	0.292	2	0.565	0.692	Do Not Test
Row 3 vs. Row 11	0.819	6	1.229	0.454	Do Not Test
Row 3 vs. Row 2	0.740	5	1.198	0.458	Do Not Test
Row 3 vs. Row 8	0.601	4	0.974	0.537	Do Not Test
Row 3 vs. Row 6	0.491	3	0.796	0.601	Do Not Test
Row 3 vs. Row 4	0.0263	2	0.0427	0.976	Do Not Test
Row 4 vs. Row 11	0.793	5	1.189	0.462	Do Not Test
Row 4 vs. Row 2	0.713	4	1.156	0.464	Do Not Test
Row 4 vs. Row 8	0.575	3	0.931	0.541	Do Not Test
Row 4 vs. Row 6	0.465	2	0.753	0.598	Do Not Test
Row 6 vs. Row 11	0.328	4	0.492	0.756	Do Not Test
Row 6 vs. Row 2	0.248	3	0.402	0.792	Do Not Test
Row 6 vs. Row 8	0.110	2	0.178	0.901	Do Not Test
Row 8 vs. Row 11	0.218	3	0.327	0.830	Do Not Test
Row 8 vs. Row 2	0.139	2	0.224	0.875	Do Not Test
Row 2 vs. Row 11	0.0797	2	0.120	0.933	Do Not Test

A result of "Do Not Test" occurs for a comparison when no significant difference is found between two means that enclose that comparison. For example, if you had four means sorted in order, and found no difference between means 4 vs. 2, then you would not test 4 vs. 3 and 3 vs. 2, but still test 4 vs. 1 and 3 vs. 1 (4 vs. 3 and 3 vs. 2 are enclosed by 4 vs. 2: 4 3 2 1). Note that not testing the enclosed means is a procedural rule, and a result of Do Not Test should be treated as if there is no significant difference between the means, even though one may appear to exist.