In vitro optimization of antioxidants for total parenteral nutrition for newborns

by © Kandeepan Karthigesu

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Abstract

Newborns with gastrointestinal anomalies or premature birth often require lifesaving nutritional support in the form of total parenteral nutrition (TPN). However, TPN administration is associated with several complications in newborns. Several studies have suggested that it could be due to oxidant generation in the TPN admixture due to various factors, including light exposure. Therefore, these studies aimed to assess the peroxidation of the commercially available TPN for newborns by increasing levels of antioxidants and decreasing levels of prooxidants and optimizing the environmental conditions, leading to the development of a new admixture to be tested in a cell culture model. Standard TPN, similar to that in use in the neonatal intensive care unit (NICU) of Janeway Children Hospital, St. John's, Newfoundland, Canada (control diet) and the antioxidant-fortified all-in-one (AIO)-TPN (test diet), were prepared immediately before experiments commenced by optimizing each selected antioxidant. Both TPNs were light-exposed (LE) and light-protected (LP) for 24 hours. Standard and optimized AIO-TPN, both LE and LP, were treated to THP-1 human monocytes for further assessment of peroxidation, antioxidant status, and endoplasmic reticular (ER) stress. Peroxide levels in the AIO-TPN were gradually increased with increasing light intensity (at 0 Lux=1.020 (±0.07) vs. at 3000 Lux=1.588 (±0.088) mM H_2O_2 equivalents; $p < 0.05$). L-ascorbic acid at 60 mg/kg/day (2.27 mM), DL-α-tocopherol acetate at 8.8 mg/kg/day (124.1 μM), selenium at 4 μg/kg/day (0.24 μM), glutathione at 4 mg/kg/day (40 μM), copper at 40 μg/kg/day (4.2 μM), and zinc at 300 μg/kg/day (30.6 μM) in TPN significantly decreased peroxide levels ($p < 0.05$). The peroxide levels of optimized LE AIO-TPN were significantly decreased compared to standard LE AIO-TPN (1.165 (±0.042) vs. 1.439 (±0.074) mM H_2O_2 equivalents, $p < 0.05$). L-ascorbic acid and DL-α-tocopherol acetate at 20 μM fortified SMOFlipid® treated to the THP-1 cells significantly decreased lipid peroxidation

compared to the control (1.16 (±0.04) vs. 1.56 (±0.15) μM thiobarbituric acid reactive substances (TBARS), respectively; $p < 0.001$). Optimized AIO-TPN treated THP-1 cells generated less reactive oxygen species (ROS) than standard TPN. TBARS levels in LE-standard AIO-TPN (3.484 (±0.247) μM), for 24 hours treated THP-1 cells were significantly higher than LP 24 hour-AIO-TPN treated cells (1.257 (±0.153) μM); $p < 0.05$. The optimized LP AIO-TPN for 24 hours treated THP-1 cells had the highest cellular antioxidant activity (CAA) activity (36.97 (±9.63) %), followed by LE-optimized AIO-TPN for 24 hours (33.93 (±9.1) %), whereas the CAA of standard AIO-TPN was 13.37 (±5.069) %; $p < 0.05$. Though LP-optimized AIO-TPN for 24 hours treated THP-1 cells exhibited a higher ferric reducing antioxidant power (FRAP) activity (49.5 (±7.73) Trolox equivalents (TE) μg/mL), the FRAP activity of LE-optimized AIO-TPN treated cells was low (23.63 (±2.67) TE μg/mL). Even though the oxidized form of glutathione (GSSG) at 40 μM concentrations for TPN decreased the peroxide levels, the reduced form of glutathione (GSH)/GSSG ratio was low, indicating low redox potential in THP-1 cells. ER stress protein, GRP78 expression was high in THP-1 cells treated with LE AIO-TPN compared to LE standard TPN. However, it did not significantly differ. These studies concluded that peroxide levels were significantly increased when TPN was exposed to light. Optimizing TPN using antioxidants demonstrated lowering lipid peroxidation, better antioxidant capacity, and improving ER stress. Overall, these studies give novel insight into a promising strategy to develop a new TPN formula for newborns, particularly preterm babies, to decrease *in vitro* oxidants and eventually minimize the complications associated with oxidative stress in neonates.

Co-authorship statement

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List of abbreviations

AAPH: 2,2'-azobis (2-amidinopropane) dihydrochloride

AIO-TPN: All-in-one TPN

ASPEN: American Society for Parenteral and Enteral Nutrition

ATF6: activating transcription factor 6

BPD: Bronchopulmonary dysplasia

CAA: Cellular antioxidant activity

CHOP: C/EBP homologous protein

DCF: 2', 7'-dichlorofluorescein

DHA: Docosahexaenoic acid

EPA: Eicosapentaenoic acid

ER: Endoplasmic reticulum

ESI: Electrospray ionization

EVA: Ethylene-vinyl acetate

FF-TPN: Fat-free TPN

FOX: Ferrous oxidation-xylenol orange

FRAP: Ferric reducing antioxidant power

GPx: Glutathione peroxidase

GRP78: Glucose regulated protein 78

GSH: Reduced form of glutathione

GSSG: Oxidized glutathione

H_2O_2: Hydrogen peroxide

IFALD: Intestinal failure-associated liver disease

LE TPN: Light-exposed TPN

LP TPN: Light-protected TPN

MAT: Methionine adenosyltransferase

MCTs: Medium-chain triacylglycerols

MDA: Malondialdehyde

MVP: Multivitamin preparation

$N_{2(g)}$: Nitrogen gas

NICU: Neonatal intensive care unit

NO: Nitric oxide

NOS: Nitric oxide synthase

NOXs: NADPH oxidases

NRF2: Nuclear factor erythroid 2-related factor 2

PN: Parenteral nutrition

PNALD: Parenteral nutrition associated liver disease

PVC: polyvinyl chloride

ROS: Reactive oxygen species

SOD: Superoxide dismutase

TBARS: Thiobarbituric acid reactive substances

t-BHP: *tert*-butylhydroperoxide

TE: Trolox equivalents

TEs: Trace elements

TPN: Total parenteral nutrition

UPR: Unfolded protein response

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CHAPTER 1: INTRODUCTION

1.1 Preterm birth and its complications

A preterm or premature infant is a baby born before maturity. They are defined as babies born before 37 weeks of gestation age. Based on the gestational age, prematurity can be categorized into moderate to late preterm (32 to 37 weeks), very preterm (28 to less than 32 weeks), and extremely preterm (less than 28 weeks). The World Health Organization reported that 13.4 million babies were born prematurely in 2020. Not surprisingly, 0.9 million newborns died in 2019 due to the complications associated with prematurity (Ohuma *et al.*, 2023; Perin *et al.*, 2022). Hence, preterm birth is a leading cause of death in newborns in the world. Indeed, preterm or premature birth and its complications pose an enormous economic burden to a country. For instance, Canada spends nearly $587.1 million for all premature infants (Johnston *et al.*, 2014). In Canada, an estimated 8% of newborns are born prematurely at 37 weeks or less (Shah *et al.*, 2018). Moreover, over the last 18 years, the proportion of low birth weight babies (<2.5 kg) increased from 5.9% in 2003 to 7.0% in 2022 (Statistics Canada, 2021). In consideration of prematurity, neonates born at 36 weeks of gestation or earlier had a significant number of low birth weights (55.7%) in 2022 (Statistics Canada, 2021). Premature and low birth weight neonates may encounter several complications, including respiratory, hepatocellular, and cardiovascular complications, and have a higher risk of chronic health conditions later in life (Luu *et al.*, 2016).

The lungs and respiratory system of immature babies are affected by respiratory distress syndrome, bronchopulmonary dysplasia (BPD), and apnea. The hepatocellular system is crucial for the synthesis and detoxification of various molecules. In premature babies, the hepatocellular

system is not fully developed, leading to diminished liver function. The excessive metabolic demands placed on their immature liver can overwhelm its capacity, resulting in weakened overall function in such infants (Mawson, 2016). Preterm babies can also be affected by several cardiovascular disorders, including morphological defects, such as underdeveloped aortas, restricted vascular beds, and narrowed and stiffer arteries in blood vessels (Lewandowski *et al.*, 2020), which can impair blood circulation to tissues. These abnormalities also contribute to endothelial dysfunction, further disrupting nutrient delivery to the tissues of such babies. Several studies have reported that the majority of such complications in neonates are related to oxidative stress that develops early in life (Lavoie and Chessex, 2019; Saugstad, 2003).

1.2 Oxidative stress in preterm or term newborns

Oxidative stress occurs when there is an imbalance between the generation and accumulation of reactive oxygen species (ROS) in a body, and the capacity to detoxify those reactive compounds by the antioxidant system in neonates (Pizzino *et al.*, 2017). Superoxide radicals ($O_2^{\bullet-}$), hydrogen peroxide (H_2O_2), hydroxyl radicals ($^{\bullet}OH$), and singlet oxygen ($^1O_2$) are collectively known as ROS, which are generated as by-products during metabolic activity (Navarro-Yepes *et al.*, 2014). Newborns are at a higher risk of oxidative stress due to the exposure of oxidants at an early growing period, from various endogenous and exogenous sources (Jankov *et al.*, 2001). For instance, numerous endogenous sources of oxidative stress are from birth trauma, reperfusion injury from hypoxia, oxygen therapy, acidosis, phototherapy, mechanical ventilation, infection, and inflammation (Bhatia *et al.*, 1981; Lunec, 1990). The exogenous sources of peroxides are from diet infused to newborns. Besides diet, if oxygen therapy was used during the neonatal period or exposure of diet to light, those can be additional sources of oxidant load exacerbating oxidative stress (Saugstad, 2005; Vento *et al.*, 2009).

Premature babies often require an intravenous diet, which refers to parenteral or total parenteral nutrition.

1.2.1 The composition of the TPN for neonates

1.2.1.1 Carbohydrates

Carbohydrates are the primary source of energy for neonates, usually provided as a dextrose solution, which generates 3.4 kcal/g in its hydrated form. Indeed, preterm babies have low glycogen storage in the liver. Therefore, they need an adequate amount of glucose (Johnson, 2013). The commonly available forms are 25%, 50% or 70% of dextrose for parenteral administration. It is then diluted to 10% or a maximum of 12.5% for peripheral venous administration, depending on the neonate's needs and tolerance. In hypoglycemic conditions, a higher dose of dextrose can be given (>12.5%) via the central vein because of the tendency to cause thrombophlebitis in peripheral veins (Mirtallo, 2001). The amount of dextrose is determined by the total calorie requirement per day. For instance, a total of 90-120 kcal/kg/day and 85-105 kcal/kg/day are recommended from carbohydrates, lipids, and proteins for preterm and term neonates under six months, respectively. A total of 17.19 g of dextrose provides 55% (58.446 kcal/day) of the total calorie requirement per day/kg neonates.

1.2.1.2 Proteins

Essential and non-essential amino acids are provided in a balanced mixture for TPN to support the growth and development of neonates (see *Appendix I*) (Prolla *et al.*, 2022). Amino acids in the TPN have several functions, such as protein synthesis and non-protein functions, including glutathione and nitric oxide (NO) biosynthesis. Even though several intravenous amino

acid injectable solutions are available in the market, Primene 10% (*w/v*), a pediatric (< 1 year-old) amino acid injection, is typically prescribed to preterm and term neonates in Canada (see *Appendix I*). Pediatric amino acid formulations typically have high amounts of branched-chain amino acids and lower amounts of methionine and phenylalanine (Lev-Ran *et al.*, 1987; Singer *et al.*, 1992).

Taurine is a sulphur-containing amino acid that plays a vital role in various physiological processes in neonates. It is particularly important in neonates. An exogenous source of taurine is beneficial for bile acid secretion, fat absorption and overall liver function. Due to these critical functions, taurine is considered a conditionally essential amino acid for premature infants. Studies have shown that supplementing parenteral nutrition with taurine can decrease the incidence of parenteral nutrition-associated cholestasis, suggesting its beneficial role in lowering bilirubin levels (Guertin *et al.*, 1991; Spencer *et al.*, 2005).

Ornithine is a non-essential and non-proteinogenic amino acid that is crucial in the urea cycle, helping to eliminate excess nitrogen from the body. This process is vital for detoxifying ammonia, a byproduct of protein metabolism, thus preventing hyperammonemia, especially in neonates with liver dysfunction. Beyond its role in the urea cycle, ornithine serves as a precursor to citrulline and arginine, both of which are important for synthesizing NO, a molecule essential for vascular health and cellular communication. Thus, ornithine is often included in TPN formulations (see *Appendix I*) to support metabolic functions and promote overall health, particularly in neonates with compromised liver function or those requiring intensive nutritional support (Prolla *et al.*, 2022).

Crystalline amino acids provide 4 kcal/g of energy. The dose rate of amino acids should be 3.5 to 4.5 g/kg/day, providing 14-18 kcal/kg/day.

1.2.1.3 Lipids

Lipid emulsions are an integral component of parenteral nutrition. It contains essential fatty acids and triglycerides to supply essential fatty acids and energy. For instance, fat-free TPN (FF-TPN) infusion for infants for an extended period of time can lead to the development of essential fatty acid deficiencies (EFAD). Lipid emulsions can provide essential and nonessential fatty acids with balanced ratio of omega-6 to omega-3 fatty acids. Lipid is administered to neonates at a dose of 2.5-3.5 g/kg/day, delivering 25-35 kcal/kg/day.

Other components in lipid emulsions are egg yolk phospholipid (lecithin) as an emulsifier, glycerin or glycerol to render the formulation isotonic, and sodium hydroxide to adjust the final pH (5.9-6.7).

1.2.1.4 Vitamins, trace elements, and electrolytes

Fat-soluble vitamins (vitamins A, D, E, and K) and water-soluble vitamins (vitamins B complex and C) are essential for vital function in newborns. Multivitamin preparation (MVP) is a commercially available form of vitamin injection for parenteral nutrition (see *Appendix I*). Trace elements, namely copper, zinc, selenium, iodine, chromium, and manganese, are added to the TPN to prevent deficiency syndromes (see *Appendix I*).

TPN also contains sodium, potassium, calcium, magnesium, chloride, acetate, and phosphorus. These are essential for cellular function, bone development, and overall metabolic processes. The amounts are carefully monitored based on laboratory values and clinical status (see *Appendix I*).

1.2.1.5 Fluids and additives

The total volume of TPN includes the necessary water to meet hydration needs, adjusted based on the neonate's fluid status, weight, and ongoing losses. The fluid dose rate of 140-160 mL/kg/day is commonly used.

Low-dose heparin is added to the continuous TPN infusion. It is added to prevent clotting in the central line and venous thrombosis, and improve lipid digestion at the rate of 0.25 units per mL (Foinard *et al.*, 2014; Moclair and Bates, 1995).

1.3 Implication of total parenteral nutrition for the newborns

The gastrointestinal tract of premature, low birth weight or term newborn neonates is immature and inefficient with respect to digestion, assimilation, and absorption of nutrients for the newborn. Thus, neonates of preterm or term depend on adequate early nutrition, which not only safeguards life, but also provides positive health outcomes in later life (Lucas *et al.*, 1992). Starting feeds by mouth or nasogastric tube (enteral feeding) as quickly as possible to safeguard their lives or stimulates gastrointestinal tract development and function (Kennedy *et al.*, 2000). However, because of lower gastrointestinal tract capacity, immature gut function or congenital problems, severe malnutrition, and high demand for nutrients for their accelerated growth, the consumption of recommended nutrients per day may require intravenous administration of nutrients (i.e., parenteral nutrition), in addition to some enteral feeding. However, in some cases in extremely premature or low birth weight neonates, or neonates with congenital anomalies of the gastrointestinal tract or bowel obstruction, all nutrients might need to be delivered as total parenteral nutrition (TPN) (Lavoie and Chessex, 2019). The American Society for Parenteral and Enteral Nutrition (ASPEN) consensus report recommended that preterm less than 28 weeks at

birth, extremely low birth weights of less than 1,000 g, and infants with gastrointestinal perforation and severe congenital abnormalities require exclusive TPN for their life-support (Pederson, 2011). TPN is prepared by mixing elemental nutrients, including dextrose, amino acids, vitamins, minerals, and trace elements, and delivered intravenously with a lipid emulsion infused either separately, or mixed with aqueous solutions (see *Appendix I*). It is carefully formulated to achieve the specific nutritional requirements of neonates based on the neonatologist's or pediatrician's prescription, and the formulation can be adjusted based on the newborn's weight, age, and clinical condition (Rizzo *et al.*, 2022).

1.4 History of TPN development

Even though the concept of the modern development of the mode of TPN feeding was developed in the 1930s, the clinically safest and practical TPN was developed in the 1960s for global usage (Dudrick, 2009; Dudrick and Palesty, 2011). The composition of TPN has been modified from time to time to alleviate its adverse effects, mainly due to lipid and amino acid compositions. For instance, optimizing the fatty acid composition of intravenous lipid emulsions for parenteral administration has been evolving since the 1960s. The first safe lipid emulsion was approved in the 1960s to provide patients (Fell *et al.*, 2015). The first commercially available soybean oil-based lipid emulsion, Intralipid^{TM}, was developed by Swedish physician and nutrition researcher Arvid Wretlind and approved for clinical use in Sweden in 1962 (Isaksson *et al.*, 2002). Intralipid^{TM} contains 50% omega-6 linoleic acid in its total fatty acid composition (Isaksson *et al.*, 2002) and a high amount of phytosterol (see *Appendix I*), which has been associated with cholestasis and other complications. Despite these issues, it remains widely used in clinical settings due to its affordability (Buys *et al.*, 2015).

Due to the side effects associated with the high pro-inflammatory omega-6 fatty acids and phytosterol content, which can induce oxidative stress (Calder *et al.*, 2010), researchers have more recently developed new generation lipid emulsions. These newer formulations reduce the content of proinflammatory omega-6 fatty acids and phytosterols while increasing the levels of omega-3 fatty acids (Van Aerde *et al.*, 1999; Diamond *et al.*, 2017; Waitzberg *et al.*, 2006).

Plant-based lipid emulsions contain phytosterols, which are one of the major causative substances for cholestasis. As a result, companies have been prompted to develop animal-based lipid emulsions. The dietary phytosterol absorption is regulated by intestinal transporters such as ATP binding cassette (ABC) G5/8, which facilitate the excretion of free phytosterols back into the intestinal lumen. Under normal circumstances, less than 5% of ingested sterols are absorbed, with the rest released into the intestine via those transporters (Ling and Jones, 1995). Intravenous phytosterols bypass this mechanism and lead to accumulation in the circulation (Li *et al.*, 2022), likely attributed to cholestasis in infants (Clayton *et al.*, 1993; Pianese *et al.*, 2008). For instance, Clayton *et al.* (1993) reported that the children receiving phytosterols containing lipid emulsion developed cholestasis due to the uptake of phytosterols into systemic circulation. When phytosterol reaches the liver, it is metabolized to bile salts, which are less soluble than cholesterol and eventually precipitated (Clayton *et al.*, 1993). The hydrolytic action of 7α-hydroxylase, the rate-limiting enzyme in bile metabolism in the liver, on cholesterol, can be affected due to sterol precipitation in the cell membrane and leading to decrease in bile acid synthesis and secretion. Pianese *et al.* (2008) reported that TPN-related cholestasis is induced by the accumulation of phytosterols in the plasma and red blood cell membranes of newborns. Indeed, administration of phytosterols indicated its elevation in the blood, liver, and bile, causing cholestasis in animal models (Iyer *et al.*, 1998). Hence, cholestasis emerged as a concern with

long-term use of lipid emulsions or TPN when incorporating plant-based oils (Clayton *et al.*, 1993; Savini *et al.*, 2013).

To reduce the phytosterol content and increase omega 3 fatty acids, the next-generation lipid emulsion, Lipofundin®, was developed with a 50:50 composition of soybean oil and medium-chain triacylglycerols (MCTs) derived from coconut oil by B. Braun Melsungen AG, a German medical and pharmaceutical device company (see *Appendix I*) (Wanten and Calder, 2007).

Further advancements led to the development of ClinOleic® in 1990 by Baxter International Inc., which features a 20:80 mixture of soybean to olive oil. Due to its high content of monounsaturated fatty acids and a higher omega-6 to omega-3 ratio (9:1), there was a need for a lipid emulsion that better mimicked the physiological ratio of omega-6 to omega-3, which is approximately 2.5:1. This led to the creation of SMOFlipid®, a fourth-generation lipid emulsion that contains a blend of soybean oil (30%), MCTs (30%), olive oil (25%), and fish oil (15%). SMOFlipid®, rich in anti-inflammatory omega-3 fatty acids, such as docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA), may reduce several complications, including cholestasis, compared to Intralipid™ (Diamond *et al.*, 2017; Nandivada *et al.*, 2013; Unal *et al.*, 2018). However, the new generation lipid emulsion, SMOFlipid®, also has considerable amounts of phytosterols.

To further eliminate the adverse effects associated with phytosterols, a fourth-generation lipid emulsion called Omegaven®, which contains 100% fish oil, was developed in 2018 and approved for use in the USA. However, it has not been approved for routine use in Canada. Consequently, most pediatric clinics and neonatal intensive care units (NICUs) in Canada

primarily use Intralipid^{TM} and SMOFlipid$^{®}$. The latter is a newly emerging solution in many clinical settings of NICU, and the *in vitro* generation of oxidants is still not well known. Though lipid peroxidation is common in TPN, a lipid component in TPN is inevitable because it provides essential fatty acids – linoleic acid and α-linolenic acid, and conditionally essential fatty acids – DHA and EPA (Deshpande and Cai, 2020).

1.5 Limitations of parenteral nutrition for the newborns

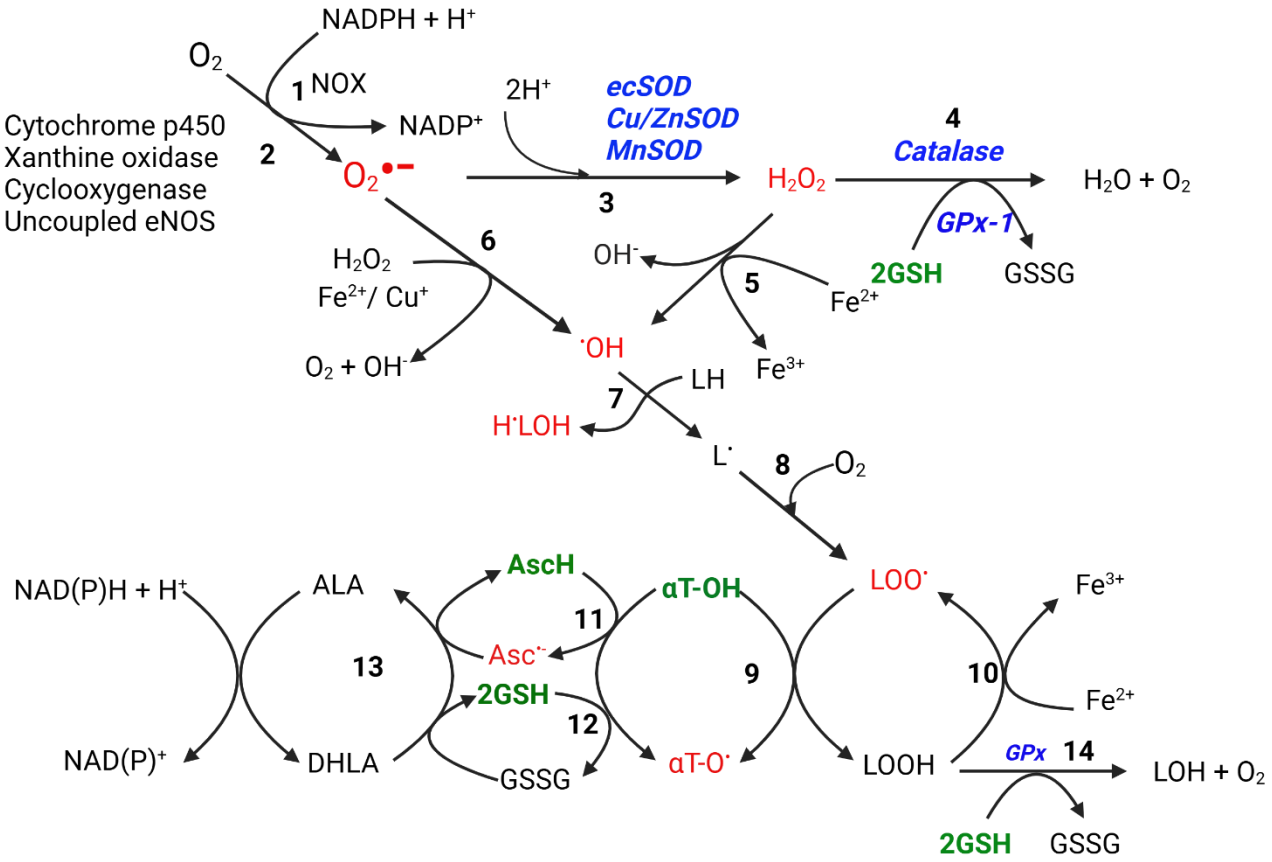
TPN is a highly advanced medical technique, yet it is not without complications in clinical settings. These complications can arise from using a central venous catheter or the TPN itself. The common complications may be due to high or low doses of nutrients, which may lead to hypo- or hyperglycemia, vitamin deficiencies, trace element deficiencies or toxicities. The catheter-related complications are pneumothorax, haemothorax, air embolism or intravenous line sepsis (Tsotsolis *et al.*, 2015). Although, TPN has become a crucial part of the clinical management of premature and newborn infants, it is subject to oxidation because of its nutrients and is thus a source of oxidant exposure to neonates as mentioned above (Calkins *et al.*, 2014). Oxidants in TPN may also lead to the production of additional oxidation products *in vivo*, which may detrimentally affect the health of the infant. The oxidant load collectively generated in human neonates from various sources could lead to liver diseases, BPD, gut atrophy, necrotizing enterocolitis, retinopathy (Koseesirikul *et al.*, 2012; Ozsurekci and Aykac, 2016), and periventricular leukomalacia (Deshpande and Cai, 2020). Moreover, several animal experiments have demonstrated that the exogenous oxidized molecules from TPN could cause hepatocellular damage, cholestasis, apoptosis, and pulmonary fibrosis (Elremaly *et al.*, 2014; Guzman *et al.*, 2020; Hoff and Michaelson, 2009; Morin *et al.*, 2019; Wang *et al.*, 2021).

1.6 Antioxidant status of newborns to balance the oxidative stress

Antioxidants are substances that inhibit or delay the oxidative process at low concentrations while often being oxidized themselves during the scavenging action (**Figure 1.1**). In the body, enzymatic or non-enzymatic antioxidants act on free radicals to counteract them by maintaining redox balance (Sen and Chakraborty, 2011). Despite *in vivo* antioxidants such as vitamin E, vitamin C, superoxide dismutase, catalase, and glutathione (Graham *et al.*, 1998) **(Figure 1.1**), which play a major role in reducing oxidative stress, neonates are even more prone to oxidative damage because antioxidant systems in newborns are immature, especially in preterm infants. Moreover, premature neonates, to whom TPN is prescribed more often, are more likely to be exposed to high amounts of peroxides. In addition, neonates just after birth are exposed to a relatively hyperoxic environment due to high oxygen bioavailability that causes the generation of free radicals (Perrone *et al.*, 2010). The antioxidant systems within neonates that remove these peroxides work at a lower capacity. Indeed, neonates have lower levels of vitamin E, β-carotene, and glutathione compared to adults (Behrman *et al.*, 1982; Lavoie *et al.*, 1997; Lindeman *et al.*, 1989). Superoxide dismutase and catalase activities are also lower in preterm neonates compared to term neonates, children, and adults (Anne *et al.*, 1976). Although the antioxidant effect of the iron transport protein, apotransferrin, and the iron oxidizing protein, ceruloplasmin, is nearly 200-fold higher than that of vitamin E (Sullivan, 1988), they are found at lower concentrations in preterm than term neonates and children (Sullivan and Newton, 1988). Therefore, exogenous antioxidants and precursors for the synthesis of antioxidants should be supplied via TPN in neonates.

Figure 1.1: Generation of reactive oxygen species (ROS), lipid oxidation, and role of enzymatic (superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx)), and non-enzymatic antioxidants (vitamin C, vitamin E, and lipoic acid) in the process of oxidative stress.

1) The superoxide radical anion ($O_2^{\bullet-}$) is generated from oxygen via reduction reaction of NADPH-oxidase (Nox) during tissue damage, **2**) Xanthine oxidase during the conversion of hypoxanthine to xanthine or xanthine to uric acid, cyclooxygenase, cytochrome p450, uncoupled reaction of endothelial nitric oxide synthase (eNOS) actions or non-enzymatic reactions at electron transport chain of mitochondria generates $O_2^{\bullet-}$. **3**) Hydrogen peroxide (H_2O_2) formed from superoxide via various reactions: extracellular SOD (ecSOD) acts on superoxide; Cu/Zn SOD (SOD_1) located in the cytoplasm, mitochondrial intermembrane space, nucleus, and lysosomes; mitochondrially localized manganese SOD (MnSOD (SOD_2)) (Andrés *et al.*, 2022); **4**) Catalase hydrolyses H_2O_2 into H_2O and O_2. Another enzyme, GPx, cleaves H_2O_2 efficiently. **5**) Soluble Fe^{2+} donates an electron to an H_2O_2 molecule, causing it to decompose into hydroxyl radicals $^{\bullet}OH + {}^{-}OH$ (Fenton reaction). The hydroxy radical can damage the biomolecules, including DNA, proteins, and lipids. **6)** Superoxide is reduced to hydroxy radical while H_2O_2 is converted to O_2 and hydroxide in the presence of Fe^{2+} or Cu^{+} (Haber–Weiss reaction). **7**) If there is any overproduction of hydroxy radical beyond the capacity of catalase, SOD or glutathione, the hydroxyl radical can abstract an electron from polyunsaturated fatty acid (LH) and generating a carbon-centred lipid radical ($L^{\bullet}$) while producing $H^{\bullet}LOH$, hydroxy lipid radical. **8)** The lipid radical ($L^{\bullet}$) can further interact with molecular oxygen to produce a lipid peroxyl radical ($LOO^{\bullet}$ radical). **9) and 10)** The lipid peroxyl radical ($LOO^{\bullet}$) is reduced within the membrane by the reduced form of alpha-tocopherol (αT-OH) to alpha-tocopheryl radical (αT-$O^{\bullet}$), resulting in the formation of a lipid hydroperoxide (LOOH); **11)**. A radical form of vitamin E is reduced back to alpha-tocopherol by ascorbic acid while converting it to radical form of vitamin C (ascorbyl radical). **12)**. The radical form of vitamin E can be also regenerated by a reduced form of glutathione (GSH). **13)**. The formed ascorbyl radical can be reduced back to ascorbic acid by dihydrolipoic acid (DHLA) and form lipoic acid (ALA). Lipid peroxide can be converted to lipid hydroxide and release O_2 by GSH.


NADPH + H+
O2
1 NOX
NADP+
2H+
ecSOD
Cu/ZnSOD
MnSOD
4
Catalase
H2O + O2
Cytochrome p450
Xanthine oxidase
Cyclooxygenase
Uncoupled eNOS
2
O2•−
3
H2O2
GPx-1
2GSH
GSSG
6
OH-
5
Fe2+
H2O2
Fe2+/ Cu+
O2 + OH-
·OH
Fe3+
LH
7
H·LOH
L·
8
O2
LOO·
Fe3+
AscH
αT-OH
11
Asc·-
9
10
Fe2+
NAD(P)H + H+
ALA
13
2GSH
12
αT-O·
LOOH
GPx
14
LOH + O2
NAD(P)+
DHLA
GSSG
2GSH
GSSG

1.6.1 Deficient in precursors for the in vivo biosynthesis of glutathione

There are also antioxidant systems that depend on sufficient dietary intake of precursors. For example, glutathione, a tripeptide, γ-L-glutamyl-L-cysteinylglycine, is the most abundant non-protein thiol containing intracellular antioxidant. The biosynthesis of glutathione depends on sufficient dietary precursors, including cysteine (Morin *et al.*, 2019). In order to synthesize glutathione, the active form of methionine adenosyltransferase (MAT-SH), a key metabolic enzyme, involves in transmethylation of the first step of methionine catabolism to provide the precursor, cysteine for the synthesis of glutathione (Lu, 2013). In the context of TPN, the MAT-SH is converted to an inactive form of methionine adenosyltransferase (MAT-SOH) by H_2O_2 generated in TPN, resulting in a reduction of the S-adenosylmethionine synthesis (Elremaly *et al.*, 2016) (**Figure 1.2**). The low activity of MAT-SH is regenerated from MAT-SOH by glutathione. In addition, the detoxification process of peroxides by glutathione peroxidase (GPx) needs the reduced form of glutathione (GSH) (Lubos *et al.*, 2011). Most importantly, the availability of cysteine from methionine is a limiting step for the synthesis of glutathione in neonates because of the immaturity of the cystathionase and glutathione synthase (Morin *et al.*, 2019). Therefore, TPN contaminated with peroxides limits the use of methionine for the biosynthesis of cysteine. Hence, cysteine is deemed a conditionally essential amino acid, and may become essential during TPN feeding. However, the addition of higher amount of cysteine to TPN is limited due to various reasons.

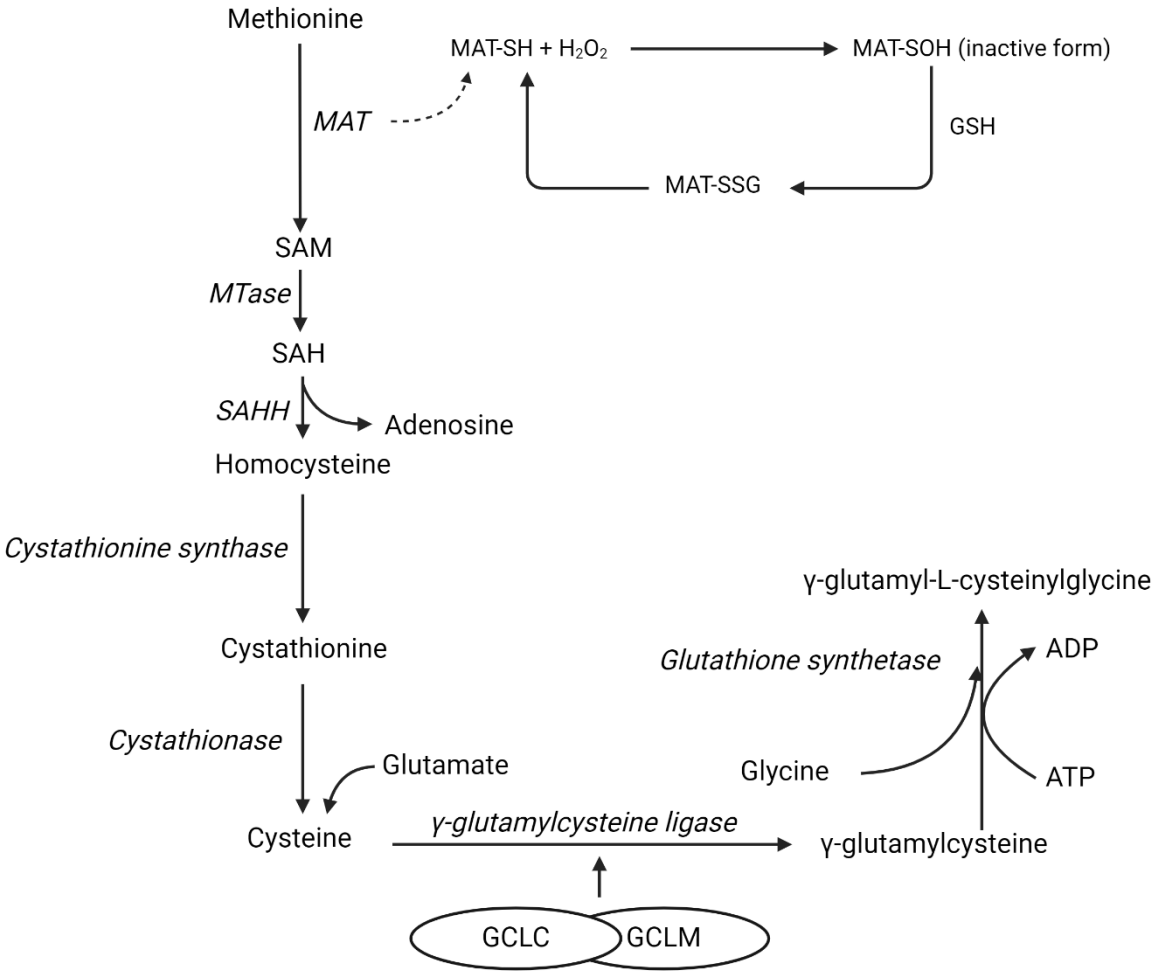
Cysteine can be oxidized quickly (Raftery, 2014). For instance, one-electron oxidation of cysteine by ROS generates thiyl radicals, while two-electron oxidation between cysteine by ROS produces sulfenic acid (Verrastro *et al.*, 2015; Zhang *et al.*, 2013), which leads to loss of a cysteine containing protein's function (Paulsen and Carroll, 2013). Though the infusion of high

doses of cysteine in TPN to preterm infants is considered safe, it was reported to not increase the amount of plasma glutathione or cystine levels (te Braake *et al.*, 2009), and it increased nitrogen retention in tissues (Soghier and Brion, 2006). It is also important to note that cysteine is unstable in TPN solutions and often undersupplied because of its poor solubility. However, according to the new guidelines from the ASPEN (Boullata *et al.*, 2014), the supply of L-cysteine as the hydrochloride (HCl) salt to TPN is beneficial, including the acidification of the TPN admixture, which can enhance the solubility of calcium and phosphate. Direct glutathione supplementation rather than cysteine supplementation could also have a beneficial effect in lowering the oxidant load (Morin *et al.*, 2019). For instance, Morin *et al.* (2019) reported that supplementation of glutathione to the TPN decreased peroxides in the lung and the liver in guinea pig models. The supplementation of glutathione prevented epigenetic modifications that are caused by endogenous H_2O_2 metabolism of TPN-fed guinea pigs (Mungala Lengo *et al.*, 2024). Incorporating the oxidized form of glutathione (GSSG) into a TPN mixture prevented the oxidized redox potential of glutathione (high GSSG), activation of caspase-3 (apoptosis marker), and loss of alveoli (Elremaly *et al.*, 2015).

Oxidants, including H_2O_2 and lipid peroxide, are reduced by the scavenging action of glutathione as a reduced form of protonated dimeric glutathione tuned to GSSG by GPx in the cellular system. The oxidized form of GSSG can be reduced back to GSH by GSSG reductase to continue the redox cycle (Lu, 2009). Therefore, the GSH to GSSG ratio is a vital redox marker to determine the intracellular redox potential.

Figure 1.2: Glutathione biosynthesis and role of glutathione on reactivation of MAT-SOH.

In the first step, methionine is converted to S-adenosylmethionine (SAM) by methionine adenosyltransferase (MAT). The limitation of this step is that hydrogen peroxide (H_2O_2) generated in the TPN reacts with the -SH group of MAT, converting it to -SOH, which is the oxidized form of thiol group, resulting in a vicious cycle. Reduced form of glutathione (GSH) reforms the MAT-SH from MAT-SOH by generating MAT-mixed disulfide (MAT-SSG). SAM is then converted to S-adenosylhomocysteine (SAH) by multiple cellular methyltransferase (MTase) reactions, which transfer the methyl group. The reversible hydrolysis of SAH by the SAH hydrolase (SAHH) reaction generates homocysteine and adenosine. Homocysteine can then be either remethylated to methionine for tetrahydrofolate (THF)-dependent methionine transmethylation or irreversibly removed by cystathionine synthase. This one-way reaction removes homocysteine synthesized from methionine and initiates the transsulfuration pathway for glutathione synthesis by incorporating other precursor amino acids, glutamate and glycine. GCLC: catalytic subunit of glutamate-cysteine ligase; GCLM: modifier subunit of glutamate cysteine ligase.


Methionine
MAT-SH + H_2O_2
MAT-SOH (inactive form)
MAT
GSH
MAT-SSG
SAM
MTase
SAH
SAHH
Adenosine
Homocysteine
Cystathionine synthase
γ-glutamyl-L-cysteinylglycine
Cystathionine
Glutathione synthetase
ADP
Cystathionase
Glutamate
Glycine
ATP
Cysteine
γ-glutamylcysteine ligase
γ-glutamylcysteine
GCLC
GCLM

Overall, it is essential to understand the components within TPN that can lead to oxidation *in vitro* as well as *in vivo*, and how TPN could be adapted to reduce the oxidant load as well as enhance antioxidant capacity in the neonatal body. Moreover, oxidative stress from TPN feeding can come from not only oxidation of nutrients *in vitro* in the mixed TPN solutions, but also from *in vivo* reactions from infusing prooxidant molecules intravenously, both of which can overwhelm immature antioxidant systems.

1.7 *In vitro* oxidation of TPN

TPN is prepared by combining individual nutrients together into a bag before administration or mixed with SMOFlipid® line at the NICU, and is referred to as all-in-one TPN (AIO-TPN). The interaction among the nutrients within the bag is a major source of oxidants, in part due to chemical reactions among elemental nutrients under various conditions. These chemically altered elemental nutrients in the bag can result in oxidant production that affects metabolism within neonates (Lavoie and Chessex, 2019). For example, the solution can generate oxidants such as H_2O_2 and organic peroxides (R-O-O-R') – (compounds possessing one or more oxygen-oxygen bonds) – as a consequence of redox reactions (Jalabert *et al.*, 2011; Miloudi *et al.*, 2012; Neuzil *et al.*, 1995). Organic peroxides can be classified into different types of peroxides that correspond to the peroxide structure. Among the classification, hydroperoxides are the major peroxide form; of these, organic (alkyl) hydroperoxides (R-O-OH), are among the more common hydroperoxides. Peroxides further non-specifically react with lipids, amino acids, vitamins, and trace elements in the TPN. For instance, the oxidative degradation of lipids generates lipid hydroperoxides (Ayala *et al.*, 2014), which are the primary products of free-radical initiated peroxidation of polyunsaturated fatty acids (Milne *et al.*, 2011). These oxidant molecules, together with amino acid oxidation and a high level of elemental prooxidant nutrients

including vitamin C, copper, and iron within the TPN, may overwhelm neonatal antioxidant systems and cause adverse outcomes in neonates.

1.7.1 Oxidation of lipid emulsions for TPN

Lipids are more prone to get oxidized by free radical attack during oxidative stress (Milne *et al.*, 2011). Lipids are oxidized by enzymatic and non-enzymatic reactions. Non-enzymatic oxidation is mediated by free radical and non-radical oxidation (Niki, 2007). In the free radical process, peroxides lead to hydrogen abstraction at sites of allylic or *bis*-allylic position of polyunsaturated fatty acids, resulting in free radical production; the free radicals eventually react with oxygen to generate lipid peroxyl radicals and hydroperoxides (Ayala *et al.*, 2014; Gardner, 1989; Yoshida and Niki, 2004) (**Figure 1.3**). The free radical form, a hydroperoxyl radical formed during lipid peroxidation, plays a crucial role in oxidant injury (Ayala *et al.*, 2014). Stored lipid emulsions that are eventually added to TPN can generate high concentrations of oxidized lipids even before administration to neonates as part of TPN (Fell *et al.*, 2015). Helbock *et al.* (1993) reported that commercial lipid emulsions (such as Intralipid™-20%) were contaminated with 300 μM hydroperoxides. In addition, hydroperoxyl radicals get protonated and generate H_2O_2, which can further react with iron or copper to produce hydroxyl radicals via the Fenton reaction (Janda *et al.*, 2015; Padayatty and Levine, 2016) (**Figure 1.4 A**). In Haber-Weiss reactions, superoxide radicals can react with H_2O_2 and generate hydroxyl radicals (**Figure 1.4 B**). Superoxide radicals further react with the oxidized form of metal ions to yield oxygen and reduced form of metal ions, which can again participate in redox reactions.

A variety of volatile, non-volatile, and polymeric secondary products, including aldehydes, alkanes, alkenes, alcohols, hydrocarbons, organic acids, epoxy compounds, and

conjugated dienes can be formed through secondary reactions during lipid peroxidation reactions (Reis and Spickett, 2012). Among the secondary products, 4-hydroxy-2-nonenal is an important oxidative molecule formed from the peroxidation of omega-6-series fatty acids, while 4-hydroxy-2-hexenal is generated from the peroxidation of omega-3-series fatty acids. However, the formation of other oxidative products has also been proposed. For example, F_2-isoprostanes, a prostaglandin F_2-like compound are produced by the free radical-mediated non-enzymatic oxidation of arachidonate (Milne *et al.*, 2011; Roberts *et al.*, 2005). With high oxygen tension, the formation of F_2-isoprostanes is limited (Fessel *et al.*, 2002). Isofuran, a similar product contains substituted tetrahydrofuran ring of F_2-isoprostanes, has been examined as a marker of oxidative stress during increased oxygen tension. In addition, malondialdehyde (MDA) is another oxidative molecule that can be produced from lipid peroxidation. MDA is highly cytotoxic and can rapidly attach to proteins or nucleic acids in the cells (Esterbauer, 1996). MDA detection is used in the thiobarbituric acid-reactive substance (TBARS) assay, but it lacks specificity because it measures MDA equivalents (Halliwell, 2000); moreover, other aldehydes, including 2-alkenals and 2,4-alkadienals, and additional molecules such as sugars, can react with thiobarbituric acid (Esterbauer *et al.*, 1991). Hence, gas chromatography-tandem mass spectrometry (GC-MS/MS) methods have been developed to detect MDA, as well as other oxidative markers (Giera *et al.*, 2012). Overall, any *in vitro* assessment of peroxides before administration of TPN would help to minimize oxidants that are infused into parenterally fed neonates.

Figure 1.3: Lipid peroxidation of unsaturated fatty acids.

Step 1: abstraction of a hydrogen atom by free radicals, and generating carbon-centered lipid radical. **Step 2**: molecular rearrangement to generate a stabilized conjugated diene. **Step 3**: oxygen reacts with unsaturated fatty acid radical to form a lipid peroxyl radical. **Step 4**: the lipid peroxyl radical abstracts H^+ from another source to generate lipid hydroperoxide. **Step 5**: lipid peroxyl radical breaks down to form aldehydes, including malondialdehyde (MDA), 4-hydroxy-2-nonenal (from omega-6 fatty acids) and 4-hydroxy-2-hexanal (omega-3 fatty acids). **Step 6**: lipid hydroperoxides can react with Fe^{2+} via Fenton-type reactions, producing $LO^{\bullet}$ radical.

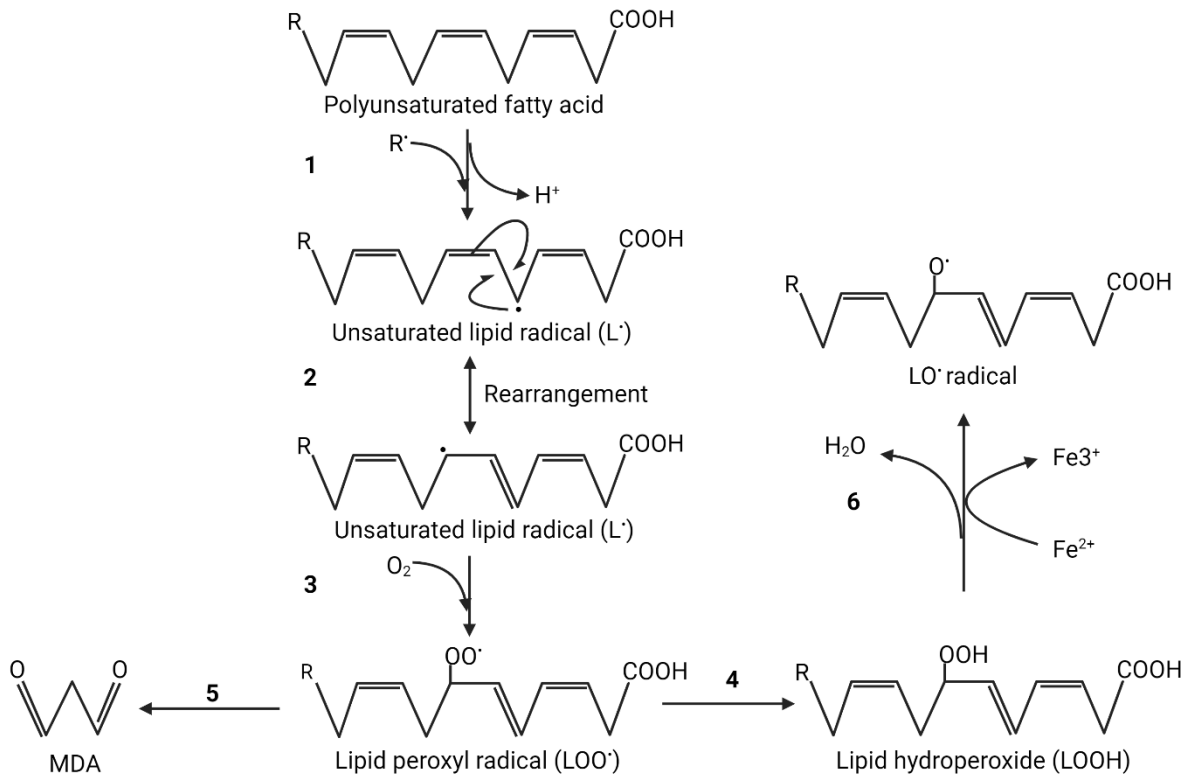
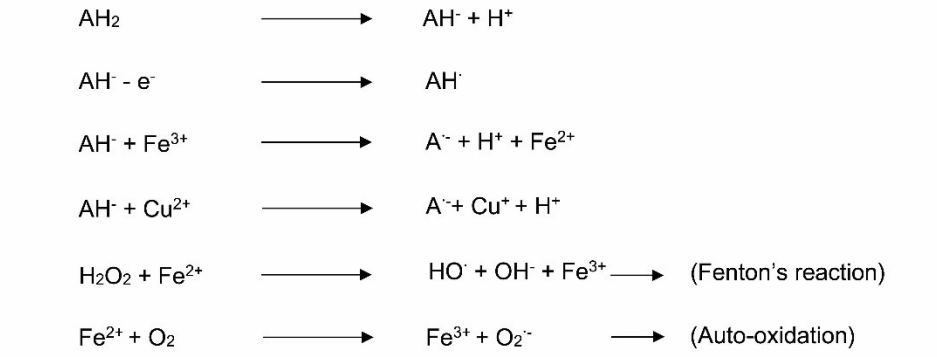

R
COOH
Polyunsaturated fatty acid
1
R·
H+
R
COOH
Unsaturated lipid radical (L·)
2
Rearrangement
R
COOH
Unsaturated lipid radical (L·)
3
O2
R
OO·
COOH
Lipid peroxyl radical (LOO·)
4
R
OOH
COOH
Lipid hydroperoxide (LOOH)
5
O
O
MDA
R
O·
COOH
LO· radical
6
H2O
Fe3+
Fe2+

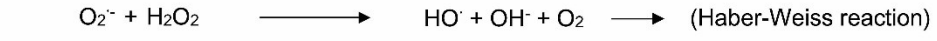
Figure 1.4: The formation of ascorbate radicals, hydroxyl radicals, and superoxide radicals generated from ascorbic acid.

(A) Hydrogen peroxide (H_2O_2) reacts with a ferrous ion and generates a hydroxyl radical according to the Fenton's reaction; **(B)** The formation of a hydroxyl radical via the Haber-Weiss reaction. AH_2: ascorbic acid. AH^-: ascorbate anion. $AH^{\bullet}$: ascorbate radical. $A^{\bullet -}$: dehydroascorbate. $O_2^{\bullet -}$: superoxide radical. $HO^{\bullet}$: hydroxyl radical.

A


AH_2 → $AH^- + H^+$
$AH^- - e^-$ → $AH^{\cdot}$
$AH^- + Fe^{3+}$ → $A^{\cdot -} + H^+ + Fe^{2+}$
$AH^- + Cu^{2+}$ → $A^{\cdot -} + Cu^+ + H^+$
$H_2O_2 + Fe^{2+}$ → $HO^{\cdot} + OH^- + Fe^{3+}$ (Fenton's reaction)
$Fe^{2+} + O_2$ → $Fe^{3+} + O_2^{\cdot -}$ (Auto-oxidation)

B


$O_2^{\cdot -} + H_2O_2$ → $HO^{\cdot} + OH^- + O_2$ (Haber-Weiss reaction)

1.7.2 Role of vitamins in oxidation of TPN solutions

Premature and low birth weight infants require vitamin supplementation to prevent deficiencies because they have low body storage and an accelerated growth (Oliver *et al.*, 2019). Among those vitamins, vitamins E and C are important antioxidants in neonates (Abdul-Razzak *et al.*, 2007). Vitamin E is an extracellular lipid-soluble antioxidant, while vitamin C is an extracellular water-soluble antioxidant (Graham *et al.*, 1998; Kaźmierczak-Barańska *et al.*, 2020). A MVP that contains all lipophilic and hydrophilic vitamins (see *Appendix I*) is added to TPN to carry out vitamins' vital functions, including antioxidant activity. However, the anti-peroxide activity of MVP-supplemented TPN or lipid emulsions is still not clear. Lavoie *et al.* (1997) claimed that six hours of light-protected incubation of a FF-TPN solution and lipid emulsion (Intralipid^{TM}-10%) without admixture of MVP did not significantly generate peroxides, whereas MVP added to FF-TPN solution and lipid emulsion generated a three-fold and a two-fold rise in peroxides, respectively. They also observed that the peroxide formation was further increased when the solutions were exposed to light for 6 hours (Bassiouny *et al.*, 2009). The peroxide activity of MVP can be explained by the presence of polysorbate, riboflavin, and vitamin C.

1.7.2.1 Polysorbate

Polysorbate is added during the preparation of multivitamin mixtures to solubilize both immiscible lipophilic and hydrophilic vitamins in the same medium. Polysorbate gets oxidized when exposed to light, as it contains fatty acid esters of polyoxyethylene sorbitan (Ha *et al.*, 2002). Laborie *et al.* (1998) demonstrated that the peroxide levels were slowly increased when TPN was incubated for 24 hours with polysorbate (polysorbate 20 (1 mg/L) and polysorbate 80

(1.6 mg/L)). However, the formation of peroxides by polysorbates is quite low compared to peroxides generated by other components in the TPN.

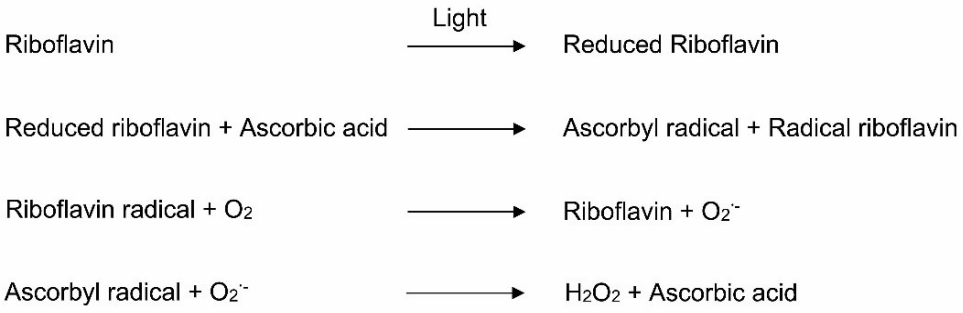
1.7.2.2 Riboflavin

Among the water-soluble vitamins, riboflavin is an important precursor for the synthesis of the biological redox molecules flavin mononucleotide (riboflavin 5'-phosphate) and flavin adenine dinucleotide (adenosine 5'-diphosphate) (Pinto and Zempleni, 2016). The source of riboflavin in MVP is riboflavin 5'-phosphate sodium, which is a highly photosensitive vitamin (Laborie *et al.*, 1998). Riboflavin in MVP-containing TPN catalyzes the oxidation of ascorbate by oxygen to generate peroxides (Kim *et al.*, 1993) (**Figure 1.5**). After exposure to light, riboflavin undergoes intersystem conversion from singlet riboflavin to a strongly oxidizing triplet riboflavin state (Khan *et al.*, 2019). The triplet riboflavin is then reduced by an electron donor, such as ascorbic acid, to generate ascorbyl radical and riboflavin radical (Kim *et al.*, 1993). The reduced riboflavin reacts with O_2 and produces superoxide, while regenerating the riboflavin. Superoxide reacts with ascorbyl radical and H_2O_2 (Kim *et al.*, 1993) (**Figure 1.5**). For instance, Kim *et al.* (1993) reported that, after 24 hours of incubation, hydroperoxide concentrations were significantly increased in TPN after mixing flavin mononucleotide and ascorbic acid. Photoprotection of riboflavin minimizes the generation of peroxides.

1.7.2.3 Vitamin E

Vitamin E has four molecular structures (α, β, γ, and δ) of tocopherols and tocotrienols, which total to eight naturally occurring forms, synthesized by plants from homogentisic acid (Rizvi *et al.*, 2014). Among the different structures, the α-molecular structure has the highest vitamin E activity.

Figure 1.5: Generation of peroxides in the TPN in the presence of riboflavin and vitamin C.


Riboflavin
Light
Reduced Riboflavin
Reduced riboflavin + Ascorbic acid
Ascorbyl radical + Radical riboflavin
Riboflavin radical + O_2
Riboflavin + $O_2^{\cdot-}$
Ascorbyl radical + $O_2^{\cdot-}$
H_2O_2 + Ascorbic acid

The natural form of the α-tocopherol stereoisomer is the RRR-α-tocopherol (D-α-tocopherol), while all-rac-α-tocopheryl acetate (DL-α-tocopherol acetate) is the usual synthetic form. Among those two forms, D-α-tocopherol shows higher bioavailability than the synthetic DL-α-tocopherol acetate (Lauridsen *et al.*, 2002). DL-α-Tocopherol acetate is abundant in new-generation lipid emulsions, including the pure fish oil containing lipid emulsion, Omegaven®. Studies have shown that the bioavailability of α-tocopherol found in those lipid emulsions was high in tissues, compared to γ-tocopherol, the principal form of vitamin E found in soybean lipid emulsions (Kathleen, 2019). Vitamin E is also known to have distinct biological functions, including the activation of bile acid and xenobiotic metabolism.

The biological activity of vitamin E is dependent on vitamin C, vitamin B3, selenium, and glutathione (Rizvi *et al.*, 2014). For instance, tocopherol efficiently prevents lipid peroxidation when another electron donor is present in TPN to reconvert a tocopheryl radical to a non-radical form. Usually, vitamin C acts as an electron donor to the tocopheryl radical to regenerate tocopherol (**Figure 1.1**). For instance, clinical and experimental studies demonstrated that vitamin E supplementation alone does not produce a beneficial effect on oxidative stress leading to atherogenesis, but it works synergistically when co-administered with vitamin C to reduce the oxidative stress effectively (Bowry *et al.*, 1995; Carr *et al.*, 2000). For example, Ng *et al.* (2016) found that vitamin E, in Omegaven® (rich in omega-3 fatty acids)-supplemented TPN or Intralipid™-containing TPN after adding extra vitamin E, prevented the elevation of biliary and lipidemic markers (direct bilirubin, gamma glutamyl transferase, serum triglyceride, low-density lipoprotein, and hepatic triglyceride) of parenteral nutrition-associated liver diseases (PNALD) of preterm piglets. The prevention was believed to be due to the protective mechanism of vitamin E and the presence of other electron donors in the solution. For instance, the inclusion

of vitamin E in newer highly polyunsaturated fish oil-based emulsions that are available in the market (i.e., Omegaven® (fish oils) and SMOFlipid®) has been shown to prevent the oxidation of fatty acids (Burrin *et al.*, 2014). On the other hand, the failure to prevent the cholestasis developed by TPN feeding is explained by presence of high plant sterols as described above and the reaction of α-tocopherol with peroxyl radicals to form α-tocopheroxyl radicals, which leads to further oxidation to α-tocopheryl quinone if lacking electron donors (Muto *et al.*, 2017; Niki, 2007). This clearly shows that a high vitamin E-supplemented diet cannot have an optimal effect unless it is fortified with other nutrients (Karthigesu *et al.*, 2021). Hence, supplementation of vitamin E and omega-3 fatty acids with vitamin C may protect from oxidative stress.

1.7.2.4 Vitamin C

The antioxidant activity of vitamin C is supported by several studies (Dennis and Witting, 2017; Esmaeilizadeh *et al.*, 2019). Beyond its antioxidant properties, vitamin C also has anti-inflammatory and immune-enhancing functions, and it acts as a cofactor for many enzymes, including hydroxylases and oxygenases (Padayatty and Levine, 2016). However, humans and only a few other mammals (e.g., primates, bats, and guinea pigs) require L-ascorbic acid in the diet daily because it cannot be synthesized due to absence of the enzyme L-gulonolactone oxidase (Pohanka *et al.*, 2012). Vitamin C scavenges free radicals, such as hydroxyl radicals, aqueous peroxyl radicals, and superoxide anion, and nonradical species, including singlet oxygen, nitroxide, and peroxynitrite (Carr and Frei, 1999). Vitamin C first releases an electron from ascorbate (AH^-) to form an ascorbyl radical ($A^{\bullet-}$), and then releases a second electron from $A^{\bullet-}$ to produce the diketone moiety of dehydroascorbic acid. Both the ascorbyl radical and dehydroascorbic acid have low reduction potentials (Buettner, 1993). Hence, these two molecules can neutralize most biologically relevant radicals and oxidants. In addition, Buettner

and Jurkiewicz (1996) reported that the ascorbyl radical has minimal reactivity because of its resonance stabilization of the unpaired electron ($k_2 = 2x10^5\ M^{-1}s^{-1}$). Dehydroascorbic acid serves as the strong reducing form of ascorbic acid and defends from oxidation. In addition to the scavenging action of vitamin C, it regenerates other antioxidants, including α-tocopherol and glutathione (Halliwell, 1996). The advantage of vitamin C is that it can be regenerated from the ascorbyl radical and dehydroascorbic acid by enzymatic and non-enzymatic pathways (Carr and Frei, 1999).

In contrast to the above beneficial effects, high doses of vitamin C could exhibit prooxidant effects because the hydroxyl groups of ascorbic acid are reactive, towards singlet oxygen, hydroxyl radicals, hydroperoxide radicals, and H_2O_2. For example, H_2O_2 generated in TPN solutions reacts with dehydroascorbate spontaneously to produce ascorbyl peroxide (Deutsch, 1998; Knafo *et al.*, 2005), which is associated with detrimental health outcomes. In the presence of iron, the ascorbate could reduce Fe^{3+} to Fe^{2+}, subsequently resulting in the generation of an ascorbate radical (Buettner and Jurkiewicz, 1996) (**Figure 1.4 A**). In addition, the electrons from ascorbate can reduce Cu^{2+} to Cu^{+}, and eventually generate superoxide as per the Haber-Weiss reactions, as mentioned above. In newborn guinea pigs, ascorbyl peroxide causes hypo-alveolarization and apoptosis of lung tissue as well as higher glutathione redox potential with increasing ascorbyl peroxide concentration (Elremaly *et al.*, 2014). Mohamed *et al.* (2017) observed that higher urinary ascorbyl peroxide in infants who were TPN fed for seven days.

The stability of ascorbic acid in TPN is also another reason for its efficient protection from oxidants. For example, ascorbic acid is more stable in an acidic pH. The stability of ascorbic acid is altered when the solution is exposed to oxygen, light, high temperature, and prooxidants such as iron and copper. Burge *et al.* (1994) reported that vitamin C measured by the

spectrophotometric method of dinitrophenylhydrazine in a TPN solution containing 10% amino acids, 50% dextrose, and multivitamin (100 mg ascorbic acid) was relatively stable for the first eight hours, and after that, the vitamin C level dropped gradually such that by 32 hours, the concentration of ascorbic acid decreased to 74% of the original concentration. Burge *et al.* (1994) also claimed that the loss of ascorbic acid was higher (60% of the original concentration) when the added trace element solution contained 1.2 mg copper sulfate, compared to TPN without copper. The prooxidant activity of vitamin C can be avoided by maintaining the optimum concentration of vitamin C and providing other electron donors (Burge *et al.*, 1994).

1.8 Role of trace elements in oxidation of TPN solutions

Trace elements are vital micronutrients in TPN. They play an important role in physiological and metabolic functions, including enzymatic reactions. Substantial studies on trace elements in TPN solutions focused on prevention of micronutrient deficiencies (Frankel, 1993; Jin *et al.*, 2017; Stehle *et al.*, 2016) although toxicity is also a concern with intravenous infusion of minerals, which bypasses key excretory regulation mechanisms for many minerals, especially iron, copper, and zinc (Bertolo *et al.*, 2014). However, studies evaluating the oxidative effect of trace elements in TPN are limited. Steger and Mühlebach (2000) reported that peroxide levels were significantly increased when mixing the trace elements (2.79 mg iron, 3.27 mg zinc, 0.27 mg manganese, 0.32 mg copper, 0.026 mg chromium, 0.024 mg selenium, 0.019 mg molybdenum, 0.95 mg fluoride, and 0.13 mg iodine per 10 mL) with TPN and incubated at room temperature for 14 days. This higher peroxide levels could be explained by the presence of prooxidants, such as zinc, iron, copper, and manganese.

1.8.1 Zinc

Zinc is a stable divalent cation (Zn^{2+}), unlike iron (ferric state (Fe^{3+}) or ferrous state (Fe^{2+})) or copper (cuprous state (Cu^{+}) and cupric state (Cu^{2+})). Hence, it does not directly undergo oxido-reduction reactions. The possible reasons for decreasing ROS with zinc are 1) it acts as a cofactor for Cu/Zn-specific superoxide dismutase; 2) it induces metallothionein, which scavenges oxidants; and 3) it protects sulfhydryl groups in proteins from oxidants (Bray and Bettger, 1990). Conversely, an increase in zinc levels is associated with prooxidant effects, explained by the elevation of ROS production. In addition, zinc stimulates the expression of NADPH-oxidase (Nox) (Salazar *et al.*, 2017). Hence, zinc is the portent prooxidant at higher concentrations. ASPEN or European Society for Paediatric Gastroenterology, Hepatology and Nutrition (ESPGHAN)/the European Society for Clinical Nutrition and Metabolism (ESPEN) guidelines on pediatric nutrition reported that giving 400-500 μg/kg/day in preterm infants or 250 μg/kg/day in term newborns zinc is recommended (Domellöf *et al.*, 2018).

1.8.2 Iron

Intravenous administration of iron has been proposed in preterm neonates because the intestinal absorption of iron is poor during the first weeks of life (Meyer *et al.*, 1996; Pollak *et al.*, 2001), however caution is warranted. Free iron stimulates the formation of free radicals, whereas conjugated iron (e.g., iron dextran, iron gluconate) prevents the formation of peroxides in TPN solutions. Conversely, Grand *et al.* (2011) used iron saccharate in TPN, and they reported that the lipid peroxide levels were increased. This is supported by Grand *et al.* (2011) who showed that the concentration of MDA in an all-in-one TPN solution with iron was high, suggesting that the generation of lipid peroxides formed quickly when iron was present in TPN

solutions. Another aspect to consider is that prolonged iron infusion via TPN can lead to dangerously high iron levels, as the body does not have an effective excretory system for absorbed iron (Bertolo *et al.*, 2014); this high iron status could prolong oxidative damage well beyond TPN feeding. Even though iron is a vital element for various functions in newborns, it is free from or released from highly reactive complexes that react with ROS or molecular oxygen, producing more oxygen radicals through the Fenton reaction. Thus, iron is not supplemented with TPN for preterm or term neonates until 28 days after birth. However, the intramuscular or intravenous iron infusion is necessary if the newborn develops anemia or iron deficiency after 28 days (National Guideline Alliance, 2020).

1.8.3 Copper

The vital function of copper includes functional components of several enzymes such as cytochrome oxidase, superoxide dismutase, monoamine oxidase, and lysyl oxidase. Preterm and term newborn babies require 20 and 40 μg/kg/day copper, respectively (Domellöf *et al.*, 2018). Copper in TPN can also further catalyze oxidative reactions by interacting with other nutrients. For instance, ascorbic acid readily induces H_2O_2 generation in the presence of Cu^{2+} (Jansson *et al.*, 2005). Though the iron is a highly reactive metal, also being a strong biological oxidant and a reducing agent, the prooxidant activity of copper is more pronounced than iron (Jansson *et al.*, 2005).

1.8.4 Manganese

Manganese is added to the TPN at a dose of 1 μg/kg/day (Domellöf *et al.*, 2018). Even though manganese is a cofactor for several enzymes, including mitochondrial superoxide dismutase, it stimulates the generation of ROS (HaMai and Bondy, 2004). Moreover, manganese

administration via TPN coupled with decreased biliary manganese excretion, secondary to cholestasis, induces manganese intoxication (HaMai and Bondy, 2004). However, the prooxidant activity of zinc and manganese has been highly contentious. It has been suggested that they may have similar effects as iron and copper on generation of peroxides, but less is known about those interactions.

1.8.5 Selenium, chromium, and iodine

Other trace minerals found in the neonatal TPN are selenium, chromium, and iodine. Selenium is an essential component of selenoenzymes such as GPx, thioredoxin reductase, and selenoprotein P (Tindell and Tipple, 2018). Among those enzymes, GPx involves in antioxidation activity. It also plays a role in the enzymatic conversion of thyroxine to its more active metabolite, triiodothyronine, and serves as a cofactor for protein and DNA synthesis. Animal studies have shown that selenium acts in a protective role in oxidative stress (Bhandari *et al.*, 2000; Kim *et al.*, 1992; Lavoie *et al.*, 1999). No studies have documented any adverse effects related to selenium supplementation in preterm infants (Tindell and Tipple, 2018). ASPEN and ESPEN recommended to provide a dose of 2 μg/kg/day selenium to preterm and a maximum of 7 μg/day to newborns (ASPEN, 2019a; Domellöf *et al.*, 2018).

Chromium is a vital element that is required for carbohydrate metabolism. A daily intravenous intake of 0.2 μg/kg/day with a maximum of 5 μg/day of chromium has been recommended for infants on TPN by ASPEN and ESPGHAN/ESPEN (Domellöf *et al.*, 2018). Hence, the dose rate of such trace elements should be carefully considered, and it should be within the range of no deficient, toxic or prooxidant levels.

Iodine is a vital element for neonates for the synthesis of thyroid hormones, which are crucial for the development of the central nervous system (Kanike *et al.*, 2020). Maternal iodine deficiency during the early postnatal period causes iodine deficiency in neonates. One of the causes of neonatal hypothyroidism is iodine deficiency. A dose of at least 1 μg/kg/day is required with TPN (Domellöf *et al.*, 2018). Few studies have addressed iodine toxicity in neonates. For example, iodine toxicity in newborns can develop from exposure to iodine-rich skin preparations, consumption of breast milk from a lactating mother who has high iodine exposure, or direct ingestion (Aitken and Williams, 2014; Chung *et al.*, 2009).

1.9 Parenteral nutrition and environmental conditions

In addition to the nutrient composition of TPN, its preparation and storage environment can also contribute to the oxidation of its components. As briefly discussed above, light exposure is a key factor that can increase oxidant levels, but TPN can also generate additional oxidants when exposed to oxygen or air during preparation, storage, and infusion at bedside. Moreover, temperature in the NICU can induce the formation of oxidative molecules. It is essential to minimize these conditions to limit the production of oxidants in TPN.

1.9.1 TPN exposure to light

As briefly mentioned, the exposure of TPN to ambient light, day light or to light during phototherapy of a neonate induces the generation of peroxides. Indeed, the oxidation of lipid emulsions is particularly immense when exposed to ambient light or phototherapy in a clinical setting. In particular, premature babies in the NICU often require TPN in the first weeks of life or longer, when they are voluntarily exposed to natural or artificial light to prevent neonatal jaundice (Mehta *et al.*, 2017). Laborie *et al.* (2000a) found that the peroxide concentration in

light-exposed TPN was between 190 and 300 μM, compared to 60 and 130 μM when TPN was protected from light. Some of the identified peroxides generated *in vitro* from light exposure are 4-hydroxy-2-nonenal, MDA (Grand *et al.*, 2011; Picaud *et al.*, 2004) and 4-hydroxy-2-hexenal, in the lipid emulsions (Miloudi *et al.*, 2012). Moreover, many light-induced reactive oxidative species can interfere with endogenous NO levels, resulting in increased vasoconstriction and exacerbating physiological effects of TPN feeding.

FF-TPN, including amino acids and vitamin mixtures, can also be contaminated with H_2O_2 after mixing with light-exposed riboflavin (Brawley *et al.*, 1993; Shattuck *et al.*, 1995). In another study, a light-exposed mixture containing 10% dextrose, amino acids, and electrolytes generated 25 μM peroxides, but photo-protection over six hours at room temperature did not result in peroxide production (Lavoie and Chessex, 2019). Interestingly, the concentration of peroxides jumped three-fold (75 μM) after adding a lipid emulsion. Notably, the addition of 1% MVP induced the generation of peroxides to 350 μM, even after two hours incubation; however, the concentration of peroxides dropped to 250 μM when protecting the solution from light (Lavoie and Chessex, 2019). So, the generation of peroxides in TPN is dependent on numerous combinations of nutrients, but light protection is critical to minimize many of these interactions.

The protection of TPN from photooxidation in a clinical setting is challenging to achieve (Lavoie *et al.*, 1997) because it is difficult to protect the TPN bag and its connected tubing efficiently. However, some studies have demonstrated effective photo-protection by covering it with aluminum foil or opaque plastic polythene. For instance, Laborie *et al.* (1998) examined the peroxide levels in the TPN bag, which was shielded from light using a black garbage bag, and by using different colored tubing including orange, yellow, and black. They observed that the yellow tubing is as effective as a completely opaque black tube or tube covered with aluminum foil in

preventing further oxidation when exposed to light. Indeed, the yellow tube is more suitable to see air bubbles, or precipitation than the black tube (Laborie *et al.*, 1998). Moreover, the protection from light during product preparation must also be considered. The generation of free radicals in any solution can be lowered simply by shielding TPN from light during preparation (Chessex *et al.*, 2017), although how practical that is in a manufacturing plant or hospital pharmacy needs to be evaluated.

1.9.2 TPN exposure to oxygen

A TPN solution exposed to oxygen or air during its preparation and infusion to a neonate gains oxidant molecules at each step. Oxygen is the basis of the aerobic life of living organisms, but it is frequently reactive by itself (Vivekanandan-Giri *et al.*, 2011). It reacts with an electron from a donor, such as a monounsaturated or polyunsaturated fatty acid, certain amino acid residues such as tyrosinyl- or tryptophanyl-residues, or vitamin C, to generate a superoxide anion. Laborie *et al.,* (2000b) demonstrated that the removal of oxygen by nitrogen from a test solution of neonatal TPN inhibited the generation of peroxides, but they also observed that the effect of the oxygen washout was lost when the solution was subsequently infused via an intravenous infusion set. The removal of oxygen from preparation to infusion would be a difficult task. For example, even if all the air was removed from TPN bags, the effect to minimize the peroxides is lost because the chance of oxygenation is high when administering the TPN to neonates (Hoff and Michaelson, 2009). A recent study has shown that oxygen levels present in the mitochondria of platelet induced DNA damage in patients receiving parenteral nutrition (Dąbrowska *et al.*, 2023). Interestingly, the molecular oxygen level was high in patients with TPN compared to controls (Dąbrowska *et al.*, 2023).

1.9.3 Effect of storage on oxidants in TPN solutions

Within TPN, amino acids are typically stable for about four months at 2-8 °C, except for cysteine, which slowly dimerizes to yield cystine, giving a yellow discoloration of parenteral solutions with storage time (Yailian *et al.*, 2019). Pitkäken (1992) reported that lipid degradation and the production of oxidants were high during the storage of lipid emulsions. They determined that levels of pentane, generated from peroxidation of omega-6 fatty acids (Pincemail *et al.*, 1990), were significantly higher after six months of storage after adding Intralipid^{TM} to phosphate-buffered saline (Pitkäken, 1992). They also found that the infusion of lipid emulsions increased the concentration of exhaled pentane during the first week of life in premature infants, suggesting significant peroxidation of lipid in TPN during storage. Intravenous infusion of TPN contaminated with oxidants *in vitro* can overwhelm the antioxidant capacity of neonates and cause adverse effects.

1.10 *In vivo* impact of oxidized TPN after infusion

Inhaled or generated oxygen via metabolic processes is the final acceptor of highly energized electrons generated through xanthine oxidoreductase or Nox, NO synthase (NOS), and the mitochondrial oxidative phosphorylation process. Under normal physiological circumstances, approximately 5% of the total oxygen metabolized is not reduced, leading to the generation of ROS (Buonocore *et al.*, 2010). The ROS in the reduction process uses one electron to form a superoxide anion ($O_2^{\bullet-}$), whereas the reduction of oxygen with two or three electrons generates H_2O_2 or a hydroxyl radical ($^{\bullet}OH$), respectively. Even though the half-life of ROS is short, it quickly reacts with biomolecules such as proteins, DNA, RNA, glucose or free fatty acids, thus altering their structure and function (Kalyanaraman, 2013; Maltepe and Saugstad, 2009; Wu *et*

al., 2015). Eventually, damaged biomolecules dysregulate the cellular environment, which can lead to inflammation, cell death, and tissue injury (Lunec, 1990; Vitetta and Linnane, 2014).

TPN is linked to harmful complications after prolonged administration (Calkins *et al.*, 2014). For instance, in *in vivo*, oxidants present in the TPN can diffuse into tissues, generating reactive free radicals that can further react with metal ions and produce unstable molecules in the presence of oxygen. Numerous *in vivo* studies have been carried out to examine the effect of oxidant molecules from contaminated TPN after infusion. For instance, Chessex *et al.* (2005) reported that light-exposed MVP-supplemented TPN was associated with an elevated urinary excretion of nitrogen (by 40%) compared to control TPN. Another study reported that guinea pigs on light-exposed TPN had more hepatic steatosis, higher liver weight, and elevated isoprostane $F_{2\alpha}$ concentrations compared to animals fed light-protected TPN (Chessex *et al.*, 2002; Hoff and Michaelson, 2009). In addition, metabolic complications, including glucose intolerance, may arise due to extensive use of TPN (Calkins *et al.*, 2014). However, the most established complications due to prolonged TPN feeding are PNALD, gut atrophy, and BPD.

1.10.1 Parenteral nutrition, oxidant load and liver diseases

PNALD is defined as a heterogeneous injury of the liver, characterized by cholestasis, steatosis, and, eventually, fibrosis and cirrhosis (Ng *et al.*, 2016; Żalikowska-Gardocka and Przybyłkowski, 2020). Several animal experiments in piglet, guinea pig, and rat models have demonstrated that a continuous infusion of oxidized TPN solution for some days affects hepatobiliary function. Bhatia *et al.* (1993) claimed that 10 days of infusion of light-exposed TPN to rats leads to hepatobiliary disease. Morin *et al.* (2019) also observed liver steatosis after five days of TPN in one-month-old guinea pigs. TPN feeding for seven days to male one-month

old Sprague-Dawley rats also led to development of liver diseases (Wang *et al.*, 2021). And in humans, newborn infants (> 1000 g) who received TPN for a longer period (> 7 days) developed PNALD (Koseesirikul *et al.*, 2012). Although TPN-induced PNALD is a well-established consequence of intravenous feeding, its etiology is still unclear.

The reasons for the development of PNALD in neonates may be due to composition of omega-3 and omega-6 fatty acids, amount of phytosterols, parenteral lipid load, and different composition of non-lipid nutrients in TPN (Burrin *et al.*, 2014; Nandivada *et al.*, 2013; Waitzberg and Torrinhas, 2009). For example, soy-based parenteral lipid emulsions, containing high concentrations of phytosterol and omega-6 fatty acids, have become known as risk factors for cholestasis and hepatocellular damage as mentioned above (Muto *et al.*, 2017). This association manifests as a significant association between the accumulation of circulating phytosterols and the elevation of liver enzymes in neonates (Kurvinen *et al.*, 2012). The accumulation of phytosterols also leads to higher bile acid secretion and causes cholestasis in TPN-fed piglets (Ng *et al.*, 2016). In fact, the long-chain polyunsaturated fatty acids are more prone to damage by peroxidation, resulting in free radical peroxide production, which can contribute to the liver injury detected in PNALD (Biesalski, 2009; Nandivada *et al.*, 2013). Lipid emulsions with vitamin E undoubtedly reduce the risk of peroxidation due to their antioxidant capacity, and protects lipid membranes from oxidation (Diamond *et al.*, 2011). Omegaven®, a lipid emulsion containing purely fish oil, also has hepatoprotective effects (Le *et al.*, 2011; Premkumar *et al.*, 2014), which may be due to its rich content in vitamin E and/or the lack of phytosterols. In addition to the above number of etiologies hypothesized to explain PNALD, the lack of enteral feeding is also one of the key reasons for PNALD, because it leads to gut atrophy and disruption of the enterohepatic circulation of bile acids (Guzman *et al.*, 2020; Jain *et al.*, 2012). Hence, liver

disease may also be due to intestinal failure, as result of exclusive parenteral nutrition feeding, which is referred to as intestinal failure-associated liver disease (IFALD) (Pironi and Sasdelli, 2019; Rochling and Catron, 2019). Because development of PNALD in neonates is multifactorial, the term intestinal failure-associated liver disease is preferred to explain liver disease due specifically to intestinal failure as a result of lack of enteral feeding (Lacaille *et al.*, 2015).

The absence of enteral feeding prevents the stimulation of receptors, hormones, and growth factors. It also blocks the normal gut-liver cross talk by reducing the downstream signaling to the liver via the portal circulation (Madnawat *et al.*, 2020). For example, farnesoid X receptor (FXR), a ligand-activated transcription factor is expressed in the terminal ileum and is regulated by bile acids. Lack of enterohepatic circulation suppresses the FXR and reduces hepatic bile acid production by modulating cholesterol 7-α-hydroxylase (CYP7A1). Reduced FXR expression subsequently reduces the activation of fibroblast growth factor 19 (FGF19), which reduces protein synthesis in the liver and may exacerbate liver injury due to PNALD (Kir *et al.*, 2011). Bile acid absorption in the ileum during enterohepatic circulation is linked with the stimulation of FXR (Inagaki *et al.*, 2005). Thus, the enteral administration of chenodeoxycholic acid can serve as a ligand for FXR, thus preventing hepatic injury (Jain *et al.*, 2012). The gut microbiota also performs a vital function in the health of infants. It has been suggested that administration of TPN alters the composition of gut microbiota because of the lack of enteral feeding and starvation of bacteria, which leads to bacterial translocation. Exclusive TPN can also encourage the growth of gram-negative, endotoxin producing bacteria, which can exacerbate systemic bacterial infection. These events can result in the suppression of bile acid transporters and eventually hepatic injury by endotoxin- and cytokine-mediated suppression (Madnawat *et*

al., 2020). Although the mechanisms are complex, lack of enteral stimulation during TPN feeding can lead to impaired enterohepatic metabolism of bile acids, leading to profound liver injury and potentially life-threatening sepsis.

1.10.2 Parenteral nutrition and gut atrophy

In growing neonates, exclusive TPN leads to significant functional and morphological gut atrophy; however, the mechanisms and consequences of atrophy are poorly understood. The atrophied gut leads to a reduction of intestinal metabolic capacity, diminished absorptive capacity, and compromised de novo synthesis of many nutrients, including polyamines and amino acids such as arginine (Bertolo *et al.*, 2003). Moreover, prolonged gut atrophy also leads to intolerance to reintroduction of oral feeds and complicates the transition from parenteral to enteral feeding. Niinikoski *et al.* (2004) reported that the gut atrophy was a direct result of a parenteral feeding-induced rapid suppression of blood flow in the superior mesenteric artery (by 30% in under eight hours), which preceded small intestinal tissue atrophy and lowered protein synthesis. As a result, clinical practice during TPN feeding often includes minimal enteral nutrition, which involves small volumes of oral feeding to stimulate gastrointestinal function and growth to prevent atrophy. The mechanism by which enteral stimulation improves enteral feeding tolerance is still unclear, but the primary physiological outcome necessary for improving gut atrophy and recovery during TPN is improved mesenteric blood flow (Niinikoski *et al.*, 2004). Previously reported work had shown that mesenteric blood flow can predict the early feeding tolerance of preterm infants (Fang *et al.*, 2001; Pezzati *et al.*, 2004). Notably, small intestinal blood flow is regulated by NO, which is synthesized from arginine. In neonates, because arginine synthesis depends on small intestinal metabolism (Bertolo *et al.*, 2003), gut atrophy exacerbates arginine availability and NO synthesis, further reducing intestinal blood

flow. Hence, arginine-supplemented TPN can help maintain the integrity of the small intestine through increased rate of protein synthesis and migration of enterocytes and serves as a precursor of NO synthesis (Dinesh *et al.*, 2014).

As already discussed, NO availability is also sensitive to oxidative stress. Huber *et al.* (2019) conducted a study to examine the effects of light-protection of TPN and of N-acetyl cysteine (NAC), the limiting amino acid for the synthesis of glutathione (i.e., the primary intracellular antioxidant), on the superior mesenteric artery blood flow, gut morphology, and oxidative status of piglets. They found that the superior mesenteric artery blood flow rate declined over six days for all treatment groups (light-protected TPN, light-protected NAC-enriched TPN, light-exposed TPN, and light-exposed NAC-enriched TPN), consistent with previously observed effects of TPN (Niinikoski *et al.*, 2004). However, by day six of TPN feeding, the light-protected TPN group showed only a 34% reduction of blood flow from baseline, which was significantly better than the 45 to 63% reduction of blood flow in the other groups. They concluded that the photoprotection of the TPN solution effectively ameliorated the TPN-associated drop of the superior mesenteric artery blood flow. However, NAC supplementation surprisingly offset this amelioration. They also observed a 25% reduction in hepatic lipid peroxidation when TPN was protected from light. Therefore, TPN redox status can affect functional outcomes in neonatal gut and minimizing *in vitro* oxidation in TPN would have clinical impacts.

The mechanisms behind lack of enteral stimulation and gut atrophy may also involve enterohepatic pathways. For example, some animal studies have shown that the protein-coupled bile acid-activated receptor, also called Takeda G protein-coupled receptor (TGR5), rich in the crypts of the intestine, is involved in regulation of gut atrophy (Burrin *et al.*, 2013; Pathak *et al.*,

2018; Ticho *et al.*, 2019). Expression of TGR5 is regulated by primary and secondary bile acids (Keitel *et al.*, 2019). Jain *et al.* (2016) observed that the administration of an agonist of TGR5, namely oleanolic acid, which stimulates TGR5 expression, reduces villous atrophy by increasing the villous height/crypt depth ratio of TPN-fed piglets, and doubling small intestinal weight. Guzman *et al.* (2020) also reported that TPN feeding for 14 days to piglets resulted in a significant elevation of serum bilirubin (a biomarker of cholestatic liver injury), serum bile acids, bile acid deposition within intraparenchymal cells, and an increased hepatic cholestasis score, compared to enterally fed piglets. They also showed significant villous atrophy and reduction of the thickness of muscularis mucosa with TPN feeding. Molecular studies on gut-systemic signaling regulators revealed that TPN-fed piglets had a downregulation of liver FXR expression, liver constitutive androstane receptor (CAR), gut FXR, G-coupled bile acid receptor, epidermal growth factor (EGF), organic anion transporter (OAT), mitogen-activated protein kinase-1 (MAPK1), and sodium glucose-linked transporter (SGLT-1), compared to enteral fed piglets (Guzman *et al.*, 2020). These studies together suggest that gut atrophy could be due to reduced blood supply as well as interruption of hepatobiliary circulation.

1.10.3 Parenteral nutrition and BPD

BPD is a chronic pulmonary disease of preterm neonates. The etiology of BPD is multifactorial, including ventilatory injury, prenatal inflammation and infection, and hyperoxia. Oxidative stress is one of the important factors associated with BPD in neonates (Mohamed *et al.*, 2017). Infants who excreted higher concentrations of urinary ascorbyl peroxides were significantly more likely to develop BPD (Mohamed *et al.*, 2017). A guinea pig model demonstrated that the oxidant molecules in TPN cause adverse effects on biochemical and histological parameters in the lungs (Elremaly *et al.*, 2014). Indeed, low birth weight infants fed

TPN contaminated with ascorbyl peroxides were more likely to develop BPD (Mohamed *et al.*, 2017). Lavoie *et al.* (2002) reported that the peroxides from TPN caused increased collagen deposition in the alveoli and increased gene expression of procollagen mRNA. Animals infused with MVP or TPN had reduced alveolarization, even when these solutions were adequately light-protected (Lavoie *et al.*, 2004). Adding the antioxidant glutathione to TPN may have beneficial effects on lung health via the reduction of apoptosis, maintenance of redox potential, and elevation of alveolarization index of lung tissue (Elremaly *et al.*, 2015; Morin *et al.*, 2019). From these animal and clinical studies, it is clear that oxidant load is a key predictor of lung function in rapidly developing neonates.

1.10.4 Biomolecules in the body that could be affected by oxidized TPN

1.10.4.1 Lipids, proteins, and DNA

Generally, outcomes of oxidative stress occur as a result of an imbalance between prooxidant and antioxidant levels. An infusion of hydroxyl radicals and hydroperoxyl radical-contaminated TPN attacks cell membrane lipids. Indeed, reactive hydroperoxides further react with polyunsaturated fatty acids in cell membranes and produce lipid peroxyl radicals and eventually lipid peroxides, through three steps: initiation, propagation, and termination (Ayala *et al.*, 2014) (**Figure 1.3**). Brain tissue is extremely susceptible to oxidative injury because it consumes a relatively high amount of oxygen compared to other tissues (Shichiri, 2014), and it has an abundant amount of polyunsaturated fatty acids. For instance, neuroprostane, which is produced from the free radical-mediated oxidation of DHA in nervous tissue, is highly concentrated in the neuronal membrane. Cholesterol and linoleate are abundant lipids *in vivo*, and their free radical-mediated oxidation yields 7-hydroperoxycholesterol (7-OOHCh) and

hydroperoxyl octadecadienoate (HPODE), respectively. The aldehydes in TPN, including 4-hydroxy-2-nonenal are highly reactive, and they damage proteins and DNA to produce carbonylated proteins and 8-hydroxy-2'-deoxyguanosine, respectively (Perrone *et al.*, 2019). Similarly, oxidative lipid products in the circulation can cause the oxidation of low-density lipoproteins, which ultimately leads to cardiovascular diseases (Heinecke, 2006). The research on oxidized lipoprotein levels in infants receiving TPN are limited, but likely play a key role in metabolic perturbations, which may have long term consequences.

1.10.4.2 Endoplasmic reticular stress

The endoplasmic reticulum (ER) is an important cellular organelle, which is responsible for protein folding and trafficking in eukaryotes. Hence, it serves a vital role in protein biosynthesis and post-translational modification. Recent studies have confirmed that the association of ER protein folding pathways and ROS are highly correlated (Bhattarai *et al.*, 2021; Plaisance *et al.*, 2016). The protein biosynthesis and modification processes are significantly affected under oxidative stress, resulting in the generation of misfolded proteins. The accumulation of misfolded proteins leads to ER stress (Chong *et al.*, 2017).

Upon ER stress, a homeostatic signaling network, the unfolded protein response (UPR) is activated due to the adaptive mechanisms. The UPR restores the ER function via multiple mechanisms, such as decreasing protein synthesis, increasing protein folding mechanisms, and removing terminally misfolded proteins via initiating UPR signal transducers of ER-associated degradation (ERAD) (Hetz, 2012). When cells undergo irreversible ER stress, the UPR signaling pathway removes stressed cells by apoptosis.

The UPR signalling pathway is mediated by the activation of three main ER transmembrane-associated stress sensors, such as inositol requiring kinase 1 (IRE1), protein kinase-like ER Kinase (PERK), and activating transcription factor 6 (ATF6) (Ron and Walter, 2007). ER stress triggers the dimerization of IRE1α, and phosphorylation induces the formation of spliced X box-binding protein 1 (XBP1s), which controls ERAD, protein quality control, and phospholipid synthesis. PERK phosphorylates the eukaryotic translation initiator factor 2α (eIF2α), signalling the formation of ATF4 that controls apoptosis. ATF6 is responsible for releasing its cytosolic domain fragment (ATF6f), which controls the upregulation of genes encoding ERAD and XBP1 (Grootjans *et al.*, 2016). In addition, during ER stress, ER resident chaperones and foldases are increased due to ER stress, inducing the folding ability of the ER, and eventually eliminate the unfolded protein burden (Read and Schröder, 2021).

1.10.4.2.1 Glucose regulated protein 78 (GRP78)

GRP78 or immunoglobulin heavy chain binding protein (BiP), a major ER-resident chaperone, is a crucial component of the UPR process. It is located on the ER membrane of eukaryotes and is essential for the quality control of protein biosynthesis, including protein folding and assembly. Equilibrium is disrupted due to the accumulation of unfolded proteins, which sense the GRP78 dissociation from the ER stress sensors, resulting in high expression of GRP78. The molecular structure of GRP78 indicates the property of its soluble nature of protein with a small number of hydrophobic units. These hydrophobic units are responsible for detecting unfolded proteins, which are refolded back to normal or degraded by GRP78 (Ibrahim *et al.*, 2019). In addition, release of GRP78 due to increased unfolded proteins also activates the UPR signalling pathway through IRE1α, leading to a decrease in protein synthesis (Read and Schröder, 2021; Ron and Walter, 2007).

1.10.4.2.2 The C/EBP homologous protein (CHOP)

In response to ER stress, phosphorylation of eIF2α reduces protein biosynthesis, which serves to decrease the ER protein-folding load and simultaneously preferentially increase translation of adaptive response mRNAs, including the specific transcripts, such as ATF4, to restore ER homeostasis. Subsequently, ATF4 triggers the transcription of CHOP. *In vitro* studies have shown that CHOP is a master regulator of ER stress-induced apoptosis (Cao and Kaufman, 2014). Hence, expression of CHOP is observed in response to the ER stress-induced apoptosis pathway of PERK/activating transcription factor 4/C/EBP-homologous protein (PERK/ATF4/CHOP) pathway, which then stimulates apoptotic proteins, including Noxa, caspase, Bax, Puma, and Bim (Iurlaro and Muñoz-Pinedo, 2016). Akhter *et al.* (2023) found that the expression of *DDIP3* (that encodes for CHOP) in THP-1 macrophages was significantly increased in response to lipid treatment.

In summary, many of the clinical complications in TPN-fed neonates can be related to oxidative stress in early life. Newborns have an immature and inadequate antioxidant system to neutralize the oxidants generated in TPN, and administered via intravenous feeding regimens. Moreover, typical TPN solutions contain high concentrations of prooxidant nutrients, with few antioxidants that could limit oxidant formation. Furthermore, typical environmental conditions can induce the formation of oxidants in TPN, including light exposure and/or clinical phototherapy while receiving TPN, exposure to oxygen during preparation and infusion of TPN, and inadequate long-term storage conditions leading to instability of nutrients. Major peroxides found in the TPN are hydroxyl and hydroperoxyl radicals, MDA, F_2-isoprostane, and 4-hydroxy-2-nonenal. Infants exposed to contaminated TPN for an extended period of time develop clinical complications, including PNALD, BPD, hepatobiliary dysfunction, and gut atrophy. Moreover,

the oxidation of amino acids limits some essential amino acids for vital functions, including NO synthesis, which is required to maintain blood flow to compromised organs. Evidence from various animal and clinical studies recommends the supplementation of antioxidants, and the reduction of prooxidants in TPN, as well as the optimization of environmental conditions to limit oxidant formation during TPN manufacture and delivery. These modifications also need to consider the dietary adequacy of the nutrients for TPN-fed neonates. The evidence suggests that some potentially beneficial modifications include higher antioxidant levels and reduction of zinc and copper concentrations, and protection from light; such modifications to optimize TPN to minimize oxidant load also need to be systematically tested in a clinical setting.

1.11 Rationale

TPN is an essential medical therapy for preterm or term neonates who cannot tolerate enteral feeding or absorb the required nutrients through their gastrointestinal tract. While it is a lifesaving solution in clinical settings, TPN administration can cause several complications (see *section 1.5*). One of the potential causes for the complications is the harmful oxidant species generated in the TPN bag or the further reaction of such infused oxidants with *in vivo* biomolecules (Karthigesu *et al.*, 2021; Lavoie and Chessex, 2019). Numerous studies have suggested that the ingredients in the TPN bag react with each other and generate oxidants (Bassiouny *et al.*, 2009; Laborie *et al.*, 1998; Lavoie *et al.*, 1997). Furthermore, the formations of oxidants are accelerated due to favourable conditions such as exposure to oxygen and light, and storage conditions (**Figure 1.6**).

Newborns, especially premature babies, may require oxygenation to overcome compromised lung function, and babies with neonatal jaundice often need photography. Notably,

preparation of TPN at the pharmacy, transportation to the NICU or light intensity in the NICU can cause potential light exposure. Hence, newborns are more vulnerable to exogenous oxidants. Several studies have indicated that the low light intensity in the NICU is crucial for preventing the oxidation of TPN. However, sufficient brightness is necessary for both neonates and working staff at the NICU. Indeed, light plays a crucial role in the post-natal development of vision, including visual processes and the maturation of the visual cortex of babies and regulates circadian function. It also influences the performance of doctors and nurses (Bullough and Rea, 1996; Rea and Figueiro, 2016; Rodríguez *et al.*, 2016).

Protecting bags, tubes, or syringes from light has several disadvantages. For instance, air bubbles, contamination, color change, coagulations, and emptiness of the bag may not be noticeable. This can pose a challenge for pharmacists and clinicians in addressing such issues by protecting medical equipment.

Moreover, premature or newborn babies have immature enzymatic and non-enzymatic antioxidant systems to counteract overwhelming oxidant exposure. In addition, precursors for the synthesis of endogenous antioxidants could be limited (see *section 1.6*) (Lembo *et al.*, 2021). Several studies have shown that adding antioxidant vitamins to the TPN may improve the antioxidant status of newborns and eventually reduce complication (Ng *et al.*, 2016). However, the effectiveness of adding antioxidants to the TPN is still not well-proven.

Moreover, the levels of vitamins C and E for TPN are a significant concern as they can act as prooxidants at high concentrations. Certain trace elements, such as zinc and copper, can interact with other reactive components in TPN, leading to the generation of oxidants. Therefore, it is imperative that the levels of antioxidants and prooxidants be determined to minimize the

overall oxidant burden on the neonates. So far, no studies or guidelines have been well established on the recommended daily allowance of nutrient mixture for intravenous administration to preterm or term neonates.

The interconnection between oxidative stress in early life and metabolic syndrome in adult life, such as obesity, diabetes, hypertension, cancer, and dyslipidemia, is well documented (Masenga *et al.*, 2023). Indeed, exposure to free radicals in early life, especially in fetal life, is associated with several adverse effects. For instance, it is also intensely involved in the fetal programming of adult diseases through gene expression (Perrone *et al.*, 2016). TPN given to newborns represents a source of peroxides in early life, which may irreversibly alter cellular mechanisms (Kleiber *et al.*, 2010). When growing newborns are exposed to such oxidants, their metabolic systems adopt programming through epigenetic changes or down-regulation/alteration of endocrine, metabolic, and organ function, which could create adverse complications in later life (Mandy and Nyirenda, 2018). Hence, TPN feeding is a challenging therapeutic solution for patients nowadays. However, optimizing the TPN by minimizing oxidants would provide a beneficial effect.

1.12 Study hypothesis

This investigation hypothesizes that light and air exposure will increase the peroxidation in FF-TPN, SMOFlipid®, and AIO-TPN, and increasing levels of antioxidant vitamins (C and E), adding glutathione and selenium, and decreasing levels of prooxidants (copper and zinc) will optimize AIO-TPN solutions by reducing the oxidant load in the light-exposed or light-protected TPN solutions, and eventually testing these solutions in a cell model to assess the oxidative and

antioxidant status compared to the control TPN solution that is the standard TPN solution routinely used for neonates in NICU.

1.13 Study objective

1.13.1 General objective

The overall objective of the study was to assess the peroxidation of the commercially available AIO-TPN for newborns with increasing levels of antioxidants and decreasing levels of prooxidants and optimizing the environmental conditions, and to subsequently develop a new admixture to be tested in a cell model, and eventually in neonates.

1.13.2 Specific objectives

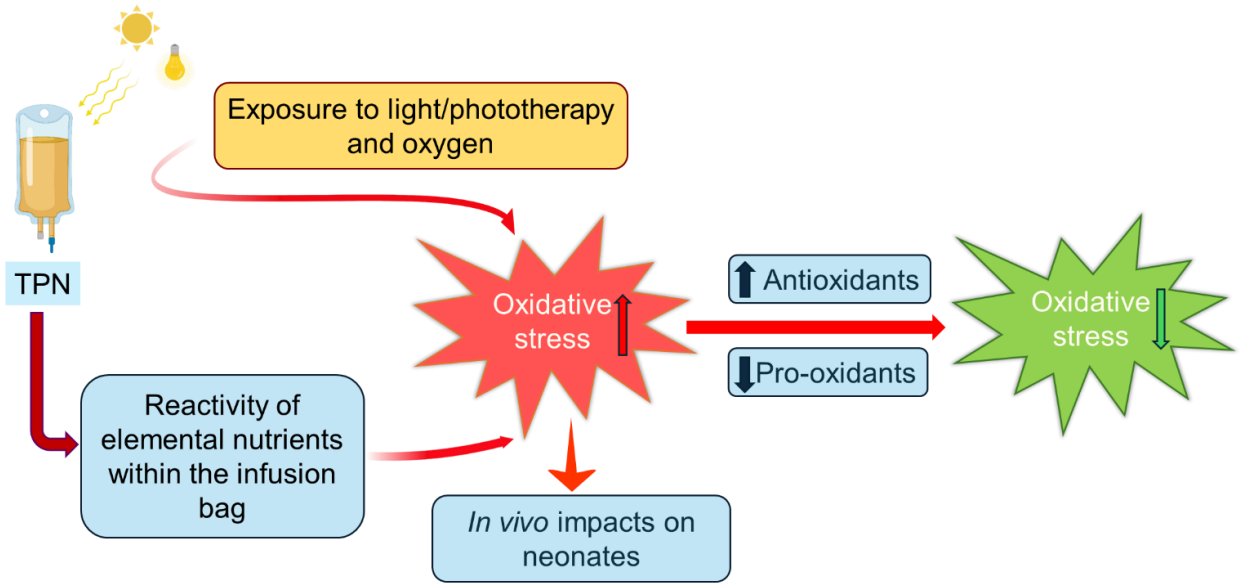
i. To evaluate the peroxidation of FF-TPN, SMOFlipid®, and AIO-TPN with or without light or air/$N_{2(g)}$ exposure.

ii. To determine the optimal concentration of vitamins C and E, zinc, copper, selenium, and glutathione added TPN that will minimize the *in vitro* generation of oxidants.

iii. To determine the ROS, TBARS, ferric reducing antioxidant power (FRAP), GSH/GSSG, cellular antioxidant activity (CAA), and ER stress of optimized TPN treated to human THP-1 monocytic cells and oxidized fatty acid levels in the TPN using LC-MS/MS.

Figure 1.6: Diagrammatic illustration depicting the generation of oxidative stress due to total parenteral nutrition (TPN) for neonates.

The process begins with oxygen permeation, light exposure or interaction between nutrients in TPN solution, leading to chemical reactions that form reactive oxygen species (ROS) and free radicals. This process leads to oxidative stress and potential complications for neonates. The illustration also highlights the role of antioxidants, including vitamins C and E, in neutralizing such free radicals and reducing oxidative damage in newborns.



CHAPTER 2: MATERIALS AND METHODS

2.1 Preparation of total parenteral nutrition (TPN) solutions

The materials used to prepare the TPN solutions were purchased from the Janeway Children's Hospital pharmacy (St. John's, NL, Canada). Briefly, 70% *w/v* dextrose, 10% *w/v* amino acids, Multi-12/K_1® Pediatric vitamins, MICRO+®6 pediatric trace elements, 1,000 units/mL heparin sodium, 20% magnesium sulfate (200 mg/mL), 32.8% sodium acetate, potassium phosphate (phosphorus 3 mmol/mL and potassium 4.4 mEq/mL), 10% calcium gluconate (100 mg/mL), 20% *w/v* lipid injectable emulsion (SMOFlipid®), sterile water, Baxter exactaMix® 500 mL ethylene-vinyl acetate (EVA) bags, and intravenous infusion tubes were purchased.

FF-TPN and AIO-TPN were prepared by mixing the above-mentioned ingredients based on the guidelines used in the NICU of Janeway Children Hospital's NICU, St. John's, NL, Canada (**Table 2.1**). The preparation of FF-TPN is typically carried out under sterile conditions in the pharmacy and then transported to the NICU. At the NICU, the FF-TPN line is combined with the SMOFlipid® line at the Y-junction, creating a single tube that delivers AIO-TPN to the neonates. Briefly, first, 40 mL of 10% amino acid solution was added into the exactaMix® 500 mL EVA bag under sterile conditions. Subsequently, 24.56 mL of 70% dextrose, 0.1 mL of MICRO+®6, 1.36 mL of potassium phosphate injection, 0.750 mL of sodium acetate injection, 0.250 mL of magnesium sulfate injection, and 0.344 mL of heparin (100 units/mL), and 50 mL of sterile water were added. The lid of the bag was closed immediately and mixed gently. Secondly, 1.98 mL calcium gluconate injection was added and mixed gently. The bag was inspected for any precipitation.

Table 2.1: Preparation of TPN solutions.

FF-TPN: fat-free TPN (no lipid emulsion was added); AIO-TPN: all-in-one TPN, all ingredients were added. The AIO-TPN was prepared based on per kg body weight and per day requirement. The total volume required for newborns was 150 mL/kg/day.

Ingredients	Manufacturer	Rate to achieve 150 mL/kg	TPN solution	
10% amino acids (*w/v*) (Primene)	Baxter Corporation, Toronto, ON, Canada	40 mL	**FF-TPN (137.5 mL)**	**AIO-TPN (150 mL)**
70% dextrose	Baxter Corporation, Toronto, ON, Canada	24.56 mL		
Potassium phosphate, USP	Fresenius Kabi, Toronto, ON, Camada	1.36 mL		
Sodium acetate, USP 32.8%	Omega Laboratories Limited, Montreal, QC, Canada	0.75 mL		
Magnesium 20%	Sandoz Canada Inc., Boucherville, QC, Canada	0.250 mL		
Trace elements (Micro +6)	Sandoz Canada Inc., Boucherville, QC, Canada	0.1 mL		
Heparin diluted (100 units/mL)	Fresenius Kabi, Mississauga, ON, Canada	0.344 mL		
Sterile water		50 mL		
Ca-gluconate	Fresenius Kabi, Mississauga, ON, Canada	1.98 mL		
Multivitamin preparation (Multi-12/K_1® Pediatric)	Sandoz, Boucherville, QC, Canada	Total volume 1.5 mL Vial 1=1.2 mL Vial 2=0.3 mL		
Sterile water		16.56 mL		
SMOFlipid®	Fresenius Kabi, Mississauga, ON, Canada	12.5 mL	**Lipid emulsion (12.5 mL)**	

Thirdly, 1.2 mL of vial 1 of MVP and 0.3 mL of vital 2 of MVP (see *Appendix I*) was added into the bag. Lastly, 16.56 mL of sterile water was added and mixed gently. After preparation of FF-TPN (137.5 mL), the bag was wrapped with aluminum foil immediately. Finally, commercially available 20% SMOFlipid® (see *Appendix I*) was added to the FF-TPN at the ratio of 1:11, just before the experiment commenced and it was referred to as AIO-TPN. The pH of the solution was tested, and it was maintained within 5.6-6.7 (Pertkiewicz *et al.*, 2009; Watrobska-Swietlikowska and Szlagatys-Sidorkiewicz, 2015). The FF-TPN, SMOFlipid®, and AIO-TPN solutions were all prepared under sterile conditions within a biosafety cabinet-II (BSCII), with minimum light exposure (Lux 5-10).

2.2 Measurement of peroxides using a ferrous oxidation-xylenol orange (FOX) assay

Peroxides generated in the TPN solutions were measured using a modified FOX assay (version II assay) for lipid emulsion by (Silvers *et al.* (2001), adapted from the FOX version I assay by Wolff and Woff (1994). The modified version II assay was used because the FOX version I assay contains sorbitol, which can stimulate the chain reaction of Fe^{2+}, resulting in extensive generation of lipid peroxidation, leading to false positive results (Rubio and Cerón, 2021). Briefly, FF-TPN, SMOFlipid®, and AIO-TPN were prepared freshly on the day of experiments. The FOX version II reagent was also freshly prepared using 90% methanol (HPLC grade), 25 mM H_2SO_4, 4 mM 99.5% butylated hydroxytoluene (BHT) (#ICN10116290, MP Biomedicals™, Fisher Scientific, Ottawa, ON, Canada), 250 µM ammonium ferrous sulphate (#FX0245-1, Millipore Sigma, Oakville, ON, Canada), and 100 µM xylenol orange (#3618-43-7, Millipore Sigma, Oakville, ON, Canada) for each experiment. To make up 1 L of the FOX version II reagent, 880 mg of BHT was added to 900 mL methanol and mixed thoroughly, and

100 mL of 250 mM of H_2SO_4 was then added and mixed well using a magnetic stirrer. To the mixture, 98 mg of ferrous ammonium sulphate and 76 mg xylenol orange were added. The mixture was then sealed under nitrogen gas and stirred 1 hour. TPN samples were diluted at a ratio of 1:1 with Milli-Q® ultrapure water, and 50 μL of standards, samples, positive control (100 μM H_2O_2 added TPN samples), and blank (water) was placed into their own 1.5 mL microcentrifuge tube (#14-666-318, Fisher Scientific, Toronto, ON, Canada) followed by the addition of 950 μL of FOX-II reagent. Tubes were vortexed well, and incubated at room temperature for 30 minutes. Following the incubation, 200 μL of blank, standards, samples, and positive controls were placed into a 96 well plate (#21-377-203, Fisher Scientific, Ottawa, ON, Canada). The absorbance at 560 nm was immediately read using a Synergy Mx plate reader (BioTek, Winooski, VT, USA). A standard curve was prepared using 30% H_2O_2 solution (#H1009, Sigma Aldrich, Oakville, ON, Canada), which was diluted to 0, 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, and 0.7 mM (see *Appendix II*).

2.3 Measurement of light intensity

Light intensity was measured using a Traceable™ Dual-Display Light Meter (#06-662-64, Fisher Scientific, Ottawa, ON, Canada). For the light source, dimmable LED bright white light (Ultra definition; 60 to 8 W) (Philips) was used. The light intensity was adjusted from 0, 250, 500, 1000, 1500, and 3000 Lux to mimic the night and day NICU light, and phototherapy light conditions.

2.4 Preparation of air and $N_{2(g)}$ exposed AIO-TPN and SMOFlipid®

Air- and $N_{2(g)}$-exposed TPN solutions were prepared according to the method described by Laborie *et al.* (2000b), with slight modifications. Briefly, 10 mL of AIO-TPN and SMOFlipid® were added to a reusable glass tube (14-932A, Corning™ PYREX™). Air or $N_{2(g)}$ was bubbled into the liquid with a continuous flow of 100% air or nitrogen at the rate of 0.25 L/minute, exposed to light at 3000 Lux or no light for 24 hours. All tubes were continuously incubated on ice. The peroxide levels were measured at 0, 6, 12, 18 and 24 hours.

2.5 Preparation of vitamins C and/or E added TPN samples

L-ascorbic acid (>99%) (vitamin C) (#AAA1561322, Thermo Scientific, Oakville, ON, Canada) or esterified ascorbic acid (esterified vitamin C) (6-*O*-palmitoyl-L-ascorbic acid) (#76183-25G, Sigma Aldrich, Oakville, ON, Canada) was added to the SMOFlipid® to prepare 5 μM (1.7 μg), 10 μM (3.5 μg), 20 μM (7.0 μg), 40 μM (14.0 μg), and 80 μM (28.0 μg) concentrations at cellular levels. The baseline vitamin C content in 150 mL AIO-TPN for newborns was 24 mg (or 0.91 mM). To such solution, 12, 24, 36, 48, and 60 mg vitamin C was added to prepare 36 mg (1.36 mM), 48 mg (1.81 mM), 60 mg (2.27 mM), and 72 mg (2.73 mM). The pH was monitored after adding vitamin C to the SMOFlipid® or AIO-TPN. The pH was maintained within 5.9-6.7 range using 1 M sodium hydroxide or 1 M hydrochloric acid.

The baseline vitamin E level in SMOFlipid® was 20 mg/100 mL (or 5 μM). DL-α-tocopherol acetate (vitamin E) (#47786, Millipore Sigma, Oakville, ON, Canada) was added to the SMOFlipid® to prepare 10 μM (9.4 μg), 20 μM (18.9 μg), and 40 μM (37.8 μg) concentrations at cellular levels. As the cell culture medium has a polar nature, polysorbate 80%

(#P1754, Millipore Sigma, Oakville, ON, Canada) at 0.5% concentration was used to solubilize the vitamin E in cell media. Various concentrations of polysorbate levels (0.015, 0.03125, 0.0625, 0.125, 0.25, and 0.5%), which were used to solubilize the vitamin E, were used to assess the cell viability or metabolic activity.

The sources for total vitamin E in AIO-TPN (150 mL) were from SMOFlipid® (2.5 mg) and MVP (2.1 IU (2.1 mg)). Hence, the baseline amount of vitamin E found in the AIO-TPN was 4.6 mg/150 mL (or 64.87 μM). To such solution, 2.1, 4.2, and 6.3, and 7.2 mg vitamin E was added to prepare 6.7 mg (94.48 μM), 8.8 mg (124.10 μM), and 10.9 mg (153.7 μM).

2.6 Vitamin C and E combinations to the *in vitro* bench-top work and the cell work

Combinations of vitamin C at 24 mg (0.91 mM) (baseline), 36 mg (1.36 mM), 48 mg (1.81 mM), 60 mg (2.27 mM), and 72 mg (2.73 mM) and vitamin E at 4.6 mg (64.87 μM) (baseline), 6.7 mg (94.48 μM), 8.8 mg (124.10 μM), and 10.9 mg (153.7 μM) were used for *in vitro* bench-top experiments. A total of 20 combinations with various concentrations of vitamins C and E were prepared to select the best combinations to lower the peroxidation in the AIO-TPN.

Vitamins C and E at 0, 10, 20, and 40 μM were used for the various combinations of both vitamins for SMOFlipid®. A total of nine combinations of different concentrations of vitamins C and E were prepared. SMOFlipids® fortified with vitamins C and E combinations were treated to THP-1 human monocytic cells (see *section 2.15*) to assess the effectiveness of those antioxidants.

2.7 Preparation of copper, zinc, or selenium added TPN samples

Standard AIO-TPN for newborns was optimized for copper, zinc, and selenium levels. Briefly, zinc-free AIO-TPN (150 mL) was prepared. To the latter, zinc in the form of zinc sulfate (#Z0251-100G, Millipore Sigma, Oakville, ON, Canada) was added to yield an AIO-TPN with 10.19 μM (0.1 mg/150 mL), 15.29 μM (0.15 mg/150 mL), 20.39 μM (0.2 mg/150 mL), 25.49 μM (0.25 mg/150 mL), or 30.59 μM (0.3 mg/150 mL). The baseline concentration of zinc in the standard AIO-TPN was 30.59 μM. Similarly, a copper-free AIO-TPN (150 mL) was prepared. To the latter, copper in the form of copper sulfate (#C8027-500G, Millipore Sigma, Oakville, ON, Canada) was added to yield 1.05 μM (0.01 mg/150 mL), 2.09 μM (0.02 mg/150 mL), 3.15 μM (0.03 mg/150 mL), or 4.19 μM (0.04 mg/150 mL). The baseline concentration of copper in the standard AIO-TPN was 4.19 μM. To assess the prooxidant status of zinc and copper, 41.96 μM (0.4 mg), 419.64 μM (4 mg), 4196.44 μM (40 mg), and 41964.35 μM (400 mg) concentration of copper for AIO-TPN (150 mL) and 305.9 μM (3 mg), 3059.04 μM (30 mg), and 30590.39 μM (300 mg) concentration of zinc for AIO-TPN (150 mL) were prepared regardless of toxic levels.

2.7.1 Combination of copper and zinc

Combinations of zinc at 0.1 mg (10.2 μM), 0.25 mg (25.5 μM), and 0.3 mg (30.6 μM) (standard concentration) and copper at 0.01 mg (1.04 μM), 0.02 mg (2.09 μM), 0.03 mg (3.15 μM), and 0.04 mg (4.20 μM) (standard concentration) were used for *in vitro* experiments. A total of 12 combinations with various concentrations of zinc and copper were prepared to select the best combinations to lower the peroxidation in AIO-TPN.

2.7.2 Preparation of selenium for AIO-TPN

After the optimization with vitamins C and E, as well as zinc and copper, selenium in the form of selenium dioxide (#200107, Millipore Sigma, Oakville, ON, Canada) was added additionally. The baseline concentration of selenium in the AIO-TPN solution was 0.12 μM (2 μg/150 mL). To obtain the best concentration of selenium for the AIO-TPN, concentrations of 0.18 μM (3 μg), 0.24 μM (4 μg), 0.30 μM (5 μg), and 0.36 μM (6 μg) were prepared. Peroxide levels were then assessed.

2.8 Preparation of glutathione for AIO-TPN

Following the best levels of vitamin C, vitamin E, zinc, copper, and selenium for AIO-TPN, various concentrations of glutathione were added and examined for peroxide levels. Glutathione is not generally added to the TPN for neonates (Karthigesu *et al.*, 2021). The oxidized form of glutathione (GSSG) (#G4376-1G, Sigma-Aldrich, Oakville, ON, Canada) was added at the concentrations of 0, 10, 20, 30, 40, and 50 μM to the AIO-TPN.

2.9 THP-1 human monocyte culture

THP-1 human monocytes were used in these studies because they are highly prone to generating reactive oxygen species (ROS) when exposed to substances that exacerbate oxidative stress, making them particularly sensitive to agents that provoke an oxidative reaction (Ayesh *et al.*, 2014; Bekeschus *et al.*, 2016; Currò *et al.*, 2023; Foucaud *et al.*, 2010; Gatto *et al.*, 2018; Loeffler *et al.*, 2020). This study utilized TPN solutions developed for preterm and term infants. To facilitate the clinical translation of the findings, human monocytes would be a suitable model to assess the oxidative and antioxidant-related parameters.

The human monocytic THP-1 cell line was purchased from American Type Culture Collection (ATCC), Rockville, MD, USA. THP-1 cells were grown with Roswell Park Memorial Institute (RPMI)-1640 media (#SH30255.01, HyClone^{TM}, Cytiva, Marlborough, MA, USA) comprising of 25 mM HEPES and 0.3 mg/L L-glutamine in T75 vent cap culture flasks (#Falcon^{TM}353136, Corning Life Sciences, Canada). The medium was further supplemented with 1% *v/v* antibiotic/antimycotic (A/A) (#15240062, Invitrogen, Ottawa, ON, Canada) and 10% *v/v* heat-inactivated fetal bovine serum (FBS) (#SH30396.03HI, HyClone^{TM}, Cytiva, Marlborough, MA, USA), and the cells were incubated at 37°C with 5% $CO_{2(g)}$.

2.10 Cell counting and seeding

Cell counting was performed to quantify the seeding density using Trypan Blue. Briefly, the THP-1 cells were mixed by pipetting up and down for five times and added into a 15 mL Fisherbrand™ Easy Reader™ conical polypropylene centrifuge tube (#05-539-12, Fisher Scientific, Ottawa, ON, Canada). The cells were centrifuged at 200 × g for 5 minutes at room temperature, and the supernatant was discarded carefully without disturbing the pellets. Following this step, the cell pellets were resuspended with 10 mL of supplemented RPMI media. In a 1.5 mL Fisherbrand™ Microcentrifuge tube with locking snap cap (#14-666-318, Fisher Scientific, Ottawa, ON, Canada), 30 µL of cell sample was mixed with 30 µL of 0.4% (*w/v*) Trypan Blue (#15250061, Gibco^{TM}, Thermo Fisher Scientific, Oakville, ON, Canada). A total of 10 µL of mixture was loaded into a hemocytometer, as described by Pioli (2019). The live cells were counted in a specified area under the microscope. Seeding density was calculated based on the following Equation 1 (Pioli, 2019):

$$\text{Seeding density} = \left[\frac{\text{Cells in four quadrants}}{4} \right] \times 2 \times 10^4 \quad \text{(Equation 1)}$$

2.11 Maintenance of THP-1 cells

For cell maintenance, approximately every four days, when cells reached 80-90% confluency, the 75 cm^2 flask was taken out from the incubator at 37 °C with 5% $CO_{2(g)}$ (see *section 2.11*) and the cell suspension was mixed by pipetting up and down five times, and 3 mL (seeding density at 0.8×10^6 cells/mL) of the cell suspension was transferred to a new T75 flask containing 12 mL of supplemented RPMI-1640 growth medium. Cells were then incubated at 37 °C with 5% $CO_{2(g)}$.

2.12 Cell viability

Cell viability was assessed based on the method described by Riss *et al.* (2016). Briefly, for the exclusion assay, THP-1 cells treated with TPN solutions were taken out of the incubator and pipetted up and down five times to mix. Cells were dyed and loaded into the hematocytometer, as described above (see *section 2.10*). The total number of dead and live cells was counted in a defined area under the microscope. The entire procedure was repeated to get the average for a sample. The cell viability was calculated based on the following Equation 2:

$$\text{Cell viability \%} = \left[\frac{\text{Average of viable cells per treatment}}{\text{Average of total cells per treatment}} \right] \times 100 \quad \text{(Equation 2)}$$

where, a total cell per square of haematocytometer is the sum of viable and dead cells.

2.13 MTT assay

The MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) tetrazolium reduction assay is often used to determine metabolic activity of living cells (Ghasemi *et al.*, 2021). The MTT assay was performed, as described by Tobin *et al.* (2021), to assess the metabolic activity of THP-1 cells. Briefly, MTT (#M6494, Molecular Probes™, Thermo Fisher Scientific, Oakville, ON, Canada) was dissolved in phosphate-buffered saline (pH 7.4) to make a 5 mg/mL solution and filtered using a 0.2-micron syringe sterile filter (#09-719C, Fisherbrand™, Fisher Scientific, Ottawa, ON, Canada) and stored -20 °C in the dark. After 16 hours incubation of TPN solution treated cells and controls from 12 well cell culture plates (#07-200-81, Corning™ Costar™, Millipore Sigma, Oakville, ON, Canada), 100 µL TPN solution treated cells were transferred into 96-well plates (#21-377-205, Fisherbrand™, Fisher Scientific, Canada) after mixing, and 10 µL of MTT was added to each well (the final concentration of MTT in a well was 0.45 mg/mL (Riss *et al.*, 2016). The procedure was performed under minimum light exposure, and the plate was incubated for 4 hours at 37 °C in the dark. After 4 hours, formazan crystals formed in the viable cells were dissolved by adding 100 µL of 0.1 N HCl in isopropanol (#AC167880025, Fisher Scientific, Ottawa, ON, Canada) to each well with thorough mixing. The absorbance of each well was read at 570 nm and 630 nm using a Synergy Mx microplate spectrophotometer (BioTek, Winooski, VT); the 630 nm values were subtracted from the 570 nm data to account for background noise. To obtain the corrected absorbance value, the sample blank (respective sample wells without cells) was subtracted from each sample. The absorbance is directly proportional to the metabolic activity of the cell.

2.14 Preparation and selection of an appropriate concentration of positive controls for cellular treatment

H_2O_2 30% (*w/w*) in H_2O (#H1009, Sigma-Aldrich, Oakville, ON, Canada) and *tert*-butyl hydroperoxide 70 wt. % in H_2O (#458139, Millipore Sigma, Oakville, ON, Canada) at 0, 50, 100, 150, 200, and 250 μM were prepared. Human THP-1 cells (undifferentiated monocytes) were seeded at the density of 0.4×10^6/mL using a 12 well plate (#07-200-82, Corning™ Costar™, Millipore Sigma, Oakville, ON, Canada) and allowed to equilibrate for 2 hours at 37 °C with 5% $CO_{2(g)}$. Following the equilibration, the cells were treated with positive controls at various concentrations mentioned above and incubated for 16 hours at 37 °C with 5% $CO_{2(g)}$. Following the incubation, the cell viability and MTT assay (see *sections 2.12 and 2.13*) were performed to select the concentrations of positive controls that would not affect the cell viability and metabolic activity of the cells.

2.15 Preparation of SMOFlipid® and AIO-TPN to treat the THP-1 cells

One mL of cells was seeded into each well of 12-well plate (#07-200-82, Corning™ Costar™, Milllipore Sigma, Oakville, ON, Canada) at the seeding density of 8×10^5 cells/well, and equilibrated for 2 hours. Serial dilutions of SMOFlipid® of 4% (1:25), 2% (1:50), 1.34% (1:75), 1% (1:100), 0.5% (1:200), and 0.25% (1:400) *v/v* (Harvey *et al.*, 2015; Isesele *et al.*, 2022) and AIO-TPN of 50% (1:2), 20% (1:5), 10% (1:10), 6.7% (1:15), 5% (1:20) and 4% (1:25) *v/v* were prepared. After equilibrium for 2 hours, 1 mL of the diluted SMOFlipid® and AIO-TPN at the respective concentrations were treated to their own wells and incubated for 16 hours at 37 °C with 5%$CO_{2(g)}$. After the incubation, cell viability and MTT assay was performed.

2.16 Measurement of reactive oxygen species (ROS)

ROS formation by THP-1 monocytes after treating with TPN solutions was measured using a DCFDA / H_2DCFDA - Cellular ROS Assay Kit (#ab113851, Abcam, Waltham, MA, USA) and performed according to the manufacturer's instructions. Briefly, THP-1 cells were seeded into 96 well black/clear bottom plates (#165305, Thermo Scientific™ Nunc, Oakville, ON, Canada) at a seeding density of 1×10^4 cells (in 100 µL) per well and incubated for 24 hours. After incubation, the plate was centrifuged at 200 × g for 5 minutes (#75004260, Sorvall™ Legend™ X1 Thermo Scientific™, Oakville, ON, Canada). After centrifugation, the supernatant was discarded without disturbing the cell pellets. Pellets were resuspended with 100 µL of 1X buffer solution (provided with kit), and washed three times. After removal of 1X buffer solution at the final wash, 100 µL of 20 µM 2',7'-dichlorofluorescein diacetate (DCFDA) was added and incubated for 30 minutes at 37 °C in the dark. After a 30-minute incubation, the plate was centrifuged, supernatant discarded, and then washed three times with 1X buffer solution. The cells were resuspended with treatments, including control and positive control and incubated for 4 hours. After 4-hour incubation, the reading was obtained using a Synergy Mx fluorescent microplate reader (BioTek, Winooski, VT, USA) at excitation of 485 nm and emission of 535 nm. The blank well intensity was subtracted from each reading to obtain the corrected fluorescence intensity. The corrected fluorescence directly intensity is proportional to ROS production. Following the reading, the cells were treated with 0.1 M NaOH for 30 minutes at room temperature for cell lysis or 10 µL was used for MTT assay. After a 30-minute incubation, the protein concentration of the cell lysates was assessed using bicinchoninic acid (BCA) assay (see *section 2.17*). The ROS production was normalized by the protein concentration of each well or with MTT reading.

2.17 Bicinchoninic acid (BCA) protein assay

Total protein was determined in cell lysate preparations using the Pierce™ BCA Protein Assay Kit (#23225, Thermo Fisher Scientific, Ottawa, ON, Canada). The samples and the standards (0, 6.25, 12.5, 25.0, 50.0, 100.0, 200.0, and 400.0 µg/mL) were added to 96-well microplates (#21-377-203, Corning™ clear polystyrene, Millipore Sigma, Oakville, ON, Canada) at a volume of 25 µL, in duplicate. Subsequently, 200 µL of BCA working reagent (BCA protein assay reagent A and B at 50:1 ratio) was added, mixed, and then incubated at 37 ºC for 30 minutes. Following the incubation, absorbance was measured at 562 nm using a Synergy Mx Fluorescent Plate Reader (BioTek, Winooski, VT). Protein concentration was calculated by comparison to a standard curve plotted for bovine serum albumin (BSA) (0-400 µg/mL) that was generated for each experiment using GraphPad Prism v6.0.

2.18 Thiobarbituric acid reactive substances (TBARS) assay

The TBARS assay is commonly used as a marker of lipid peroxidation in biological fluids (De Leon and Borges, 2020), and it was used to examine lipid peroxidation in THP-1 cells. Malondialdehyde (MDA) generated by THP-1 cells was estimated by analyzing the cell supernatant using a TBARS Parameter Assay Kit (#KGE013, R&D Systems Inc., Ann Arbor, MI, USA). After 16 hours of treatment with various forms of TPN solutions, the cells were transferred to 2 mL microcentrifuge tubes (#S35859, Fisherbrand™, Fisher Scientific, Ottawa, ON, Canada) and centrifuged at 200 × g for 5 minutes using microcentrifuge (#75002559, Sorvall™ Legend™ Thermo Scientific™, Oakville, ON, Canada). The supernatant was aliquoted and stored at -80°C until further use.

TBARS levels were measured from supernatants as per the manufacturer's instruction. Briefly, MDA stock solution was prepared by mixing 100 µL TBARS with 200 µL of TBARS acid reagent. The mixture was placed in tube rotary shaker with gentle agitation for 30 minutes to produce 167 µM MDA stock solution. Following the incubation, the MDA stock solution was diluted with deionized water to produce 0, 0.26, 0.52, 1.04, 2.09, 4.18, and 8.35 µM concentrations of MDA for a standard curve construction. The samples were thawed, and 300 µL of each sample was placed in 1.5 mL microcentrifuge tubes, mixed with 300 µL TBARS acid reagent, and incubated for 15 minutes at room temperature. Following the incubation, the microcentrifuge tubes were centrifuged at ≥ 12,000 × g for 4 minutes and 150 µL supernatants and standards were transferred to the 96 well plate provided with the kit. To those wells, 75 µL of thiobarbituric acid (TBA) reagents were added. The pre-optical density (OD1) was obtained immediately using a Synergy Mx fluorescent plate reader (BioTek, Winooski, VT) at 532 nm absorbance and 37 °C. The microplate was then covered with the adhesive strip provided with the kit and was incubated for 3 hours at 47 °C. Following a 3-hour incubation, the final optical density (OD2) of each well was determined at 532 nm absorbance and at a temperature of 47 °C. The OD1 was then subtracted from the OD2 to determine the MDA formation from the standard curve.

2.19 Preparation of complete cell lysate buffer and cell lysate

A cell lysate buffer (100 µL) was prepared by mixing 29 µL distilled water, 10 µL 10X lysis buffer (#9803, Cell Signaling Technology, Danvers, MA, USA), 10 µL 10X PhosSTOP™ (#04906845001, Roche Diagnostics, Laval, QC, Canada), 50 µL of 2X cOmplete ULTRA

(#05892791001, Roche Diagnostics, Laval, QC, Canada), and 1 µL of 100 mM PMSF (#93482, Millipore Sigma, Oakville, ON, Canada).

A total of 2 mL of cells per well with or without treatment were seeded at a density of 0.4 × 10^6/mL into 12-well plate (#07-200-82, Corning™ Costar™, Millipore Sigma, Oakville, ON, Canada) (see *section 2.14*) and incubated for 16 hours at 37 °C with 5% $CO_{2(g)}$. Following the incubation, cells were transferred to 2 mL microcentrifuge tube (#02-681-271, Fisher Scientific, Oakville, ON, Canada) and centrifuged at 200 × g for 5 minutes. The cell pellet was resuspended and washed three times using ice-cold phosphate buffered saline (PBS). After the final wash, the cell pellet was resuspended using 100 µL of the cell lysate buffer, mixed well by flicking and kept on ice for 20 minutes. Lysed cells were then centrifuged at 14,000 × g for 30 minutes at 4 °C, and the supernatant was transferred to a fresh 1.5 mL microcentrifuge tube (#14-666-318, Fisher Scientific, Ottawa, ON, Canada) without disturbing the pellet. The supernatant was then aliquoted and stored at -80 °C until further analysis.

2.20 Cellular antioxidant activity (CAA)

The CAA assay, a cell-based assay that measures the antioxidant activity of cells under physiological conditions based on the balance between antioxidant and oxidant levels, was established in 2007 by Wolfe and Liu (2007). The CAA assay was carried out following the method described by Wolfe and Liu (2007) with modifications for THP-1 cells. To assess the CAA, THP-1 suspension cells and a low concentration of 3.75 µM 2',7'-dichlorofluorescin diacetate (DCFH-DA) were used. Briefly, 5 mL of human THP-1 monocyte cells at 80-90% confluency was taken from a 75 cm^2 flask, and they were washed three times using warm PBS (37 °C). Following the final washing, the cell pellet was resuspended using 5 mL of PBS,

counted, and 2.5 mL transferred into a 15 mL centrifuge tube (#05-539-12, Fisher Scientific, Ottawa, ON, Canada) for the seeding density of 0.5×10^6/well of 96 black wall/clear bottom plate. An equal volume (2.5 mL) of 7.5 μM 2',7'-dichlorofluorescin diacetate (DCFH-DA) was added and mixed by pipetting up and down five times, and 50 μL was then transferred to 96 black wall/clear bottom well plate (#165305, Thermo Scientific™ Nunc, Oakville, ON, Canada). Immediately after this step, 50 μL diluted AIO-TPN samples (1:5 dilution), blank, and positive controls were added and incubated for 1 hour at 37 °C with 5% $CO_{2(g)}$. Following the incubation, cells were washed three times with PBS, and 100 μL of 600 μM 2,2'-azobis (2-amidinopropane) dihydrochloride (AAPH (or ABAP)) was added, and the plate was immediately placed in a Synergy Mx plate reader (BioTek, Winooski, VT, USA) and real-time fluorescence was read initially and every five minutes after for 1 hour (a total of 13 readings) with an excitation wavelength of 485 nm and an emission wavelength of 538 nm. Control wells were treated with DCFH-DA and ABAP without antioxidants, while blank wells were treated with DCFH-DA without ABAP and antioxidants. Positive control wells were treated with 250 μM quercetin, DCFH-DA and ABAP.

2.20.1 Quantitation of the CAA assay

The antioxidant activity of optimized AIO-TPN solution treated to human THP-1 monocyte cells was quantified by examining the percent reduction in fluorescence. Briefly, curves were generated by the 13 fluorescence readings of each treatment over the course of the 1 hour assay. The area under each curve (AUC) was calculated via integration with the GraphPad data analysis software (Version Prism 8.0). As expected, control wells exhibited the maximum

fluorescence, as there was no inhibition of the ABAP and DCFH-DA reaction. The percent reduction (or the CAA unit) was calculated based in the following Equation 3:

$$\text{CAA unit} = \%\ \text{reduction} = \left[1 - \frac{\text{Sample's AUC}}{\text{Control's AUC}} \right] \times 100 \qquad \text{Equation (3)}$$

In addition to the AUC, CAA was also calculated by comparing the fluorescent values of control with samples at a 1 hour end point in the curved data.

2.21 Ferric reducing antioxidant power (FRAP) assay

The FRAP assay is frequently used to measure the antioxidant power of biological fluids, food samples, and plant and tissue extracts based on the rapid reduction of ferric-2,4,6-tris(pyridyl)-s-triazine (Fe^{3+}-TPTZ) to ferrous-tris(pyridyl)-s-triazine complex (Fe^{2+}-TPTZ) by antioxidants present in a sample at low pH (3.6). The ferric reducing power of standard and optimized AIO-TPN treated THP-1 cells was examined using the FRAP assay described by Pulido *et al*. (2000). Briefly, the FRAP reagent was freshly prepared for each experiment using 2.5 mL of 10 mM TPTZ (#T1253, Supelco, Millipore Sigma, Oakville, ON, Canada) solution, 40 mM HCl solution, 2.5 mL of 20 mM iron (III) chloride hexahydrate ($FeCl_3.6H_2O$) (#236489, Sigma-Aldrich, Oakville, ON, Canada), and 25 mL of 300 mM sodium acetate (#241245, Sigma-Aldrich, Oakville, ON, Canada) buffer at pH 3.6. Twenty µL of the cell supernatant was added into the wells of a Corning™ clear polystyrene 96-well microplate ((#21-377-203, Fisher Scientific, Ottawa, ON, Canada), followed by 280 µL of freshly prepared FRAP reagent. After

the incubation of the microplate at 37 °C for 10 minutes, the reading was taken at 593 nm absorbance using a Synergy Mx microplate spectrophotometer (BioTek, Winooski, VT, USA).

A standard curve was plotted using the concentrations of 0 μg/mL (0 μM), 5 μg/mL (20 μM), 10 μg/mL (40 μM), 15 μg/mL (60 μM), 30 μg/mL (120 μM), 45 μg/mL (180 μM), 60 μg/mL (240 μM), 75 μg/mL (300 μM), and 90 μg/mL (360 μM) of the positive control Trolox, an antioxidant vitamin E derivative. The reducing power of experimental samples was expressed as Trolox equivalents (TE) in μg/mL.

2.22 GSH/GSSG ratio

GSH/GSSG ratio is a good marker for assessing the redox status of cells during oxidative stress (Zitka *et al.*, 2012). GSH/GSSG was measured using a GSH/GSSG ratio assay kit (fluorometric-green) (#ab138881, Abcam, Waltham, MA, USA) as per the manufacturer's instruction. Briefly, 100 μL cell lysates (see *section 2.19*) were thawed on ice and centrifuged using a microcentrifuge at 14000 × g for 30 minutes at 4 °C, and 80 μL of supernatant was placed in a separate 1.5 mL microcentrifuge tube (#14-666-318, Fisher Scientific, Ottawa, ON, Canada). Following this step, 20 μL of deproteinization solution (100% *w/v* TCA/$NaHCO_3$) was added, vortexed briefly, and then incubated for 10 minutes on ice. After incubation, it was centrifuged at 12,000 × g for 5 minutes at 4 °C; 80 μL was transferred to a separate tube, and pH was adjusted to 4-6 using 0.1 M $NaHCO_3$ drop by drop. The mixture was centrifuged at 13,000 × g for 15 minutes at 4 °C, and the supernatant was collected and kept on ice. The dilution factor for sample dilution was calculated based on the following Equation 4:

$$\% \text{ original concentration} = \left[\frac{\text{Initial sample volume}}{\text{Initial sample vol.} + \text{TCA vol} + \text{NaHCO}_3 \text{ vol.}} \right] \times 100 \quad \text{(Equation 4)}$$

GSH standards of 0, 0.1563, 0.3125, 0.625, 1.25, 2.5, 5, and 10 μM, and GSSG standards of 0, 0.0781, 0.1563, 0.3125, 0.625, 1.25, 2.5, and 5 μM were prepared freshly for each experiment. Those standards were stable for 4 hours. To detect GSH, a GSH assay mixture was prepared by diluting the thiol green (provided with the kit) in assay buffer (provided with the kit). For determining the total glutathione levels (GSH + GSSG), a total glutathione assay mixture (TGAM) was prepared by diluting the 25X GSSG probe stock solution in the GSH assay mixture at a 1:25 dilution and mixed well by vertexing. For detection, 50 μL GSH standards, 50 μL GSSG standards, and 50 μL of sample for GSH and GSSG estimation were placed into a 96 black wall/clear bottom well plate (#165305, Thermo Scientific™ Nunc, Ottawa, ON, Canada). Then, 50 μL of GSH assay mixture was added to standards and the samples for GSH estimation; 50 μL TGAM was added to the standards and samples for total glutathione estimation, respectively. The plate was covered with aluminum foil and incubated at room temperature for 30 minutes. Following the incubation, the plate was read at an excitation wavelength of 490 nm and an emission wavelength of 520 nm using SynergyMx fluorescent plate reader (BioTek, Winooski, VT, USA). The concentration of GSSG was calculated based on the following Equation 5:

$$\text{GSSG} = (\text{total glutathione} - \text{GSH})/2 \quad \text{(Equation 5)}$$

The reading of blank fluorescent value was subtracted from GSH standards' and samples' fluorescence values to get the corrected fluorescence reading. A smooth curve was plotted through the corrected fluorescence reading. A logarithmic linear regression (log (y) = a+ $b$log (x)) was used to obtain the sample concentrations.

2.23 Western blot analysis of endoplasmic reticular (ER) stress protein

Determining the expression of ER stress proteins indicates the cells exposed to oxidative stress. Cell lysates (see *section 2.19*) were thawed on ice and were subjected to protein concentration measurement using the Pierce™ BCA Protein Assay Kit (#23225, Thermo Fisher Scientific, Oakville, ON, Canada). Briefly, 5 µL of cell lysate was diluted to 50 µL using distilled H_2O. From this diluted sample, 25 µL was used for protein estimation, as described in *section 2.17*. Thirty micrograms of total protein lysate samples (approximately 18 µL of AIO-TPN treated cell lysate samples) or positive control (tunicamycin treated cell lysate at 0.5 µg/mL) and 6.48 µL loading buffer with or without 2-mercaptoethanol (#M6250, Sigma Aldrich, Oakville, ON, Canada) were subjected to 10% sodium dodecyl sulfide polyacrylamide gel electrophoresis (SDS-PAGE). Samples were run at 120 V for 30 minutes and transferred onto 0.2 µM nitrocellulose membranes (#1620115, Bio-Rad, Mississauga, ON, Canada). Blots were blocked with 5% skimmed milk in 1X Tris-buffered saline (TBST: containing 137 mM NaCl, 2.7 mM KCl, 25 mM Tris, 0.1% Tween 20) for 1 hour at room temperature. Blots were washed with TBST for 5 minutes three times and then incubated overnight at 4 °C with specific primary antibodies (antibody against GRP78 at a 1:1,000 dilution (#3177, Cell Signaling Technology, Danvers, MA, USA), CHOP at a 1:1,000 dilution (#2895, Cell Signaling Technology, Danvers, MA, USA), and beta-actin at a 1:5,000 dilution (#NB600-501, Novus Biologicals, Toronto, ON,

Canada). Following the overnight incubation, the blots were washed with TBST for 5 minutes three times and incubated with the corresponding diluted HRP-conjugated secondary antibodies (goat anti-rabbit HRP for GRP78 at 1: 2000 (#7074, Cell Signaling Technology, Danvers, MA, USA); goat anti-mouse IgG (H+L) HRP for CHOP at 1:5000 (#62-6520, Invitrogen, Mississauga, ON, Canada); and goat anti-mouse IgG (H+L) HRP for beta-actin at 1:5000 (#62-6520, Invitrogen, Mississauga, ON, Canada). The blots were visualized using the ChemiDoc Imaging System (#12018025, Bio-Rad, Mississauga, ON, Canada). Densitometric analysis was carried out using ImageLab 6.1 (Bio-Rad, Mississauga, ON, Canada).

2.24 Analysis of fatty acid composition and oxidized fatty acids using LC-MS/MS

2.24.1 Preparation of samples for lipid extracts

Lipids from SMOFlipid® and AIO-TPN samples were extracted based on the method described by Bligh and Dyer (1959). Briefly, 50 µL of SMOFlipid® (10 mg lipid) or 600 µL of AIO-TPN) (10 mg lipid), 2.5 mL of methanol (#A4564, Optima™ LC/MS Grade, Fisher Scientific, Ottawa, ON, Canada), and 1.25 mL chloroform (#C297-4, Optima™ LC/MS Grade, Fisher Scientific, Ottawa, ON, Canada) were added into a 16 ×100 mm screw cap glass tube (tube A) (#14-959-25B, Fisher Scientific, Ottawa, ON, Canada) with a septa screw insert. After 5 minutes, the mixture was vortexed for 20 seconds, and then 1 mL Milli-Q® ultrapure water was added and then vortexed for 30 seconds. Following this step, the tube was centrifuged at 500 × g for 2 minutes at room temperature using a centrifuge (#75004260, Sorvall™ Legend™ X1 Thermo Scientific™, Oakville, ON, Canada). The chloroform (bottom) layer was carefully transferred to a separate 16 ×125 mm screw cap glass tube (tube B) (#14-959-25C, Fisher Scientific, Ottawa, ON, Canada). To tube A, 2.5 mL of chloroform (Optima™ LC/MS Grade,

Fisher Scientific) was added, vortexed for 30 seconds, and centrifuged at 500 × g for 2 minutes. The chloroform (bottom) layer was transferred to tube B, and 4 mL Milli-Q® ultrapure water was then added to tube B, vortexed for 30 seconds and centrifuged at 500 × g for 5 minutes. Following the centrifuge, the lower chloroform layer was transferred into a new 16 ×100 mm screw cap glass tube with a septa screw insert, and chloroform layer was dried under nitrogen gas. The dried lipid extract was resuspended by adding 0.5 mL of chloroform and capped under nitrogen exposure and stored at -20 °C for further analysis.

2.24.2 Determination of fatty acid composition

The fatty acid composition of SMOFlipid® and AIO-TPN was determined using electrospray ionization (ESI)-MS/MS, described by (Kerwin *et al.*, 1996). Briefly, 50 μL of lipid extract (see *section 2.24.1*) was added into a separate glass tube (#14-961-25, Fisher Scientific, Ottawa, ON, Canada). To this tube, 5 μL 0.1 μM methanolic NaOH (#LC245202, Fisher Scientific, Ottawa, ON, Canada) and 445 μL of MS-grade methanol were added and mixed using a Hamilton™ glass syringe (#111011065, Fisher Scientific, Ottawa, ON, Canada). Five hundred μL of mixture was loaded into the syringe and attached to the syringe pump of the LC-MS/MS (Thermo Scientific TSQ Quantis triple quadrupole mass spectrometer) and injected directly into the mass spectrometer without using chromatographic separation at a flow rate of 5 μL/min. The mass spectrometer conditions were set at negative ion mode with voltage at 3918.2 V, sheath gas at 5.4365 a.u., auxiliary gas at 5.691 a.u., sweep gas at 0.2035 a.u., ion transfer tube temperature at 325 °C, and vaporizer temperature at room temperature. The percentage of fatty acid composition in TPN samples was calculated based on the intensity values corresponding to the

m/z values of a fatty acid compared to the total intensity values of known fatty acids (Han and Gross, 2005; Rydlewski *et al.*, 2021).

2.24.3 Determination of oxidized fatty acid, isoprostanes

Mass spectrometry conditions to detect the isoprostanes in AIO-TPN samples were optimized using standard 8-isoprostane $F_{2\alpha}$ (#25903, Max®Spec Standards, Cayman, Ann Arbor, MI, USA). Briefly, 5 µL of 100 µg/mL 8-isoprostane standard (100 ng/µL) in ethanol was added to 495 µL chloroform and mixed (working solution; 1 ng/µL). From the working solution, 50 µL was transferred with 5 µL 0.1 µM sodium hydroxide in methanol (#LC245202, Fisher Scientific, Canada) and 445 µL of methanol into a glass tube and mixed using Hamilton™ glass syringe (#111011065, Fisher Scientific, Ottawa, ON, Canada) (the final concentration was 100 ng/mL). Following to this step, 500 µL of mixture was loaded into the syringe pump of the LC-MS/MS (Thermo Scientific TSQ Quantis triple quadrupole mass spectrometer) at the flow rate of 5 µL/minute. Mass conditions for 8-isoprostane $F_{2\alpha}$ identification was detected under negative electrospray ionization mode with optimized conditions of 3918.8 voltage, 34.63 a.u. sheath gas, 6.91 a.u. auxiliary gas, 23.98 a.u. sweep gas, 325 °C ion transfer tube temperature, and 25 °C vaporizer temperature. After these optimized conditions set in the mass spectrometry, lipid extracted AIO-TPN solutions were injected at the rate of 5 µL/minute to assess the 8-isoprostane $F_{2\alpha}$. The parent ion at *m/z* 353.3 in full scan mode and then the fragmented daughter ion at *m/z* 193 were detected (*m/z* 353.3 ⟶193).

2.25 Statistical analyses

All the results are expressed as the mean ± standard deviation (SD) of at least three biological replicates of an experiment with three technical replicates of each. Statistical analyses were performed using one-way ANOVA followed by multiple comparisons using Tukey's HSD for pairwise comparisons of categorical data, or Dunnett's post-hoc tests for comparisons of each categorical data with control. If an independent variable was numerical, ordinal or of increasing categorical condition, linear regression was performed to assess the linear relationship between independent and dependent variables. Two-factor dependent variables were analyzed using two-way ANOVA followed by multiple comparisons using Tukey's HSD. GraphPad Prism (version 8.0.1) was used for analyses and generating graphs. Differences were considered statistically significant when the p-value was < 0.05.

CHAPTER 3: RESULTS

3.1 Peroxide levels of light-protected (LP) and light-exposed (LE) TPN samples

3.1.1 Peroxide levels of TPN solutions at time zero

The peroxide levels of undiluted FF-TPN (prepared by mixing the ingredients; see *section 2.1*), newly opened bags of SMOFlipid®-20% (commercially available), and AIO-TPN (prepared by mixing the ingredients; see *section 2.1*) were determined using the FOX version II assay at time zero without light exposure. Peroxide levels at time zero in the FF-TPN were 0.093 (±0.013) mM H_2O_2 equivalents, whereas the peroxide levels of a newly opened bag of SMOFlipid® were 1.8 (±0.047) mM ($p < 0.0001$) (**Figure 3.1**). The peroxide levels significantly elevated to 1.039 (±0.116) mM after adding SMOFlipid® at the ratio of 1:11 to the FF-TPN to prepare AIO-TPN ($p < 0.0001$) according to Tukey's HSD.

3.1.2 Generation of peroxide levels in the TPN at various levels of light intensity

To assess the influence of light intensity on peroxide formations, the FF-TPN SMOFlipid®, and AIO-TPN were incubated at 34 °C for 24 hours at the light exposure of 0, 250, 500, 1000, 1500 or 3000 Lux. Increasing light intensity did not affect the peroxide formation in FF-TPN ($p > 0.05$) (**Figure 3.2 A**), whereas increasing light intensity influenced the peroxide formation in SMOFlipid® and AIO-TPN. For instance, even though the peroxide level in FF-TPN increased from 0.086 (±0.016) mM H_2O_2 equivalents that incubated at dark (0 Lux) to 0.129 (±0.012) mM H_2O_2 equivalents in 3000 Lux, it was not significantly different ($p > 0.05$) according to the Dunnett's post hoc test.

Figure 3.1: Peroxide levels of light protected (LP) unopened-SMOFlipid®, and freshly prepared FF-TPN and AIO-TPN using the FOX version II assay.

The peroxide levels, expressed as H_2O_2 equivalents, were measured from FF-TPN and AIO-TPN solutions immediately after preparation, and from newly opened bags of SMOFlipid® at time zero (0 h). Different lowercase letters indicate significant differences ($p < 0.05$) among the peroxide levels generated in TPN solutions. Bars are represented as mean ± SD (n=3). Significant differences were determined by one-way ANOVA followed by multiple comparisons using Tukey's HSD.

Figure 3.1

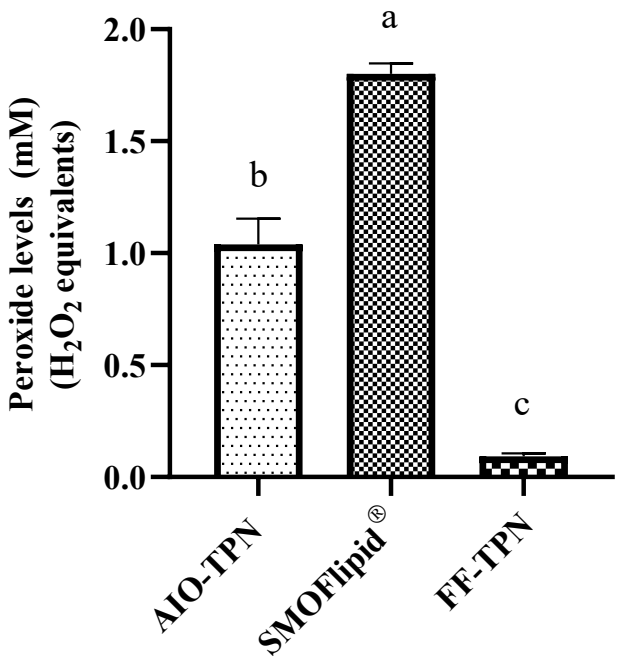
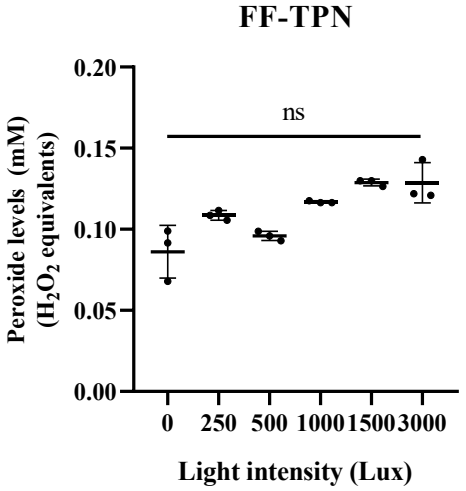

Peroxide levels (mM)
(H_2O_2 equivalents)
2.0
1.5
1.0
0.5
0.0
a
b
c
AIO-TPN
SMOFlipid®
FF-TPN

Figure 3.2: Peroxide levels of parenteral nutrition solutions exposed to increasing level of light intensity.

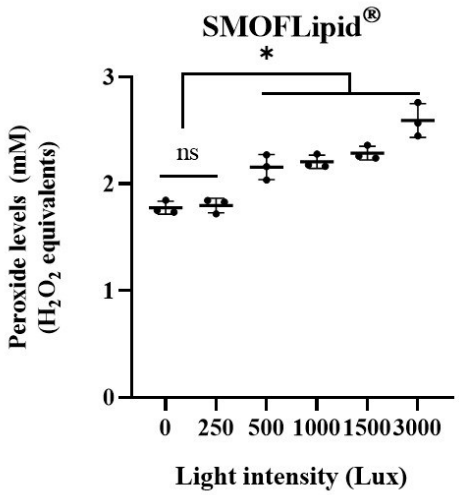
A: Fat-free TPN (FF-TPN); **B**: Intravenous lipid emulsion (ILE) (SMOFlipid®); **C**: All-in-one TPN (AIO-TPN). The light intensity was adjusted at the dark room to control the external light (see *section 2.3*); *$p < 0.05$. Error bar of column scatter dot plot graph represents the mean ± SD (n=3). Each dots represent mean of the replicated values of biological samples of three sets. Significant differences were determined by one-way ANOVA followed by the Dunnett's post hoc test.

Figure 3.2

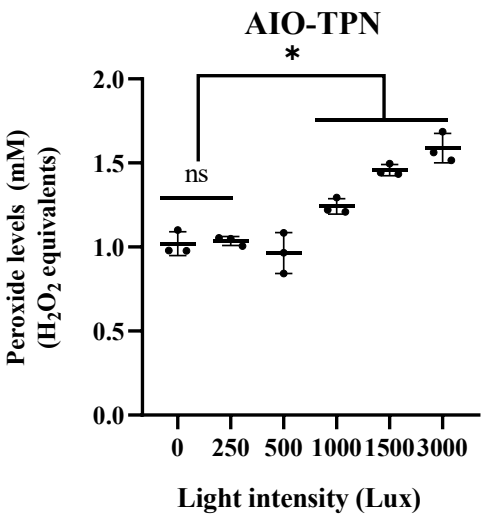
(A)



(B)



(C)



The linear regression analysis showed no significant relationship between increasing light intensity and peroxide levels of FF-TPN (R^2=0.48; $p > 0.05$). Peroxide levels generated in SMOFlipid® were not significantly increased when the light exposure was increased from 0 to 250 Lux (1.777 (±0.06) vs. 1.797 (±0.07) mM H_2O_2 equivalents) ($p > 0.05$) (**Figure 3.2 B**). On the other hand, when the light intensity was increased from 500 to 3000 Lux, the peroxide levels increased from 2.157 (±0.117) to 2.593 (±0.159) mM H_2O_2 equivalents, and those were significantly higher than the peroxide levels observed in 0 and 250 Lux according to the Dunnett's post hoc test ($p < 0.05$) (**Figure 3.2 B**). When the light intensity increased from 250 to 500 Lux, the peroxide levels in the SMOFlipid® were significantly higher ($p < 0.0001$). The linear regression analysis showed a significant relationship between increasing light intensity and peroxide levels in SMOFlipid® (R^2=0.81; $p < 0.0001$).

In AIO-TPN, light exposure from 0 to 500 Lux, peroxide levels were not significantly altered (**Figure 3.2 C**) and it was within the mean range of 0.964 to 1.02 mM H_2O_2 equivalents. On the other hand, when the light intensity was increased from 500 to 1000 Lux, the peroxide levels were significantly increased, from 0.964 (±0.121) to 1.242 (±0.046), and it was further increased to 1.588 (±0.088) mM H_2O_2 equivalents at 3000 Lux. Peroxide levels from 1000 to 3000 Lux were significantly higher compared to AIO-TPN incubated at dark (0 Lux) (1.020 (±0.07) mM H_2O_2 equivalents H_2O_2 equivalents) ($p < 0.05$) according to Dunnett's post hoc test. The linear regression analysis showed a significant relationship between increasing light intensity and peroxide levels in AIO-TPN (R^2=0.82; $p < 0.0001$). This study revealed that adding or mixing SMOFlipid® to the FF-TPN generated a significant amount of peroxides, and those were increased further by increasing the light intensity. This study has also shown that peroxide

levels were not elevated up to the light intensity of 500 Lux for AIO-TPN and 250 Lux for SMOFlipid®.

3.1.3 Peroxide generation on duration of light exposure

Peroxide formation on the duration of light exposure for FF-TPN, SMOFlipid®, and AIO-TPN was assessed. LE (6 hours) FF TPN had 0.248 (±0.013) mM H_2O_2 equivalents peroxide levels, which were significantly higher, compared to LP FF-TPN at time zero (0.099 (±0.003) mM H_2O_2 equivalents ($p < 0.0001$). However, the peroxide levels gradually declined with increasing duration of the light exposure up to 3000 Lux (at 12 hours: 0.187 (±0.008) mM; at 18 hours: 0.177 (±0.007) mM; at 24 hours: 0.149 (±0.014) mM H_2O_2 equivalents) (**Figure 3.3 A**). However, there was no significant difference in peroxide levels between time zero and 24-hour light exposure in FF-TPN ($p > 0.05$), according to Dunnett's post hoc test.

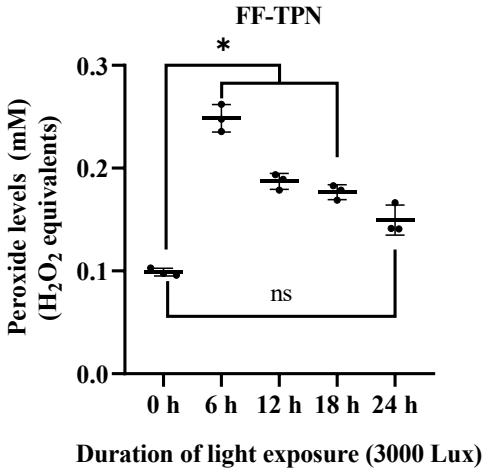
Conversely, increasing the duration of light exposure at a higher intensity from 0 to 24 hours significantly increased the peroxide levels in SMOFlipid® and AIO-TPN. The peroxide levels remained the same with up to 6 hour-light exposure on SMOFlipid®, whereas the peroxide levels were not increased until 12 hour-light exposure on AIO-TPN ($p > 0.05$). The linear regression analysis showed a significant relationship between increasing the duration of light exposure and peroxide levels in AIO-TPN (R^2=0.81; $p < 0.0001$). In SMOFlipid®, the peroxide levels jumped from 1.838 (±0.016) to 2.136 (±0.048) mM H_2O_2 equivalents ($p < 0.05$) when the duration of light exposure increased from 6 to 12 hours, and it further increased to 2.584 (±0.013) mM H_2O_2 equivalents on 24 hours light exposure (**Figure 3.3 B**).

Figure 3.3: Peroxide levels of parenteral nutrition solutions exposed to increasing exposure time.

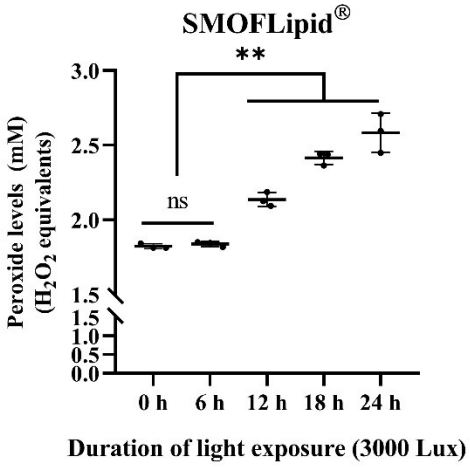
A: Fat-free TPN (FF-TPN) (n=3); **B**: Intravenous lipid emulsion (ILE) (SMOFlipid®) (n=3); **C**: All-in-one TPN (AIO-TPN) (n=6); *$p < 0.05$; **$p < 0.01$; Error bar of column scatter dot plot graph represents the mean ± SD. Significant differences were determined by one-way ANOVA followed by the Dunnett's post hoc test.

Figure 3.3

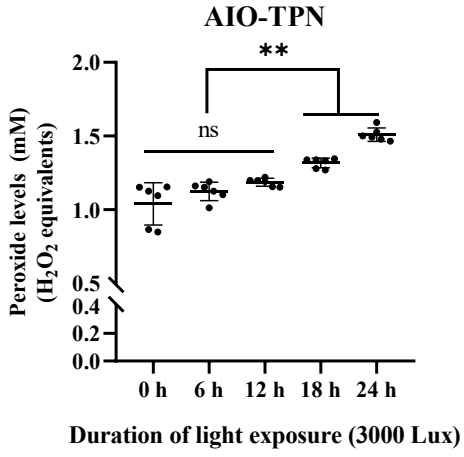
(A)



(B)



(C)



As expected, in AIO-TPN, 12-hour exposure of light did not increase the peroxide levels compared to the peroxide levels generated at time zero (**Figure 3.3 C**). The linear regression analysis showed a significant relationship between increasing light intensity and peroxide levels of SMOFlipid® (R^2=0.92; $p < 0.0001$). However, the peroxide levels were significantly increased to 1.318 (±0.033) mM H_2O_2 equivalents at 18 hours compared to time zero based on one-way ANOVA. Hence, this study has shown that the 12-hour administration of AIO-TPN and the 6-hour administration of SMOFlipid® did not increase the peroxide levels significantly. Nonetheless, the TPN solutions are recommended to given continuously to neonates for 24 hours in clinical setting under light exposure at NICU.

3.2 Peroxide levels in the TPN increased with air and light exposure

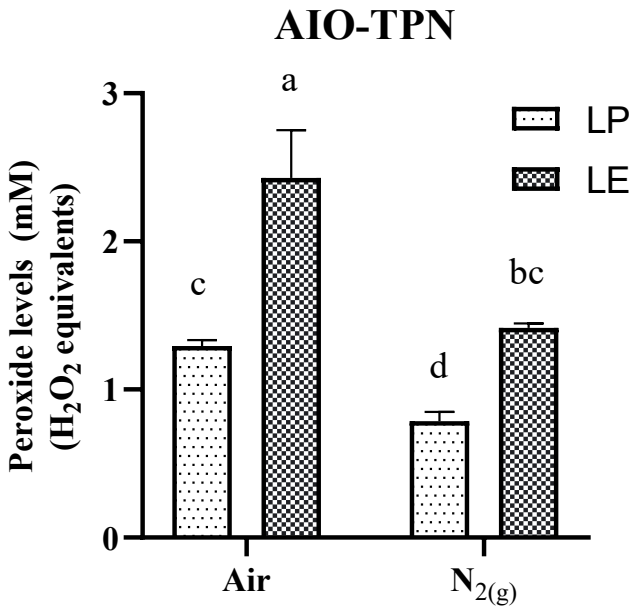
To evaluate the impact of air or oxygen on peroxide levels, AIO-TPN and SMOFlipid® were used, as polyunsaturated lipids are particularly susceptible to oxidation when exposed to oxygen. The SMOFlipid® contains rich amount of omega-3 and omega-6 fatty acids. The FF-TPNs were formulated without the inclusion of SMOFlipid® (see *section 2.1*). In clinical practice, AIO-TPN is commonly administered to neonates. Air-exposed AIO-TPN and SMOFlipid® generated a significant amount of peroxides compared to N_2 exposed AIO-TPN and SMOFlipid®. Light protected (LP)-$N_{2(g)}$ exposed AIO-TPN and SMOFlipid® for 24 hours produced minimum peroxides (0.785 (±0.063) and 0.357 (±0.064) mM H_2O_2 equivalents, respectively) compared to light and air exposed solutions (2.428 (±0.0.324) and 3.011 (±0.081) mM H_2O_2 equivalents, respectively) (**Figure 3.4 A and B**).

Figure 3.4: Peroxide levels of TPN solutions exposed to air or nitrogen ($N_{2(g)}$) for 24 hours with or without light exposure.

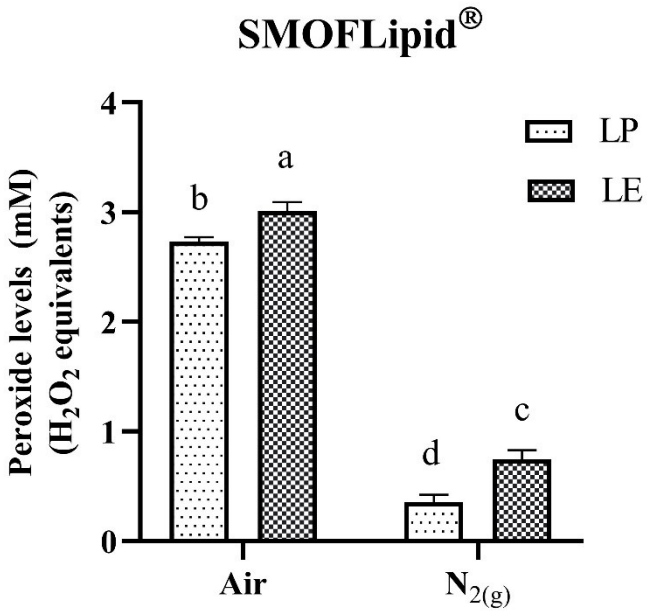
A: AIO-TPN; **B**: SMOFlipid®. Different lowercase letters indicate significant differences ($p < 0.05$) among the peroxide levels generated in TPN solutions. Bars represent the mean ± SD (n=3). Significant differences were determined by two-way ANOVA followed by multiple comparisons using Tukey's HSD. Two-way ANOVA (A): light exposure on peroxide levels, $F(1,8) = 84.20$, $p < 0.0001$; Air and nitrogen exposed AIO-TPN on peroxide levels, $F(1, 8) = 62.27$, $p < 0.0001$; Light exposure × air and nitrogen exposed AIO-TPN interaction, $F(1, 8) = 6.816$, $p = 0.0311$. Two-way ANOVA (B): light exposure on peroxide levels, $F(1,8) = 70.49$, $p < 0.0001$; Air and nitrogen exposed SMOFlipid® on peroxide levels, $F(1, 8) = 3360$, $p < 0.0001$; Light exposure × air and nitrogen exposed SMOFlipid® interaction, $F(1, 8) = 1.811$, $p = 0.2153$. LP: light-protected samples; LE: light-exposed samples.

Figure 3.4

(A)


AIO-TPN
Peroxide levels (mM)
(H_2O_2 equivalents)
3
2
1
0
a
c
d
bc
LP
LE
Air
$N_{2(g)}$

(B)


SMOFLipid®
Peroxide levels (mM)
(H_2O_2 equivalents)
4
3
2
1
0
b
a
d
c
LP
LE
Air
$N_{2(g)}$

3.3 Trace elements on peroxide generation in AIO-TPN and FF-TPN

Even though peroxide levels were lower in AIO-TPN without trace elements (1.377 (±0.067) mM H_2O_2 equivalents) than that in AIO-TPN with trace elements (1.510 (±0.170) mM H_2O_2 equivalents), it was not significantly different ($p > 0.05$). The peroxide levels generated in FF-TPN with or without trace elements were not different ($p > 0.05$) (**Figure 3.5 A and B**). Thus, this study revealed that trace elements did not influence the peroxide generation of 24-hour light-exposed (LE) AIO-TPN or FF-TPN samples.

However, in clinical settings, the preparation of TPN solution by controlling air exposure or under nitrogen exposure practically requires sophisticated methods. Hence, it is necessary to control the peroxide generation in TPN solutions for 24-hour infusion and light exposure at 3000 Lux.

3.4 Peroxide levels in light-protected TPN solutions at 0 and for 24 hours and light-exposed TPN solutions for 24 hours

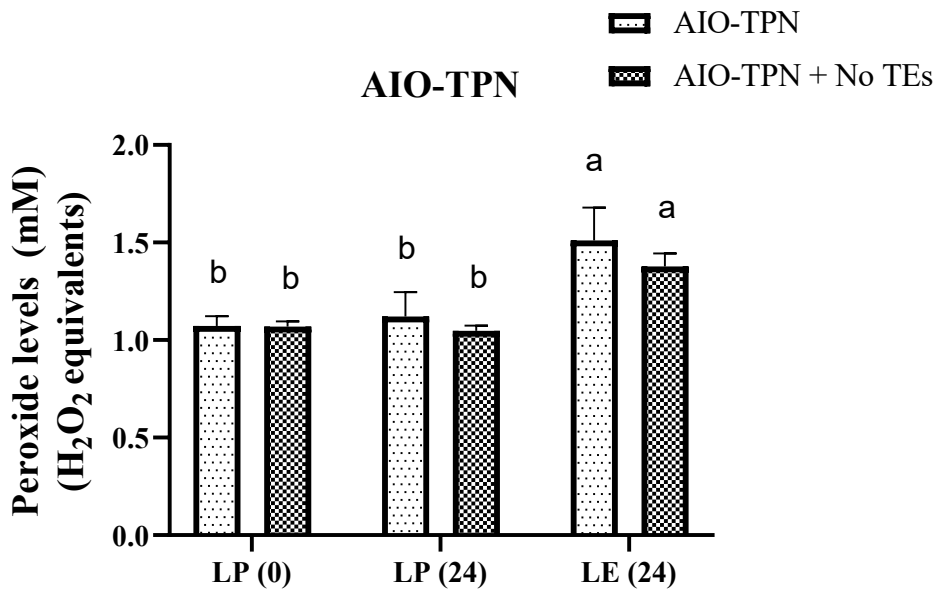
Three aliquots of 2 mL undiluted FF-TPN, SMOFlipid®, and AIO-TPN were immediately sealed under nitrogen flush after preparation. The first aliquot of each solution was wrapped aluminum foil (LP sample for 24 hours) and incubated at 4 °C for 24 hours. The second aliquot of each sample was incubated at 34 °C for 24 hours under light exposure at the intensity of 3000 Lux. The last aliquot of each solution was examined for peroxide levels immediately after preparation (at time zero).

Figure 3.5: Peroxide levels of TPN samples with or without trace elements (TEs).

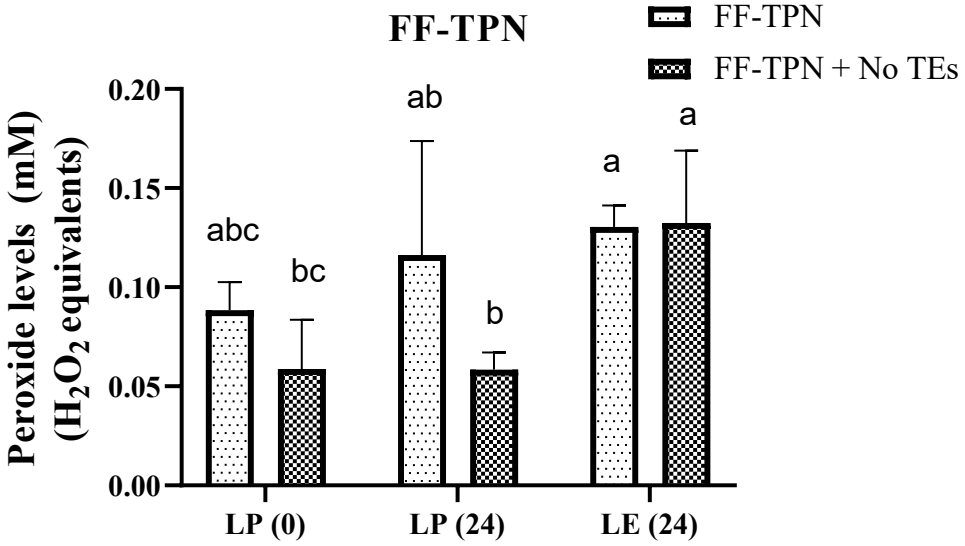
A: AIO-TPN; **B**: FF-TPN. Different lowercase letters indicate significant differences ($p < 0.05$) among the peroxide levels generated in TPN solutions. Bars represent the mean ± SD (n=4); Significant differences were determined by two-way ANOVA followed by multiple comparisons using Tukey's HSD. Two-way ANOVA (A): light exposure on peroxide levels, F (2,12) = 30.54, $p < 0.0001$; TEs added AIO-TPN on peroxide levels, F (1, 12) = 2.459, $p = 0.1428$; Light exposure × TEs added AIO-TPN interaction, F (2, 12) = 0.7194, $p = 0.507$. Two-way ANOVA (B): light exposure on peroxide levels, F (2,18) = 7.762, $p = 0.0037$; TEs added FF-TPN on peroxide levels, F (1, 18) = 5.201, $p = 0.035$; Light exposure × TEs added FF-TPN interaction, F (2, 18) = 1.901, $p = 0.1782$. LP (0 h): light protected sample at time zero; LP (24 h): light-protected sample, incubated for 24 hours; LE (24 h): light-exposed sample for 24 hours; AIO-TPN + No TEs: TPN solution without trace elements.

Figure 3.5

(A)


AIO-TPN
AIO-TPN + No TEs
AIO-TPN
Peroxide levels (mM) (H_2O_2 equivalents)
2.0
1.5
1.0
0.5
0.0
b
b
b
b
a
a
LP (0)
LP (24)
LE (24)

(B)


FF-TPN
FF-TPN + No TEs
FF-TPN
Peroxide levels (mM) (H_2O_2 equivalents)
0.20
0.15
0.10
0.05
0.00
abc
bc
ab
b
a
a
LP (0)
LP (24)
LE (24)

The formation of peroxide in FF-TPN was not significantly different between LP or LE samples (LP (0 hour) = 0.085 (±0.014); LP (24 hours) = 0.106 (±0.054); and LE (24 hours) = 0.133 (±0.112) H_2O_2 equivalents, respectively) ($p > 0.05$) (**Figure 3.6 A**).The peroxide levels generated in SMOFlipid® and AIO-TPN, exposed to light for 24 hours, were significantly increased compared to LP samples at time zero (1.838 (±0.083) vs. 2.526 (±0.159) and 1.050 (±0.055) vs. 1.475 (±0.117) mM H_2O_2 equivalents, respectively) ($p < 0.0001$). However, peroxide levels did not significantly differ between LP at time zero- and for 24-hour samples in both SMOFlipid® and AIO-TPN (**Figure 3.6 B and C**). This study has revealed that the light exposure to the SMOFlipid® or AIO-TPN for 24 hours generated a considerably higher amount of peroxides compared to LP samples.

3.5 Role of antioxidants on the peroxide levels

3.5.1 Vitamin C added AIO-TPN and peroxide levels

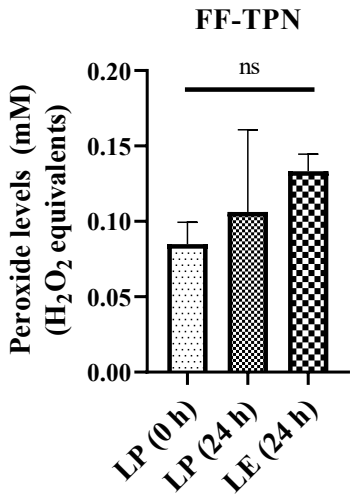
Vitamin C was added to the standard AIO-TPN, prescribed for newborns, at increasing concentration, as described in *section 2.5.* The peroxide levels of 24-hour LE samples were not significantly different with increasing concentrations of vitamin C from 0.90 to 2.27 mM (**Figure 3.7**). For instance, the peroxide levels observed in LE for 24 hour-AIO-TPN that had vitamin C at 2.72 mM were 1.304 (±0.097) mM H_2O_2 equivalents, compared to that of vitamin C at 0.90 mM concentration (1.547 (0.125)) ($p > 0.05$) (**Figure 3.7**). Even though LP for 24 hour-AIO-TPN had slightly higher peroxide levels than LP AIO-TPN at time zero, it was not significantly different ($p > 0.05$) based on one-way ANOVA (**Figure 3.7**). Linear regression analysis was performed to examine the influence of vitamin C in the LE TPN on peroxide levels.

Figure 3.6: Peroxide levels of light exposed (LE) and light protected (LP) parenteral nutrition at time zero or for 24 hours.

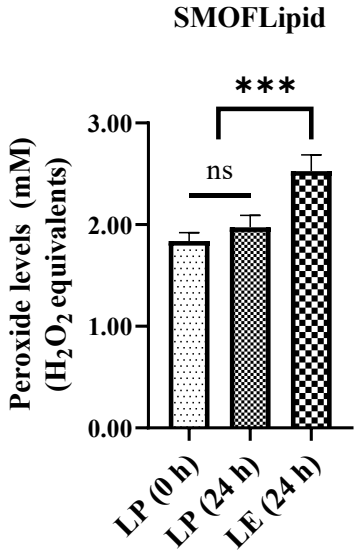
A: Fat-free TPN (FF-TPN) (*n*=4); **B**: Intravenous lipid emulsion (ILE) (SMOFlipid®) (*n*=3); **C**: All-in-one TPN (AIO-TPN) (*n*=8). Bars represent the mean ± SD; ***$p < 0.001$; Significant differences were determined by one-way ANOVA followed by multiple comparisons using Tukey's HSD. LP (0 h): light-protected sample at time zero; LP (24 h): light-protected sample, incubated for 24 hours; LE (24 h): light-exposed sample for 24 hours.

Figure 3.6

(A)



(B)



(C)

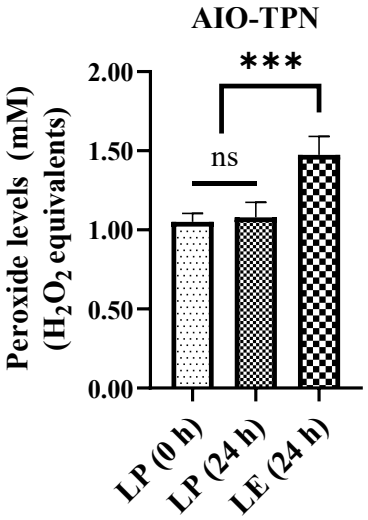
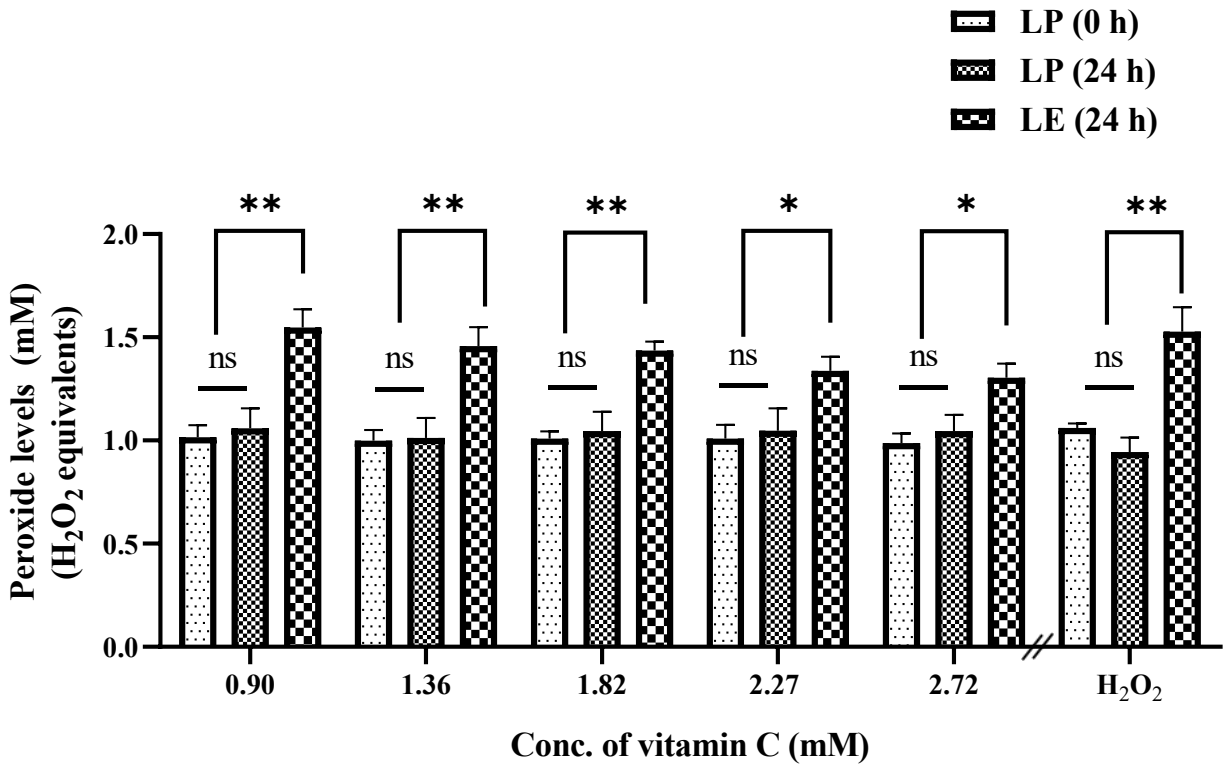


Figure 3.7: Peroxide levels of light exposed (LE) for 24 hours and light protected (LP) vitamin C-added AIO-TPN at time zero (0 hour) or for 24 hours.

Vitamin C was added to AIO-TPN to prepare 1.36, 1.82, 2.27, and 2.72 mM levels; baseline concentration was 0.9 mM; H_2O_2 at 100 μM; peroxide levels are expressed in H_2O_2 equivalents. Bars represent the mean ± SD (n=3). *$p < 0.05$; **$p < 0.01$. Significant differences were determined by one-way ANOVA followed by multiple comparisons using Tukey's HSD. LP (0 h): light-protected sample at time zero; LP (24 h): light-protected sample, incubated for 24 hours; LE (24 h): light-exposed sample for 24 hours.

Figure 3.7


LP (0 h)
LP (24 h)
LE (24 h)
**
**
**
*
*
**
ns
ns
ns
ns
ns
ns
2.0
1.5
1.0
0.5
0.0
Peroxide levels (mM)
(H2O2 equivalents)
0.90
1.36
1.82
2.27
2.72
H2O2
Conc. of vitamin C (mM)

It revealed a significant decreasing trend with increasing vitamin C concentrations from 0.90 to 2.72 mM (R^2=0.65; p = 0.0003). Though it showed a decreasing trend within the concentration range of 0.90 to 2.82 mM, it did not significantly decrease peroxide levels compared to peroxide levels generated in LP TPN solutions ($p > 0.05$).

3.5.2 Vitamin E added AIO-TPN and peroxide levels

Vitamin E (DL-α-tocopherol acetate) was added to the standard AIO-TPN at increasing concentrations, as described in *section 2.5*. The peroxide levels generated in 24 hour-LE AIO-TPN solutions did not change by increasing the concentration of vitamin E from 64.87 to 153.71 μM (the peroxide levels at 64.87 μM = 1.474 (±0.009) mM and at 153.71 μM concentration = 1.466 (±0.026) mM H_2O_2 equivalents ($p > 0.05$) (**Figure 3.8**). The peroxide levels generated in the LP for 24 hour-AIO-TPN and LP at time zero samples were not different ($p > 0.05$) (**Figure 3.8**) based on one-way ANOVA. Linear regression analysis was performed to examine the influence of vitamin E in the LE TPN on peroxide levels. It revealed no significant trend with increasing vitamin E concentrations from 64.87 to 153.71 mM (R^2=0.08; p = 0.37).

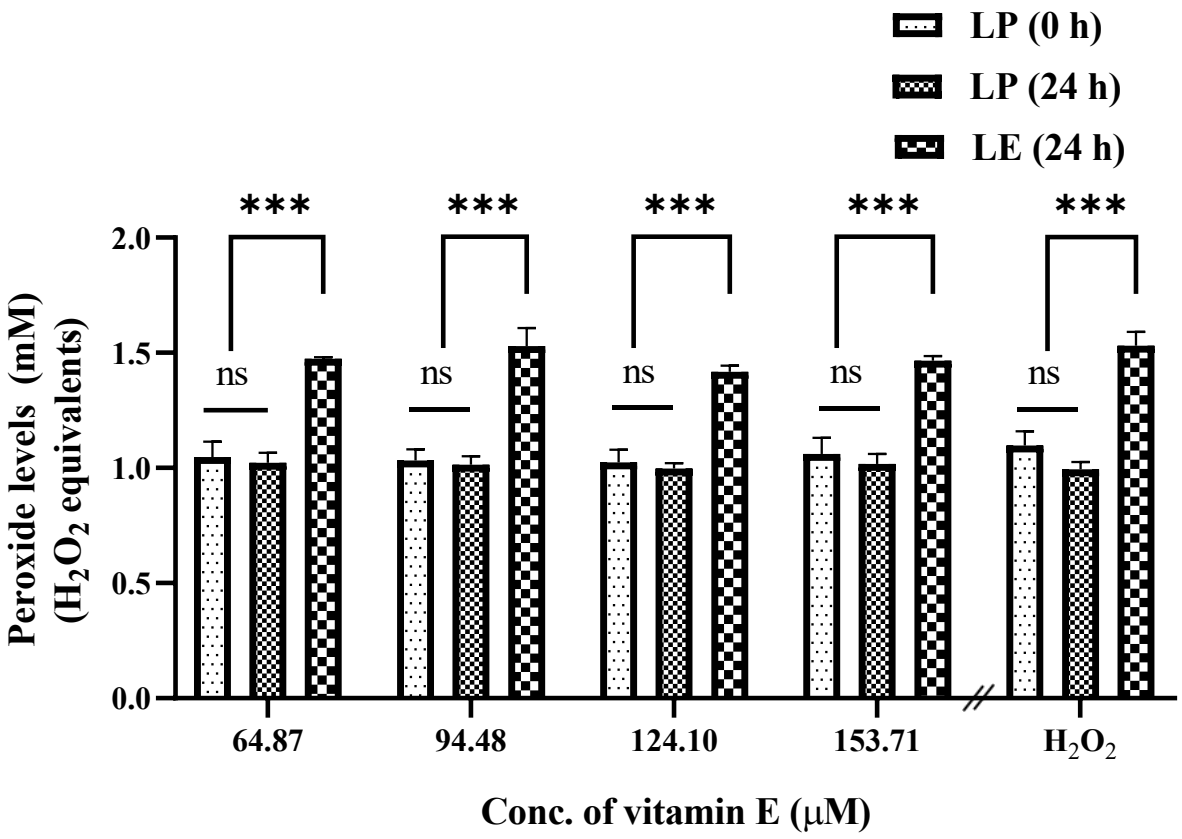
3.5.3 Combined effect of vitamins C and E on peroxidation of AIO-TPN

Vitamins C and E were added at various combinations to AIO-TPN to assess the peroxide levels, as described in *section 2.6.* Combination of vitamin C and E at increasing levels of vitamins C and E to the AIO-TPN gradually decreased the peroxide levels (**Figure 3.9 A**).

Figure 3.8: Peroxide levels in vitamin E added light protected (LP) AIO-TPN at time zero and for 24 hours and light exposed (LE) AIO-TPN for 24 hours.

AIO-TPN was supplemented with vitamin E from 94.48 to 153.71 μM (The baseline concentration was 64.87 μM). The positive control H_2O_2 was at 100 μM. Peroxide levels are expressed in H_2O_2 equivalents. Bars represent the mean ± SD (n=3); ***$p < 0.001$. Significant differences were determined by one-way ANOVA followed by multiple comparisons using Tukey's HSD. LP (0 h): light-protected sample at time zero; LP (24 h): light-protected sample, incubated for 24 hours; LE (24 h): light-exposed sample for 24 hours.

Figure 3.8


LP (0 h)
LP (24 h)
LE (24 h)
***
***
***
***
***
ns
ns
ns
ns
ns
2.0
1.5
1.0
0.5
0.0
Peroxide levels (mM)
(H2O2 equivalents)
64.87
94.48
124.10
153.71
H2O2
Conc. of vitamin E (μM)

When the vitamins C and E increased from baseline concentrations (0.90 mM of vitamin C and 0.64 μM of vitamin E) to the combination of vitamin C at 2.27 mM and vitamin E at 124.1 μM, the peroxide levels decreased significantly from 1.748 (±0.072) to 1.173 (±0.024) mM H_2O_2 equivalents ($p < 0.0001$) (**Table 3.1**). The highest peroxide concentration (1.873 (±0.146) mM H_2O_2 equivalents) was observed in AIO-TPN that had vitamin C at 0.91 mM and vitamin E at 94.48 μM concentrations. Vitamin C at 0.91 mM in all combinations of increasing vitamin E did not decrease the peroxide levels (**Figure 3.9 B**). With vitamin E at 153.7 μM concentrations, the peroxide levels were not changed when vitamin C increased from 1.36 to 2.27 mM. This could be explained by an inefficient scavenging action of vitamin E at higher concentrations and lack of sufficient vitamin C concentrations. This study also revealed that additive effect of vitamin C and E decreased the peroxide levels. In summary, vitamin C at 2.27 mM and vitamin E at 124.1 μM concentrations effectively decreased the peroxide levels.

3.5.4 Selenium added AIO-TPN and peroxide levels

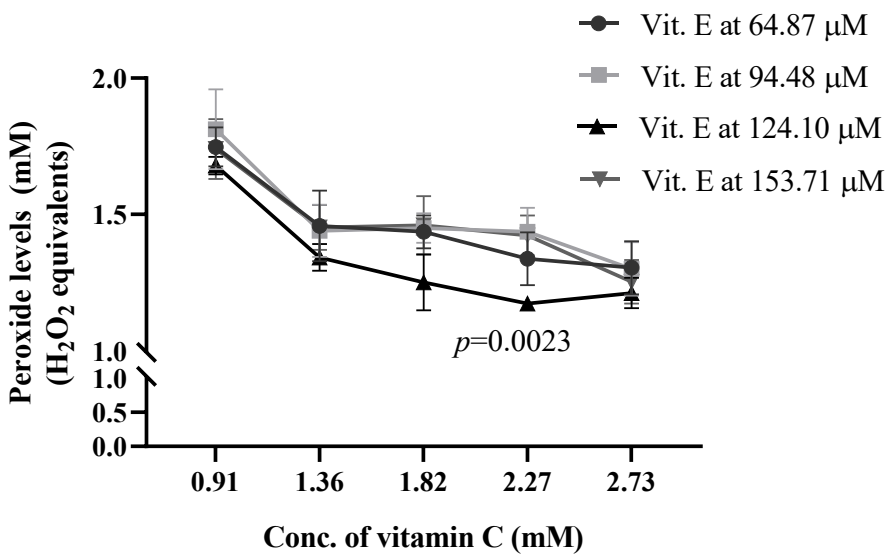
The selenium concentration of standard AIO-TPN was 0.12 μM (baseline concentration). When increased the selenium concentrations in AIO-TPN increased from 0.12 to 0.36 μM, together with light exposure for 24 hours, the peroxide levels did not change significantly (at 0.12 μM concentration =1.554 (±0.039) mM vs. at 0.36 μM = 1.543 (±0.011) mM H_2O_2 equivalents; $p > 0.05$). However, the lowest level of peroxide was observed (1.378 (±0.083) mM H_2O_2 equivalents) in the AIO-TPN, which had the selenium level at 0.24 μM concentration (**Figure 3.10**).

Figure 3.9: Peroxide levels of vitamins C- and E-added AIO-TPN exposed to light for 24 hours.

AIO-TPN was supplemented with vitamin C from 0.90 to 2.72 mM, and vitamin E from 64.87 to 153.71 μM. The positive control H_2O_2 was at 100 μM. **A**: Increasing concentration of vitamin C with each level of vitamin E in the AIO-TPN; **B**: Increasing concentration of vitamin E with each level of vitamin C in the AIO-TPN. Peroxide levels are expressed in H_2O_2 equivalents. Significant differences were determined by two-way ANOVA followed by multiple comparisons using Tukey's HSD. LP (0 h): light-protected sample at time zero; LP (24 h): light-protected sample, incubated for 24 hours; LE (24 h): light-exposed sample for 24 hours; *n*=3.

Figure 3.9

(A)


Vit. E at 64.87 μM
Vit. E at 94.48 μM
Vit. E at 124.10 μM
Vit. E at 153.71 μM
Peroxide levels (mM) (H_2O_2 equivalents)
2.0
1.5
1.0
1.0
0.5
0.0
p=0.0023
0.91
1.36
1.82
2.27
2.73
Conc. of vitamin C (mM)

(B)

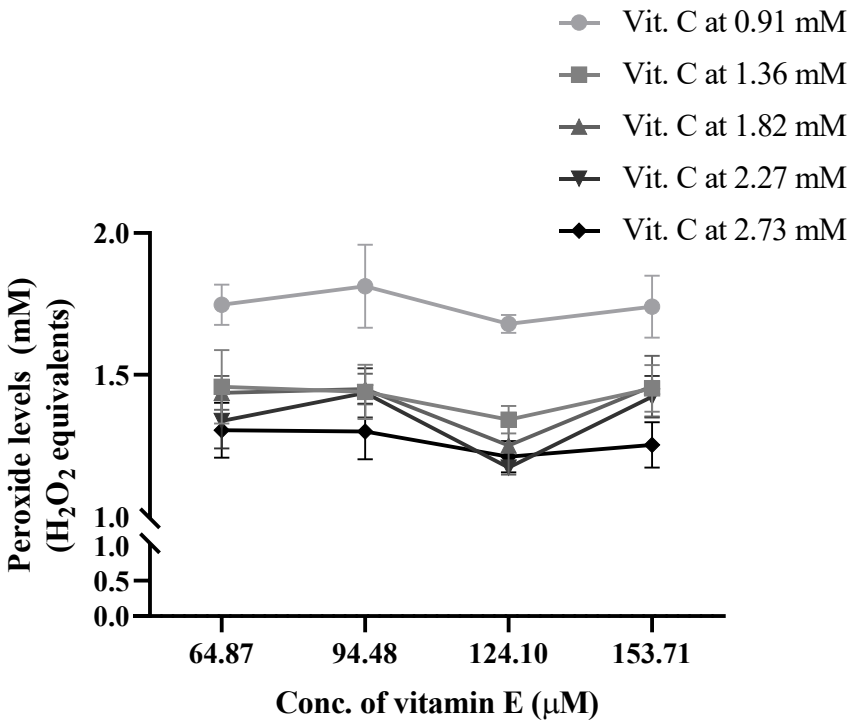

Vit. C at 0.91 mM
Vit. C at 1.36 mM
Vit. C at 1.82 mM
Vit. C at 2.27 mM
Vit. C at 2.73 mM
Peroxide levels (mM) (H_2O_2 equivalents)
2.0
1.5
1.0
1.0
0.5
0.0
64.87
94.48
124.10
153.71
Conc. of vitamin E (μM)

Table 3.1: Peroxide levels in AIO-TPN after adding various concentrations of vitamins C and E.

The vitamin C at the concentrations of 24 mg (0.91 mM) (baseline), 36 mg (1.36 mM), 48 mg (1.82 mM), 60 mg (2.27 mM), and 72 mg (2.73 mM) per day; and vitamin E at 4.6 mg (64.87 μM) (baseline), 6.7 mg (94.48 μM), 8.8 mg (124.10 μM), and 10.9 mg (153.7 μM) per day; were added.

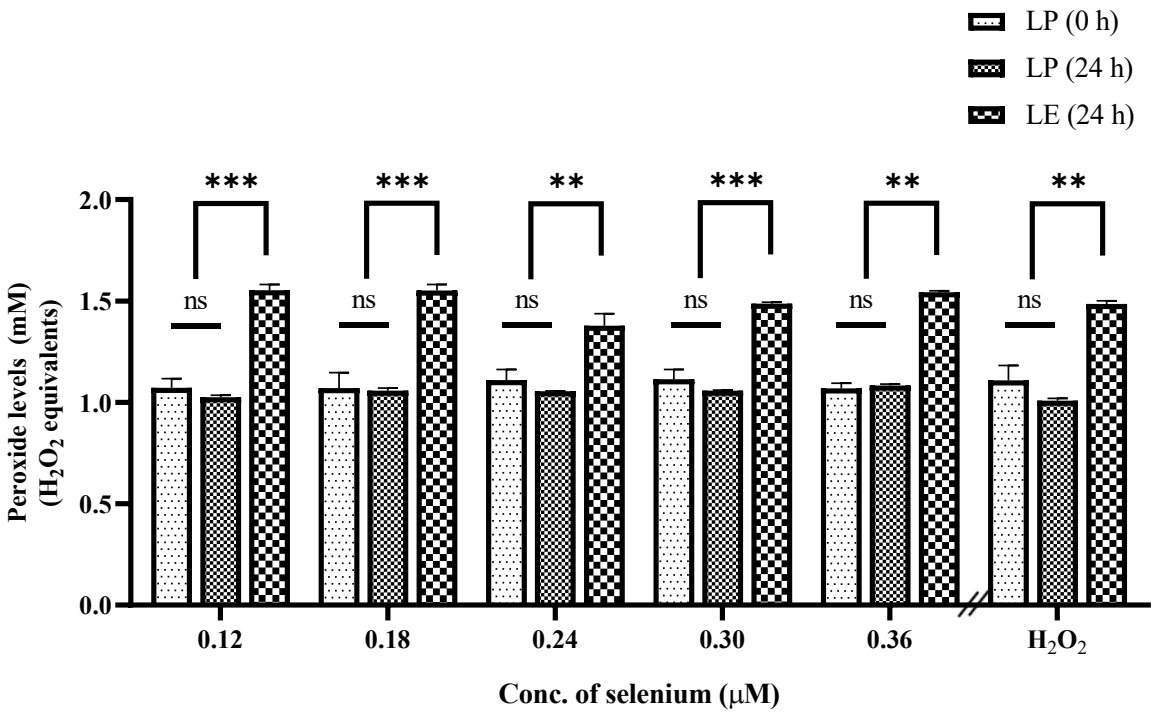
		Vitamin E mg (μM)			
		4.6 (64.87)*	**6.7 (94.48)**	**8.8 (124.10)**	**10.9 (153.71)**
Vitamin C mg (μM)	**24 (0.91)***	1.748 (0.072)	1.813 (0.146)	1.679 (0.032)	1.74 (0.110)
	36 (1.36)	1.458 (0.129)	1.440 (0.095)	1.342 (0.049)	1.452 (0.082)
	48 (1.82)	1.436 (0.060)	1.450 (0.054)	1.250 (0.102)	1.460 (0.107)
	60 (2.27)	1.337 (0.097)	1.436 (0.087)	**1.173 (0.024)**	1.422 (0.074)
	72 (2.73)	1.304 (0.097)	1.300 (0.098)	1.212 (0.056)	1.253 (0.080)

*Baseline concentration found in the AIO-TPN (standard) for newborns. Two-way ANOVA was performed to analyze the combination effect of vitamins C and E on the generation of peroxide levels. The main effect analysis of vitamin C depicted a statistically significant effect on decreasing the peroxide levels ($F(4, 40) = 52.09$, $p < 0.0001$). The main effect analysis of vitamin E showed a statistically significant effect on decreasing the peroxide levels ($F(3, 40) = 9.646$, $p < 0.0001$). The results showed that there was no statistically significant interaction between the effects of vitamins C and E ($F(12, 40) = 0.734$, $p = 0.711$).

Figure 3.10: Peroxide levels of light exposed (LE) for 24 hours and light protected (LP) selenium-added AIO-TPN that at time zero or for 24 hours.

AIO-TPN was supplemented with selenium from 0.18 to 0.36 μM (baseline concentration was 0.12 μM). The positive control H_2O_2 was at 100 μM. Peroxide levels are expressed as H_2O_2 equivalents. Bars represent the mean ± SD (n=3). ***$p < 0.001$. Significant differences were determined by one-way ANOVA followed by multiple comparisons using Tukey's HSD. LP (0 h): light-protected sample at time zero; LP (24 h): light-protected sample, incubated for 24 hours; LE (24 h): light-exposed sample for 24 hours.

Figure 3.10


LP (0 h)
LP (24 h)
LE (24 h)
***
***
**
***
**
**
ns
ns
ns
ns
ns
ns
2.0
1.5
1.0
0.5
0.0
Peroxide levels (mM)
(H_2O_2 equivalents)
0.12
0.18
0.24
0.30
0.36
H_2O_2
Conc. of selenium (μM)

3.5.5 GSSG added AIO-TPN and peroxide levels

Glutathione in any form, such as GSH or GSSG, was not in the commercially available standard AIO-TPN. GSSG at increasing concentrations at 0, 10, 20, 30, 40, and 50 μM to the AIO-TPN was added and incubated for 24 hours at the light intensity of 3000 Lux. The peroxide levels did not decrease up to the concentration of 30 μM. When the GSSG was increased from 30 to 40 μM, the peroxide levels significantly decreased from 1.612 (± 0.059) to 1.255 (± 0.120) mM H_2O_2 equivalents ($p < 0.01$), and it further decreased to 1.218 (± 0.101) mM H_2O_2 equivalents at 50 μM concentrations ($p < 0.001$) (**Figure 3.11**). AIO-TPN at 40 μM concentrations of GSSG, the peroxide levels between LP AIO-TPN at time zero (0 h) and LE for 24 hours were not significantly different ($p > 0.05$). A similar finding was observed for peroxide in AIO-TPN at 50 μM concentrations (**Figure 3.11**).

3.6 Role of prooxidants in generation of peroxides

3.6.1 Copper added AIO-TPN and peroxide levels

The concentration of copper in standard AIO-TPN, given to the neonates, was 4.2 μM. Decreasing the concentration of copper for AIO-TPN from 4.2 to 1.05 μM did not decrease the peroxide levels (1.327 (±0.137) to 1.334 (±0.052) mM H_2O_2 equivalents; $p > 0.05$) (**Figure 3.12 A**). Similarly, there was no effect on peroxide levels between LP AIO-TPN samples at time zero and LE AIO-TPN for 24 hours. On the other hand, increasing concentrations of copper from 42 to 42,000 μM decreased the peroxide levels significantly (at 42 μM = 1.385 (±0.046) mM vs. at 42,000 μM = 1.160 (±0.081) mM H_2O_2 equivalents; $p < 0.01$) **(Figure 3.12 B).**

Figure 3.11: Peroxide levels of light exposed (LE) for 24 hours and light protected (LP) reduced form of glutathione (GSSG)-added AIO-TPN at time zero or for 24 hours.

AIO-TPN was supplemented with GSSG from 10 to 50 μM. The positive control H_2O_2 was at 100 μM. Peroxide levels are expressed in H_2O_2 equivalents. Bars represent the mean ± SD (n=3); *$p < 0.05$, **$p < 0.01$, and ***$p < 0.001$. Significant differences were determined by one-way ANOVA followed by multiple comparisons using Tukey's HSD; LP (0 h): light-protected sample at time zero; LP (24 h): light-protected sample, incubated for 24 hours; LE (24 h): light-exposed sample for 24 hours.

Figure 3.11

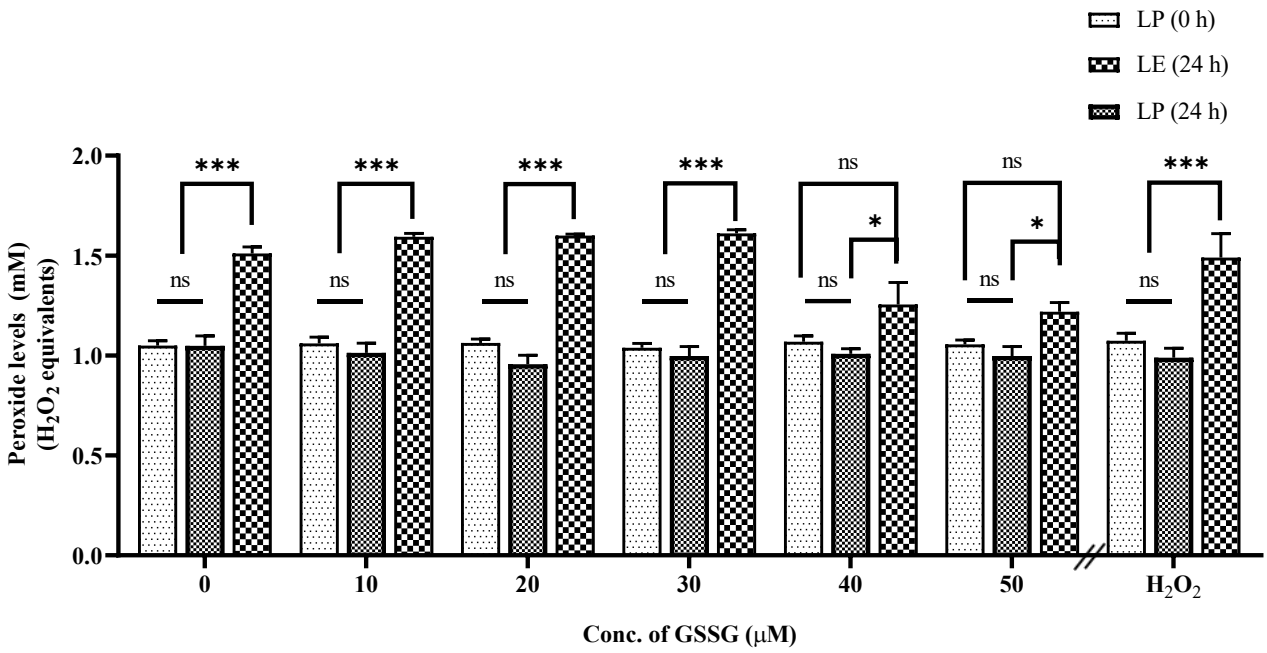
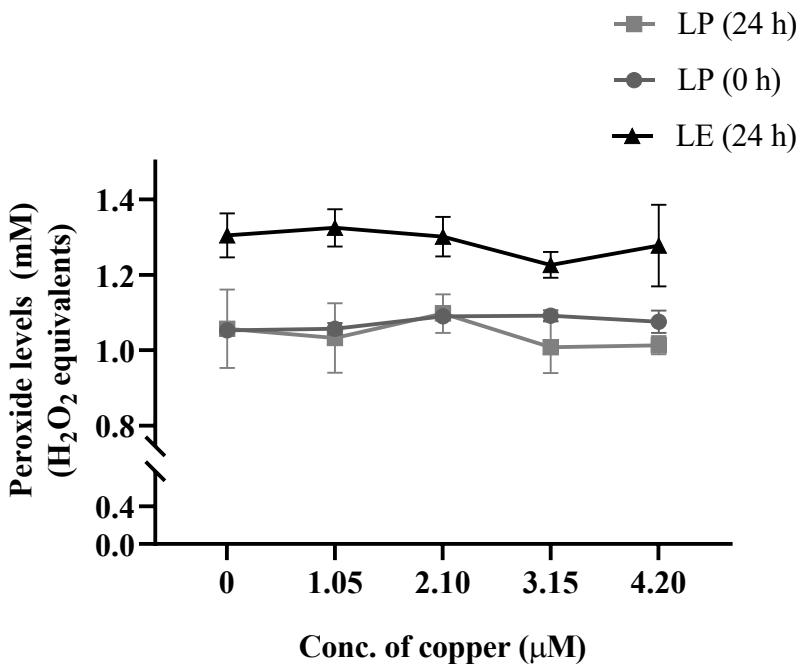

LP (0 h)
LE (24 h)
LP (24 h)
Peroxide levels (mM)
(H_2O_2 equivalents)
2.0
1.5
1.0
0.5
0.0
***
***
***
***
ns
ns
***
ns
ns
ns
ns
ns
ns
*
*
0
10
20
30
40
50
H_2O_2
Conc. of GSSG (μM)

Figure 3.12: Peroxide levels of light exposed (LE) for 24 hours and light protected (LP) copper-added AIO-TPN at time zero or 24 hours.

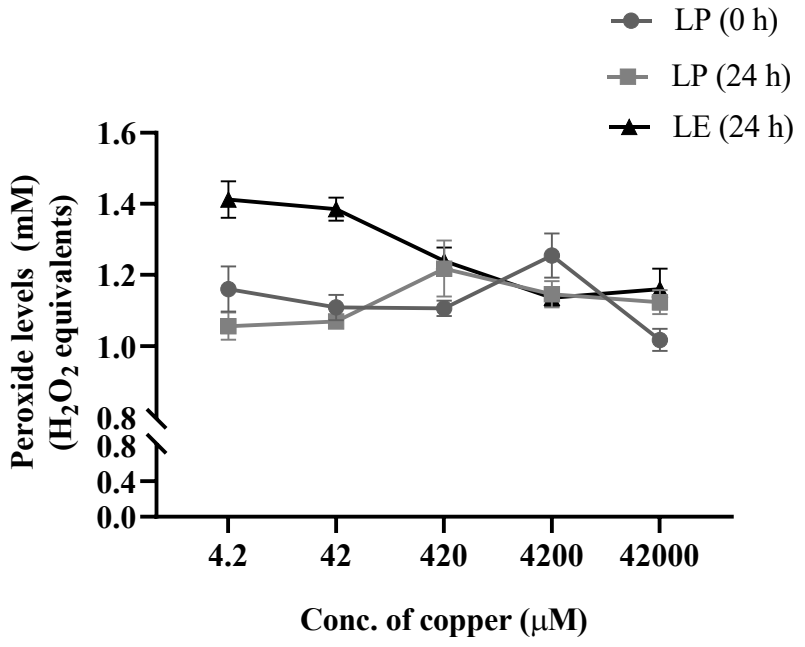
A: Copper levels in the AIO-TPN at decreasing concentrations from 4.2 to 1.05 μM; 4.2 μM: standard concentration in AIO-TPN for neonates; **B**: Copper levels in the AIO-TPN at increasing concentrations from the standard concentration to 42,000 μM (toxic level) to assess the peroxidation status. Peroxide levels are expressed in H_2O_2 equivalents; Significant differences were determined by one-way ANOVA followed by multiple comparisons using Tukey's HSD for each condition. LP (0 h): light-protected sample at time zero; LP (24 h): light-protected sample, incubated for 24 hours; LE (24 h): light-exposed sample for 24 hours; *n*=3.

Figure 3.12

(A)



(B)



3.6.2 Zinc added AIO-TPN and peroxide levels

The concentration of zinc in the standard AIO-TPN, given to the neonates, was 30.6 μM. Decreasing the concentration of zinc from 30.6 to 10.2 μM did not significantly decrease the peroxide levels, as observed in zinc-added AIO-TPN (1.373 (±0.120) vs. 1.424 (±0.053) mM H_2O_2 equivalents ($p > 0.05$) (**Figure 3.13 A**). Increasing the concentration of zinc from 30.6 to 30,600 μM did not alter the peroxide levels significantly (at 30.6 μM = 1.498 (±0.039) and at 30,600 μM = 1.417 (±0.055) mM H_2O_2 equivalents). Even though the peroxide levels were slightly elevated beyond the concentrations of 30.6 μM, the prooxidant status of zinc at higher concentration was not noticed in this study (**Figure 3.13 B**).

3.6.3 Combined effect of copper and zinc on peroxide levels

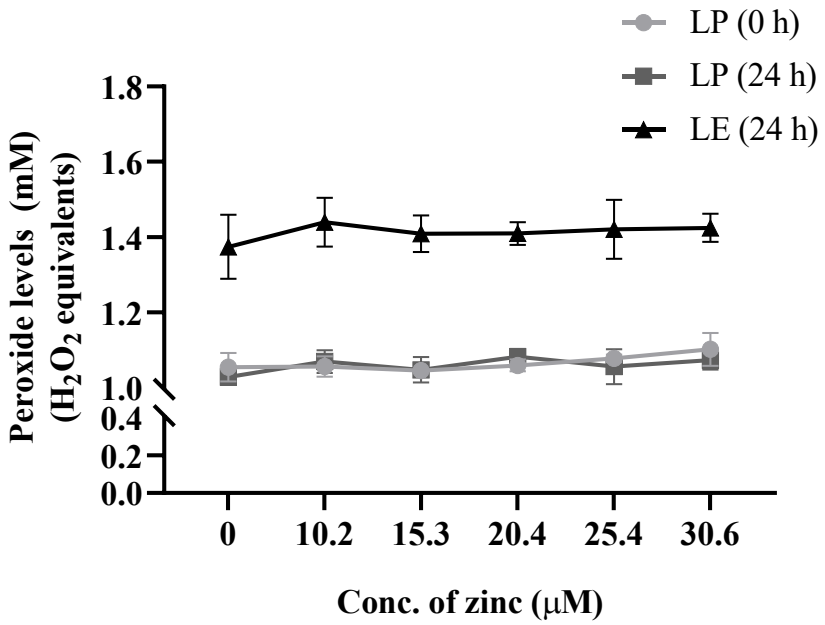
Copper and zinc were added at various combinations to AIO-TPN to assess the peroxide levels as described in *section 2.7.1.* Decreasing combinations of copper from 4.2 to 1.05 μM and zinc from 30.6 to 10.2 μM did not decrease the peroxide levels (**Figure 3.14)**. The peroxides in AIO-TPN that contains copper and zinc at various levels were within the range of 1.33 to 1.50 mM H_2O_2 equivalents (**Table 3.2**). Two-way ANOVA followed by Turkey's HSD multiple comparisons revealed that decreasing zinc levels increased the peroxide levels at fixed copper levels ($F(2, 24) = 12.91$, $p = 0.0002$), whereas decreasing copper levels did not decrease the peroxide levels at fixed zinc levels ($F(3, 24) = 0.219$, $p = 0.8822$) and no significant interactions found between zinc and copper levels ($F(6, 24) = 0.9414$, $p = 0.4843$).

Figure 3.13: Peroxide levels of light exposed (LE) for 24 hours and light protected (LP) zinc-added AIO-TPN at time zero or 24 hours.

A: Zinc levels in the AIO-TPN at decreasing concentrations from 30.6 to 10.2 μM (The standard concentration was 30.6 μM). **B**: Zinc levels in the AIO-TPN at increasing concentrations from the baseline concentration to 42,000 μM (toxic level) to assess the peroxidation status. Peroxide levels are expressed in H_2O_2 equivalents. Significant differences were determined by one-way ANOVA followed by multiple comparisons using Tukey's HSD for each condition. LP (0 h): light-protected sample at time zero; LP (24 h): light-protected sample, incubated for 24 hours; LE (24 h): light-exposed sample for 24 hours; *n*=3

Figure 3.13

(A)



(B)

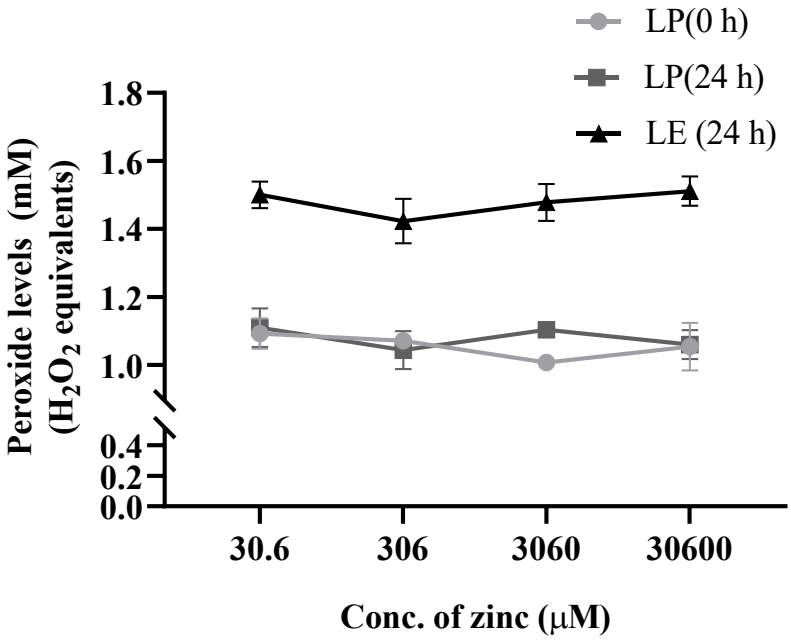


Table 3.2: Peroxide levels in AIO-TPN after adding various concentrations of copper and zinc.

Zinc at 0.1 mg (10.2 μM), 0.25 mg (25.5 μM), and 0.3 mg (30.6 μM) (baseline concentration); and copper at 0.01 mg (1.04 μM), 0.02 mg (2.09 μM), 0.03 mg (3.15 μM), and 0.04 mg (4.20 μM) (baseline concentration); were added.

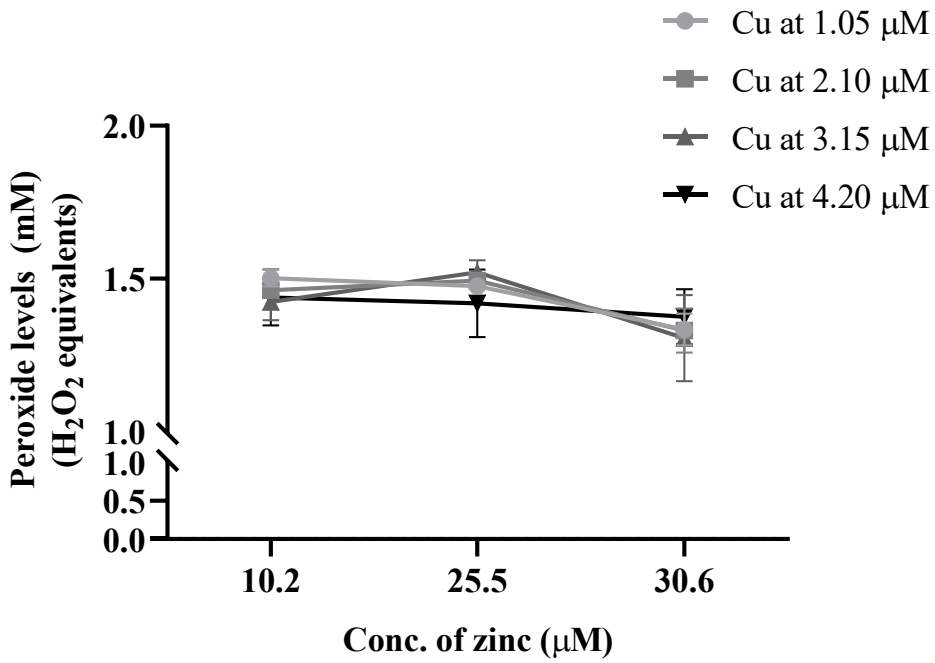
		Copper mg (μM)			
		0.01 (1.04)	**0.02 (2.09)**	**0.03 (3.15)**	**0.04 (4.20)***
Zinc mg (μM)	**0.1 (10.2)**	1.501 (0.027)	1.463 (0.026)	1.424 (0.061)	1.439 (0.092)
	0.25 (25.5)	1.475 (0.026)	1.494 (0.024)	1.520 (0.040)	1.420 (0.111)
	0.3 (30.6)*	1.334 (0.052)	1.330 (0.072)	1.305 (0.141)	1.376 (0.091)

*Baseline concentration found in the AIO-TPN (standard) for newborns. Two-way ANOVA was performed to analyze the combination effect of copper and zinc on the generation of peroxide levels. The main effects analysis of copper depicted no significant effect on decreasing the peroxide levels ($F(3, 24) = 0.219$, $p = 0.8822$). The main effects analysis of decreasing zinc showed a statistically significant effect on increasing the peroxide levels ($F(2, 24) = 12.91$, $p = 0.0002$). The results showed that there was no significant interaction between the effects of copper and zinc ($F(6, 24) = 0.941$, $p = 0.484$).

Figure 3.14: Peroxide levels of copper- and zinc-added AIO-TPN exposed to light for 24 hours.

Copper levels in the AIO-TPN was at decreasing levels from 4.20 to 1.05 μM and zinc at decreasing levels from 30.6 to 10.2 μM. Peroxide levels are expressed in H_2O_2 equivalents. Significant differences were determined by two-way ANOVA followed by multiple comparisons using Tukey's HSD. Two-way ANOVA: copper levels on peroxide levels, F (3,24) = 0.219, $p <$ 0.882; zinc levels on peroxide levels, F (2, 24) = 12.91, $p = 0.0002$; copper × zinc interaction, F (6, 24) = 0.941, $p = 0.484$. LE (24 h): light-exposed sample for 24 hours; n=3.

Figure 3.14


Cu at 1.05 μM
Cu at 2.10 μM
Cu at 3.15 μM
Cu at 4.20 μM
Peroxide levels (mM)
(H_2O_2 equivalents)
2.0
1.5
1.0
1.0
0.5
0.0
10.2
25.5
30.6
Conc. of zinc (μM)

3.7 Optimal concentration of selected nutrients in the AIO-TPN to minimize the peroxidation

Optimized AIO-TPN was prepared by adding the optimized nutrients that were developed in this study **(Table 3.3)**. The standard AIO-TPN, routinely prescribed for newborns at NICU, was also prepared as a control diet. One portion of the aliquots of optimized and standard AIO-TPNs had 100 μM H_2O_2 or *t*-BHP to induce the peroxide levels (positive controls). The optimized and standard AIO-TPNs were incubated for 0 or 24 hours with or without light exposure and examined for peroxide levels. The results revealed that LE-standard AIO-TPN for 24 hours generated 1.439 (±0.074) mM H_2O_2 equivalents compared to that of LP-standard AIO-TPN at time zero (0.993 (±0.067) mM H_2O_2 equivalents) ($p < 0.0001$) (**Figure 3.15**). Conversely, peroxide levels generated in the LE-optimized AIO-TPN for 24 hours were significantly decreased compared to LE-standard AIO-TPN for 24 hours (1.439 (±0.074) vs. 1.165 (±0.042) mM H_2O_2 equivalents ($p < 0.0001$). LE-optimized AIO-TPN for 24 hours had slightly higher peroxide levels compared to LP-optimized AIO-TPN for 24 hours (1.012 (±0.040) mM H_2O_2 equivalents), followed by LP-optimized TPN at time zero (1.005 (±0.012) mM H_2O_2 equivalents). The peroxide levels of H_2O_2-added LE-standard AIO-TPN and optimized AIO-TPN for 24 hours did not change ($p > 0.05$).

Table 3.3: Optimal concentration of antioxidants and minimal concentration of prooxidants to minimize the elevated peroxidation due to light exposure for 24 hours.
The peroxide levels were determined by using FOX version II assay. The optimization of each nutrient was based on adding such nutrients to the standard AIO-TPN for newborns at clinical settings.

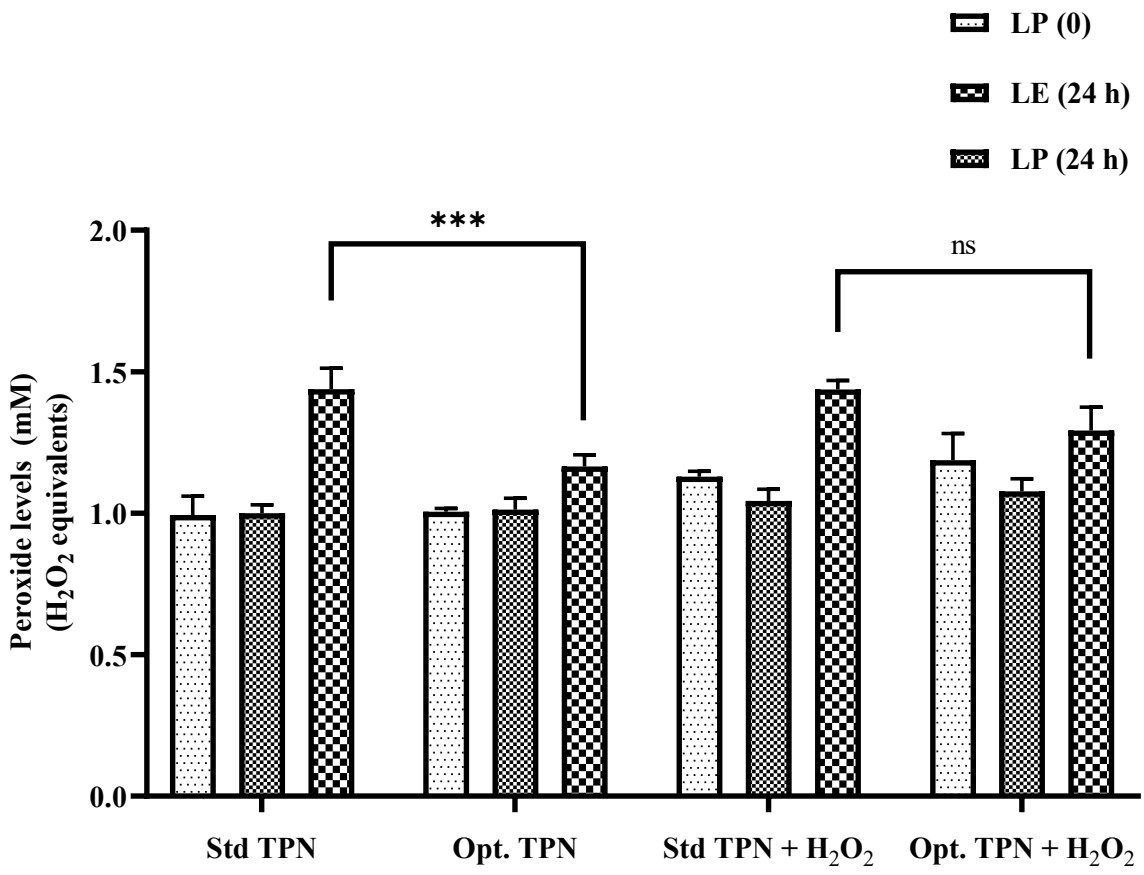
Micronutrient	Std. TPN Conc. (amount per kg/day)	Opt. TPN Conc. (amount per kg/day)
Vitamin C	0.9 mM (24 mg)	2.27 mM (60 mg)
Vitamin E	64.87 μM (4.6 mg)	124.1 μM (8.8 mg)
Selenium	0.12 μM (2 μg)	0.24 μM (4 μg)
Glutathione	0	40 μM (4 mg)
Copper	4.2 μM (40 μg)	4.2 μM (40 μg)
Zinc	30.6 μM (300 μg)	30.6 μM (300 μg)

Std. AIO-TPN: standard all-in-one total parenteral nutrition; Opt. AIO-TPN: optimized AIO-TPN. Vitamin C in the form of L-ascorbic acid; vitamin E in the form of DL-α-tocopherol acetate; selenium in the form of selenium dioxide monohydrate; glutathione in the form of the oxidized form of glutathione (GSSG), copper in the form of copper sulfate; and zinc at the form of zinc sulfate.

Figure 3.15: Peroxide levels of light exposed (LE) for 24 hours and light protected (LP) optimized AIO-TPN at time zero or for 24 hours.

AIO-TPN was supplemented with vitamin C at 2.27 mM, vitamin E at 124.1 μM, selenium at 0.24 μM, oxidized form of glutathione (GSSG) at 40 μM, copper at 4.2 μM, and zinc at 30.6 μM. Peroxide levels are expressed in H_2O_2 equivalents. Bars represent the mean ± SD (n=3). ***$p < 0.001$; Significant differences were determined by two-way ANOVA: light exposure on peroxide levels, F (2,20) = 107.5, $p < 0.0001$; Standard and optimized AIO-TPNs on peroxide levels, F (3, 20) = 11.06, $p = 0.0002$; Light exposure × standard and optimized AIO-TPNs interaction, F (6, 20) = 7.391, $p = 0.0003$. LP (0 h): light-protected AIO-TPN at time zero; LP (24 h): light-protected AIO-TPN, incubated for 24 hours; LE (24 h): light-exposed AIO-TPN for 24 hours.

Figure 3.15


LP (0)
LE (24 h)
LP (24 h)
***
ns
2.0
1.5
1.0
0.5
0.0
Peroxide levels (mM)
(H_2O_2 equivalents)
Std TPN
Opt. TPN
Std TPN + H_2O_2
Opt. TPN + H_2O_2

3.8 Cell viability and metabolic activity of THP-1 cells treated with SMOFlipid® and AIO-TPN

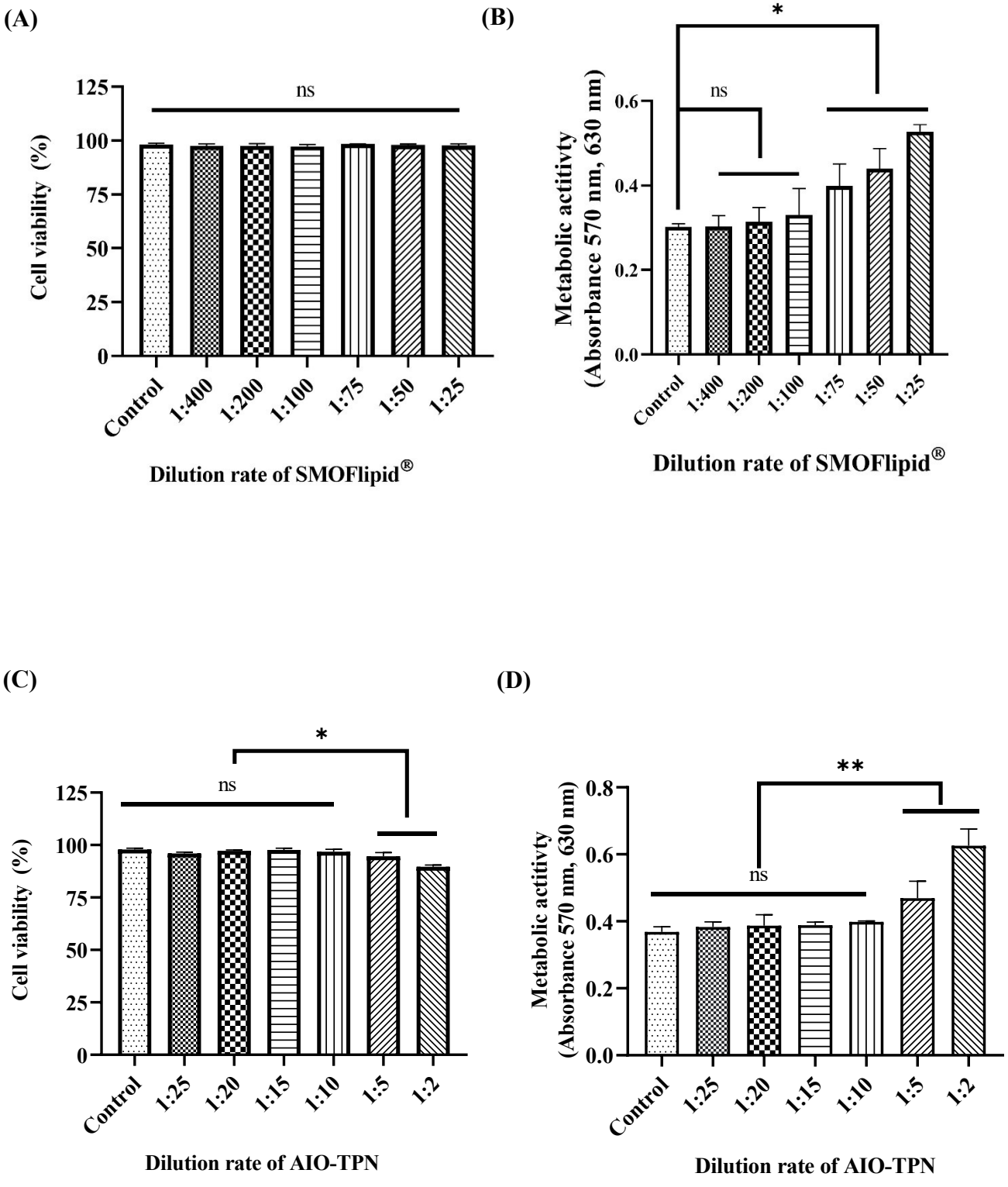
To examine the effectiveness of optimized AIO-TPN developed in the *section 3.7* or antioxidant (vitamins C and E) added SMOFlipid® at cellular levels, THP-1 human monocytic cells were used to assess the lipid peroxidation. Hence, to determine the suitable concentration of AIO-TPN or SMOFlipid® to the cells, serial dilutions of AIO-TPN and SMOFlipid® were treated to the THP-1 cells and incubated for 16 hours (see *section 2.15*). The results showed that cell viability of THP-1 cells upon treatment with 4% (1:25), 2% (1:50), 1.34% (1:75), 1% (1:100), 0.5% (1:200), and 0.25% (1:400) *v/v* of SMOFlipid® did not change the cell viability compared to control (THP-1 cells without SMOFlipid® treatment) (**Figure 3.16 A**), whereas metabolic activity based on MTT assay was gradually decreased with increasing dilution of SMOFlipid® (**Figure 3.16 B**). However, the metabolic activity was not significantly increased until the dilution from 1 to 0.25% ($p < 0.05$), while the metabolic activity was significantly increased from 1 to 4% dilution and those were significantly different compared to control (**Figure 3.16 B**). In consideration of both cell viability and metabolic activity, 1% SMOFlipid® did not alter THP-1 activity.

Cell viability of AIO-TPN diluted from 10% (1:10) to 4% (1:25) was not changed, compared to control, whereas a 20% (1:5) dilution of AIO-TPN reduced the cell viability significantly from 97.9 (±0.6) to 94.6 (±1.8) % ($p < 0.01$). The cell viability was further reduced to 89.53 (±0.86) % ($p < 0.0001$) when treated with 50% (1:2) dilution of AIO-TPN to the THP-1 cells (**Figure 3.16 C**).

Figure 3.16: Cell viability (A and C) and metabolic activity (B and D) of THP-1 cells on serial dilution of SMOFlipid® (A and B) and AIO-TPN (C and D).

Control: untreated THP-1 cells with media. Bars represent the mean ± SD (n=3); *$p < 0.05$, **$p < 0.01$. Significant differences were determined by one-way ANOVA followed by multiple comparisons using Dunnett's post hoc test. Serial dilutions of SMOFlipid® of 4% (1:25), 2% (1:50), 1.34% (1:75), 1% (1:100), 0.5% (1:200), and 0.25% (1:400) *v/v*, and AIO-TPN of 50% (1:2), 20% (1:5), 10% (1:10), 6.7% (1:15), 5% (1:20) and 4% (1:25) *v/v*, were prepared.

Figure 3.16


(A)
Cell viability (%)
125
100
75
50
25
0
ns
Control
1:400
1:200
1:100
1:75
1:50
1:25
Dilution rate of SMOFlipid®
(B)
Metabolic actitivty
(Absorbance 570 nm, 630 nm)
0.6
0.4
0.2
0.0
*
ns
Control
1:400
1:200
1:100
1:75
1:50
1:25
Dilution rate of SMOFlipid®
(C)
Cell viability (%)
125
100
75
50
25
0
*
ns
Control
1:25
1:20
1:15
1:10
1:5
1:2
Dilution rate of AIO-TPN
(D)
Metabolic actitivty
(Absorbance 570 nm, 630 nm)
0.8
0.6
0.4
0.2
0.0
**
ns
Control
1:25
1:20
1:15
1:10
1:5
1:2
Dilution rate of AIO-TPN

On the other hand, the metabolic activity of the cells was not altered until the dilutions from 10 to 4%, whereas the metabolic activity was significantly increased at the dilution of 20% and 50% ($p > 0.01$) (**Figure 3.16 D**). This study revealed that 1% SMOFlipid® and 10% AIO-TPN did not alter the cell viability or metabolic activity of THP-1 human monocytes.

3.9 Optimal concentration of H_2O_2 and *t*-BHP for the cells to induce oxidants

THP-1 cells were treated with increasing concentrations of H_2O_2 or *t*-BHP and incubated for 16 hours (see *section 2.14*) to determine their suitable concentration based on the cell viability and metabolic activity. Increasing concentrations of H_2O_2 from 0.05 to 0.4 mM did not change cell viability (at control: 96.3 (0.9) % vs. at 0.4 mM: 94.2 (0.8) %; $p > 0.05$) (**Figure 3.17 A**), while cell viability was decreased at 0.8 mM concentration (89.3 (±2.2) %, compared to control ($p < 0.01$), followed by 74.3 (±2.8) % at 1.6 mM of H_2O_2). However, the metabolic activity of H_2O_2-treated cells was significantly reduced at 1.6 mM concentration compared to the control (**Figure 3.17 B**) ($p < 0.05$).

Cell viability was not changed when the *t*-BHP concentration increased from 0.05 to 0.1 mM, while cell viability was significantly reduced beyond the concentration of 0.15 mM. At 0.25 mM concentration, the cell viability dropped by less than 50% (**Figure 3.17 C**). In consideration of metabolic activity, the concentration of *t*-BHP from 0.05 to 0.15 mM concentration on THP-1 cells did not alter the metabolic activity, whereas the metabolic activity significantly declined from an increasing concentration of 0.2 mM (**Figure 3.17 D**). H_2O_2 and *t*-BHP were selected at 100 μM concentration to induce oxidation.

Figure 3.17: Cell viability (A and C) and metabolic activity (B and D) of THP-1 cells on serial dilution of H_2O_2 (A and B) and *tert*-butyl hydroperoxide (*t*-BHP) (C and D).

Control: untreated THP-1 cells with media. Bars represent the mean ± SD (n=3); *$p < 0.05$, **$p < 0.01$. Significant differences were determined by one-way ANOVA followed by multiple comparisons using Dunnett's post hoc test. Serial dilutions of H_2O_2 and *t*-BHT at 0, 0.05, 0.1, 0.15, 0.2, and 0.25 mM were prepared.

Figure 3.17

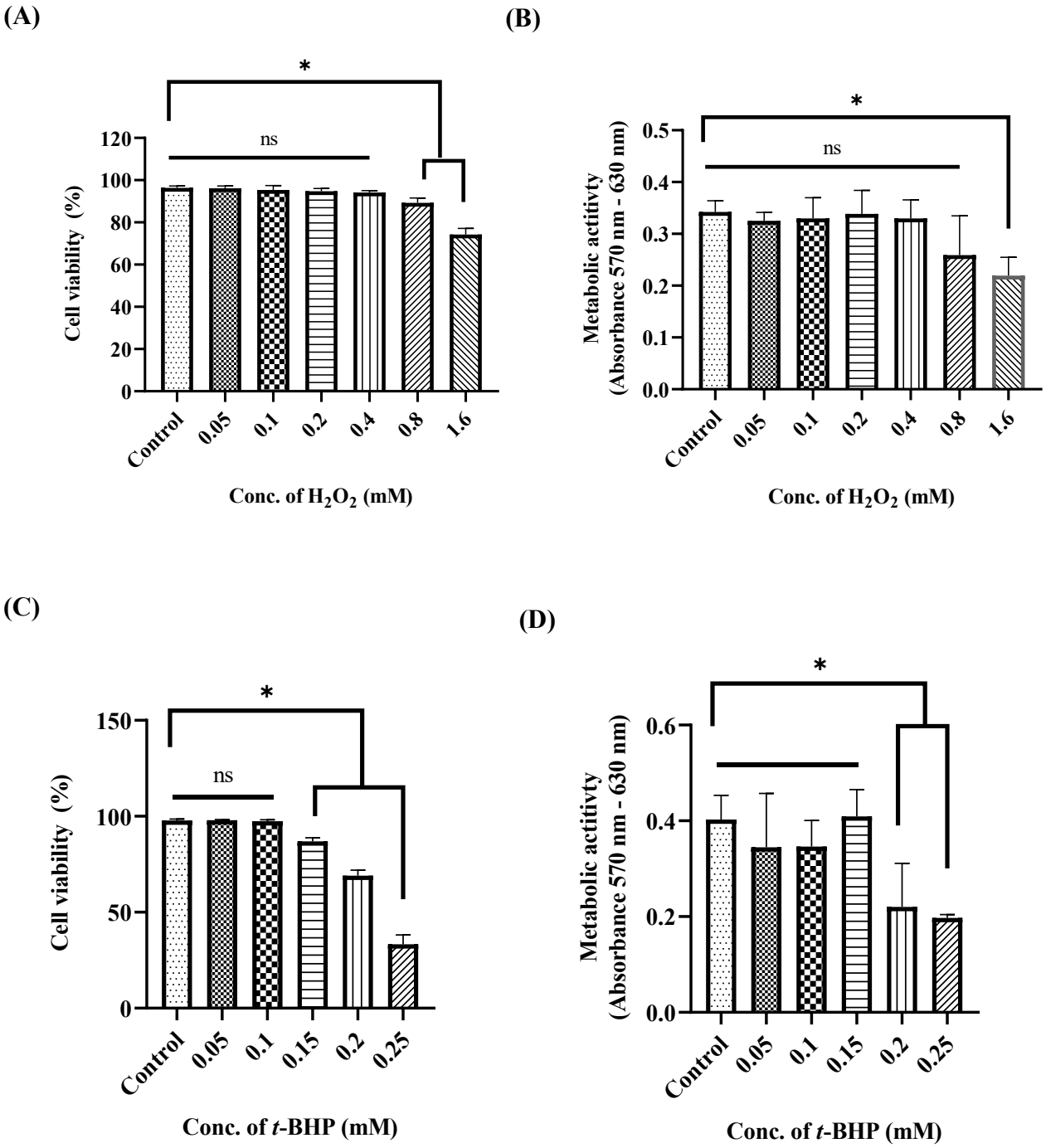
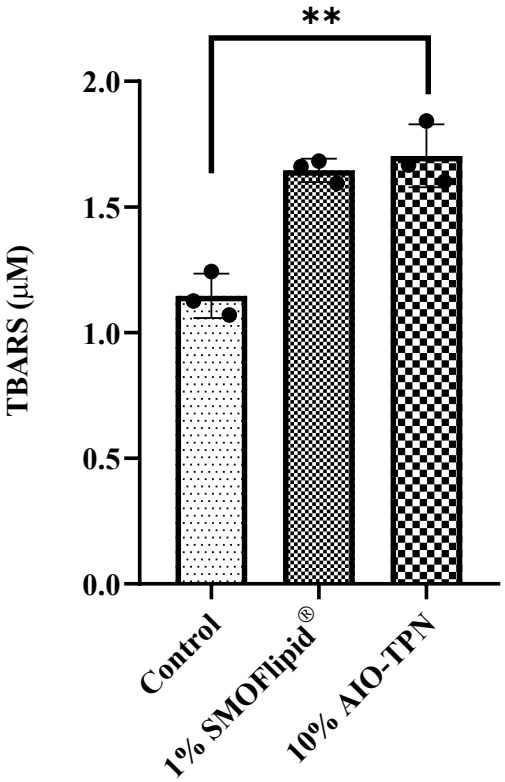

(A)
(B)
(C)
(D)
Cell viability (%)
Metabolic actitivty (Absorbance 570 nm - 630 nm)
ns
*
Control
0.05
0.1
0.2
0.4
0.8
1.6
0.15
0.25
Conc. of H_2O_2 (mM)
Conc. of *t*-BHP (mM)

Figure 3.18: TBARS level generated by THP-1 cells treated with A) SMOFlipid® and AIO-TPN B) air and $N_{2(g)}$ exposed AIO-TPN.

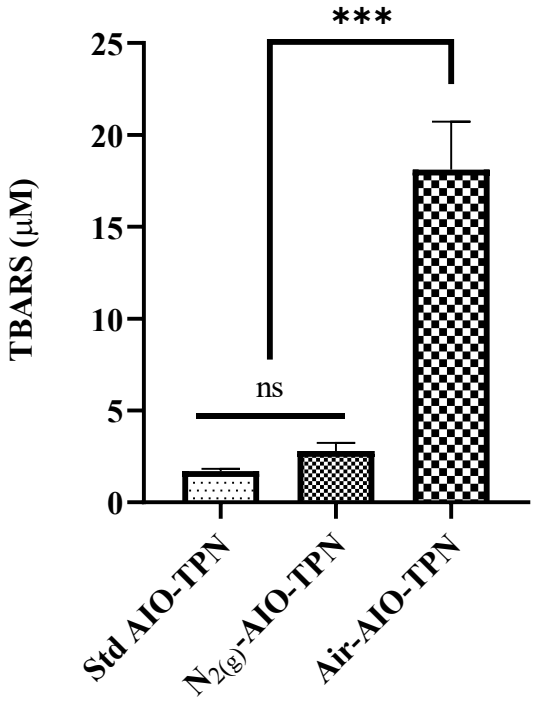
Control: untreated THP-1 cells with media. All-in-one TPN (AIO-TPN): H_2O_2 at 100 μM. Bars represent the mean ± SD (n=3); *$p < 0.05$, **$p < 0.01$. Significant differences were determined by one-way ANOVA followed by multiple comparisons using Tukey's HSD test. AIO-TPN was exposed to air and $N_{2(g)}$ for 24 hours at the rate of 0.25 L/minute prior to the treatment.

Figure 3.18

(A)



(B)



3.10 TBARS levels of SMOFlipid® and AIO-TPN

Lipid peroxidation of unopened SMOFlipid® bags and freshly prepared AIO-TPNs was assessed by measuring the TBARS level. One percent SMOFlipid® and 10% AIO-TPN were used to treat THP-1 human monocytic cells for 16 hours, as described in *section 3.8*. The supernatant was examined. TBARS level of 1% SMOFlipid®-treated THP-1 cells was significantly increased to 1.65 (±0.05) μM, compared to control (1.15 (±0.09) μM) ($p < 0.001$) (**Figure 3.18 A**). It was also observed that treating 10% AIO-TPN increased the TBARS levels to 1.7 (±0.12) μM ($p < 0.001$).

To examine the influence of air exposure on lipid peroxidation of AIO-TPN, which is the administered form of parenteral nutrition to the neonates at NICU, air- and $N_{2(g)}$-exposed AIO-TPNs were added to the THP-1 cells and incubated for 16 hours. $N_{2(g)}$- and air-exposed 10% AIO-TPN generated 2.79 (±0.44) and 18.11 (±2.61) μM TBARS, respectively ($p < 0.0001$) (**Figure 3.18 B**). The TBARS levels did not significantly change when the AIO-TPN was exposed to $N_{2(g)}$ for 24 hours compared to that of freshly prepared standard AIO-TPN.

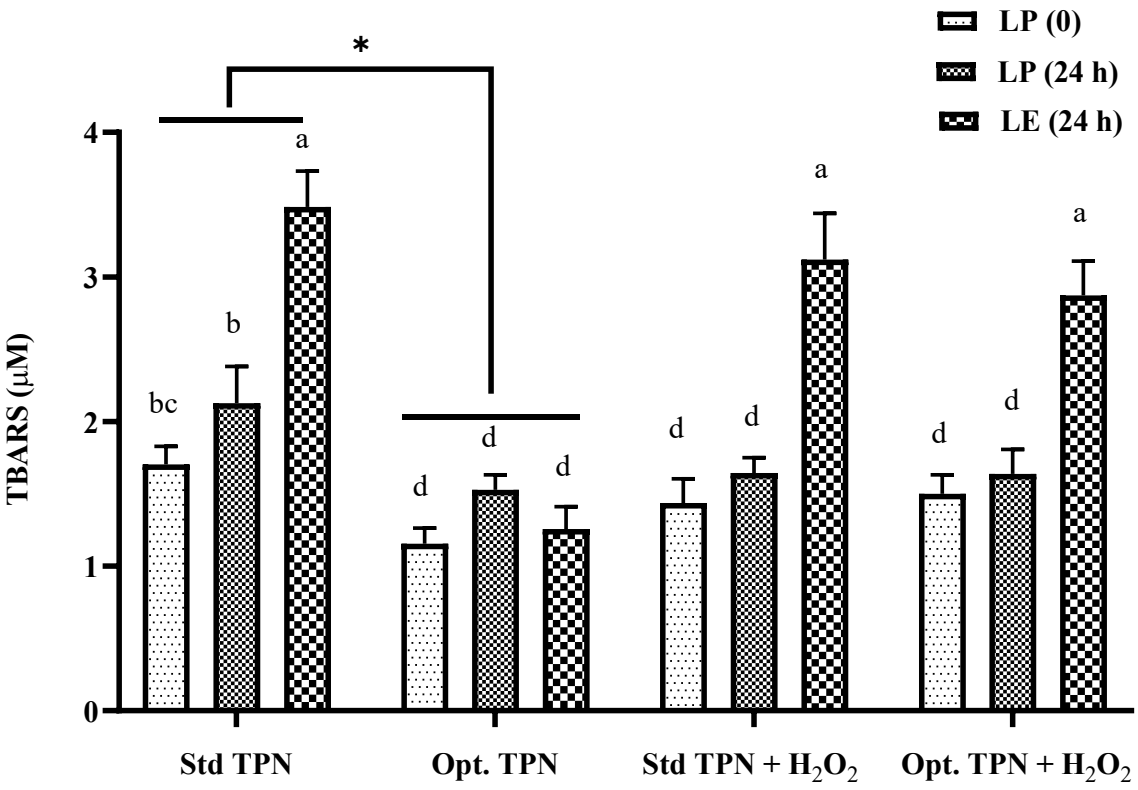
3.11 Lipid peroxidation of optimized AIO-TPN

The optimized AIO-TPN, described in *section 3.7*, was examined for cellular lipid peroxidation using THP-1 human monocytes. Optimized and standard AIO-TPNs of LP or LE for 24 hours were treated to THP-1 cells and incubated for 16 hours. The lipid peroxidation from the cellular supernatant was assessed using the TBARS assay. TBARS levels of standard AIO-TPN of LP at time zero, LP for 24 hours, and LE for 24 hours were 1.703 (±0.125), 2.127 (±0.255), and 3.484 (±0.247) μM, respectively.

Figure 3.19: TBARS levels of standard and optimized AIO-TPN generated by THP-1 cells.

Different lowercase letters indicate significant differences ($p < 0.05$) among the TBARS levels generated in AIO-TPN solutions. Bars represent the mean ± SD (n=3). Significant differences were determined by two-way ANOVA: light exposure on TBARS levels, $F(2,24) = 139.6$, $p < 0.0001$; standard and optimized AIO-TPN treated THP-1 cells on TBARS levels, $F(3, 24) = 55.11$, $p < 0.0001$; light exposure × standard and optimized AIO-TPN treated THP-1 cells interaction, $F(6, 24) = 17.96$, $p = 0.0001$. AIO-TPN was light protected (LP) at time zero, LP for 24 hours, and light-exposed (LE) for 24 hours, prior to the treatments. The THP-1 cells treated with various forms of AIO-TPNs were incubated for 16 hours at 37 °C and 5% $CO_{2(g)}$, and the respective supernatants were assessed for TBARS levels using a TBARS assay kit. Std TPN: standard AIO-TPN; Opt. TPN: optimized TPN; Std TPN/Opt. TPN + H_2O_2: 100 μM H_2O_2 added standard or optimized AIO-TPN.

Figure 3.19


LP (0)
LP (24 h)
LE (24 h)
*
TBARS (μM)
0
1
2
3
4
a
b
bc
d
d
d
d
d
a
d
d
a
d
a
Std TPN
Opt. TPN
Std TPN + H2O2
Opt. TPN + H2O2

TBARS levels of LE-standard AIO-TPN for 24 hours were significantly higher than LP at time zero or 24 hour-AIO-TPN ($p < 0.05$) (**Figure 3.19**). Conversely, the TBARS levels of optimized AIO-TPN of LP at time zero (1.156 (±0.106) μM), LP for 24 hours (1.528 (±0.103) μM), and LE for 24 hours (1.257 (±0.153) μM) were significantly lower than that of standard AIO-TPN of all forms ($p < 0.05$). The lipid peroxidation of optimized TPN decreased by 2.8-fold compared to LE-standard AIO-TPN for 24 hours (**Figure 3.19**). A similar trend was observed in lipid peroxidation of THP-1 cells treated with 100 μM H_2O_2 containing AIO-TPN.

3.12 Effectiveness of antioxidant vitamins C and E supplementation to SMOFlipid® on lipid peroxidation of THP-1 cells

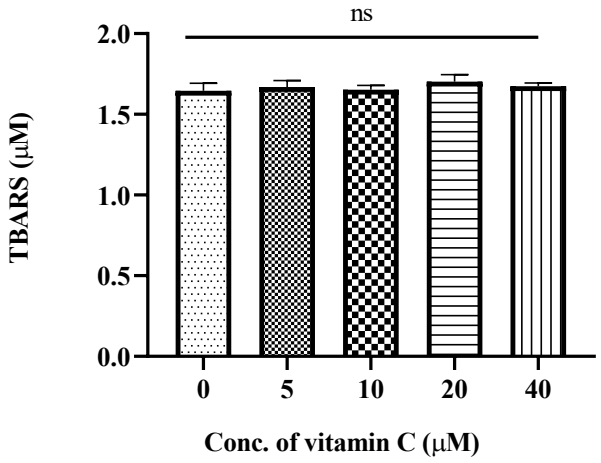
Since the optimized AIO-TPN significantly reduced lipid peroxidation following treatment to the THP-1 human monocytes (**Figure 3.19**), it is crucial to evaluate the lipid peroxidation of antioxidant-supplemented SMOFlipid® to determine the effectiveness before mixing with FF-TPN. Therefore, I assessed the lipid peroxidation in SMOFlipid® supplemented with vitamins C, E, or both. To carry out the effectiveness on antioxidant status, vitamins C and E were added to the newly opened SMOFlipid® at increasing concentrations as described in *section 2.5*. The TBARS level did not decline with increasing concentrations of vitamin C from 0 to 40 μM concentrations (**Figure 3.20 A**). A similar pattern was observed in lipid peroxidation of THP-1 cells treated with esterified vitamin C (6-*O*-palmitoyl-L-ascorbic acid) (**Figure 3.20 B**). Conversely, with increasing concentrations of vitamin E from 5 to 40 μM, the oxidation of lipids gradually decreased from 1.34 (±0.08) to 0.85 (±0.12) μM TBARS level ($p < 0.05$).

Figure 3.20: Lipid peroxidation of THP-1 cells on various concentrations of vitamins C and E with SMOFlipid®.

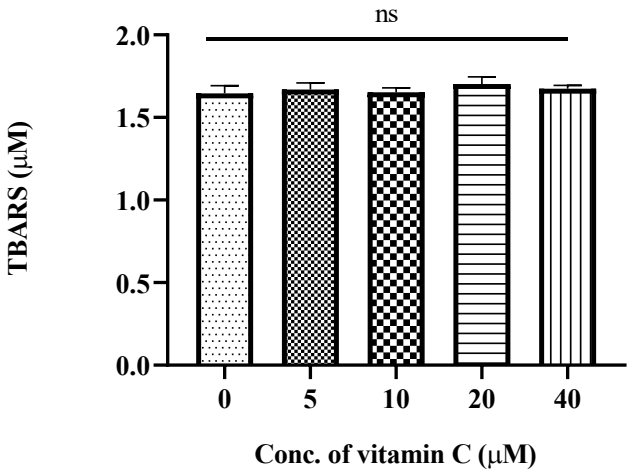
A: Vitamin C treated THP-1 cells at 5, 10, 20, and 40 μM with SMOFlipid; 0 μM vitamin C: baseline concentration in SMOFlipid®; **B**: Vitamin E treated THP-1 cells at 10, 20, and 40 μM. 5 μM vitamin E: baseline concentration in SMOFlipid®. Bars represent the mean ± SD (n=3). Different lowercase letters indicate significant differences ($p < 0.05$) among the TBARS levels generated in SMOFlipid®. Significant differences were determined by one-way ANOVA followed by multiple comparisons using Tukey's HSD test. Control: THP-1 cells without treating vitamin C or E.

Figure 3.20

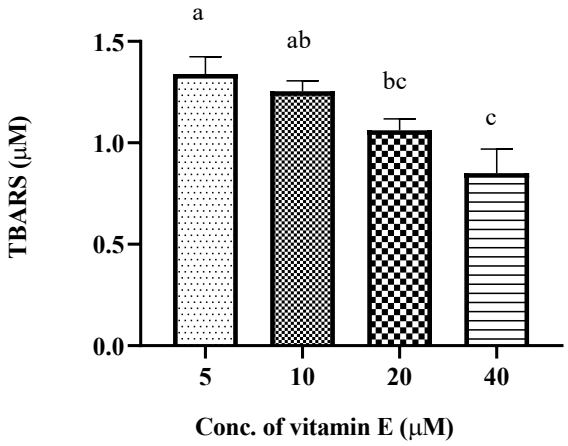
(A)



(B)



(C)



For instance, the TBARS level decreased by 0.48 μM when the vitamin E concentration increased from baseline concentration to 40 μM (**Figure 3.20 C**).

3.13 Effectiveness of the combinations of vitamins C and E supplemented SMOFlipid® on lipid peroxidation in THP-1 cells

Vitamin C, or esterified vitamin C, did not decrease the lipid peroxidation as described in *section 3.12*. Hence, vitamins C and E at various combinations were supplemented to the SMOFlipid® and incubated for 16 hours. Combined concentration of vitamin C at 20 μM and E at 20 μM levels significantly decreased the lipid peroxidation compared to control (1.16 (±0.04) vs. 1.56 (±0.15), respectively; $p < 0.001$). However, vitamins C at 40 μM and E at 40 μM combinations only tended to increase the lipid peroxidation levels to 1.23 (±0.09) ($p > 0.05$) (**Figure 3.21**).

3.14 ROS activity of standard and optimized AIO-TPN

To examine the ROS, LP- and LE-standard and optimized AIO-TPNs were added to THP-1 human monocytes and incubated for 4 hours. ROS production of standard and optimized LP AIO-TPN for 4 hours remained similar ($p > 0.05$) (**Figure 3.22**). After treating LE-standard AIO-TPN for 4 hours to the THP-1 cells, the ROS production was significantly increased fluorescence reading of 2', 7'-dichlorofluorescein (DCF) ($p < 0.05$). On the other hand, the ROS production of LE-optimized TPN did not differ significantly from its LP AIO-TPN (14312 (±803) vs. 12912 (±1042); $p > 0.05$).

Figure 3.21: Lipid peroxidation of THP-1 cells treated with various combinations of vitamins C and E added to the SMOFlipid®.

The combinations of vitamins C and E (C, E) at 5,5, 10,10, 20,20, and 40,40 μM were used. Vitamins C and E at 0,5 μM: baseline concentration in SMOFlipid®. Bars represent the mean ± SD (n=3). Different lowercase letters indicate significant differences ($p < 0.05$) among the TBARS levels generated by THP-1 cells. Significant differences were determined by one-way ANOVA followed by multiple comparisons using Tukey's HSD test.

Figure 3.21

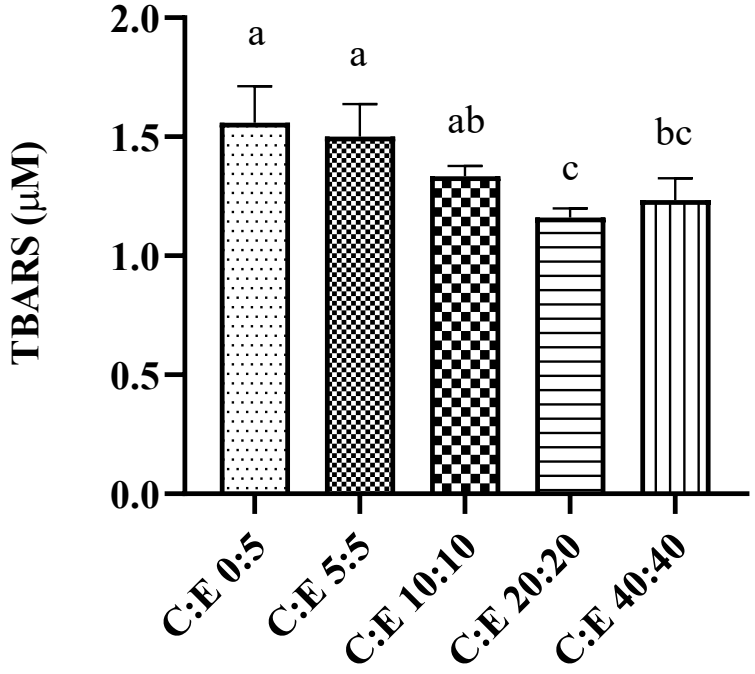
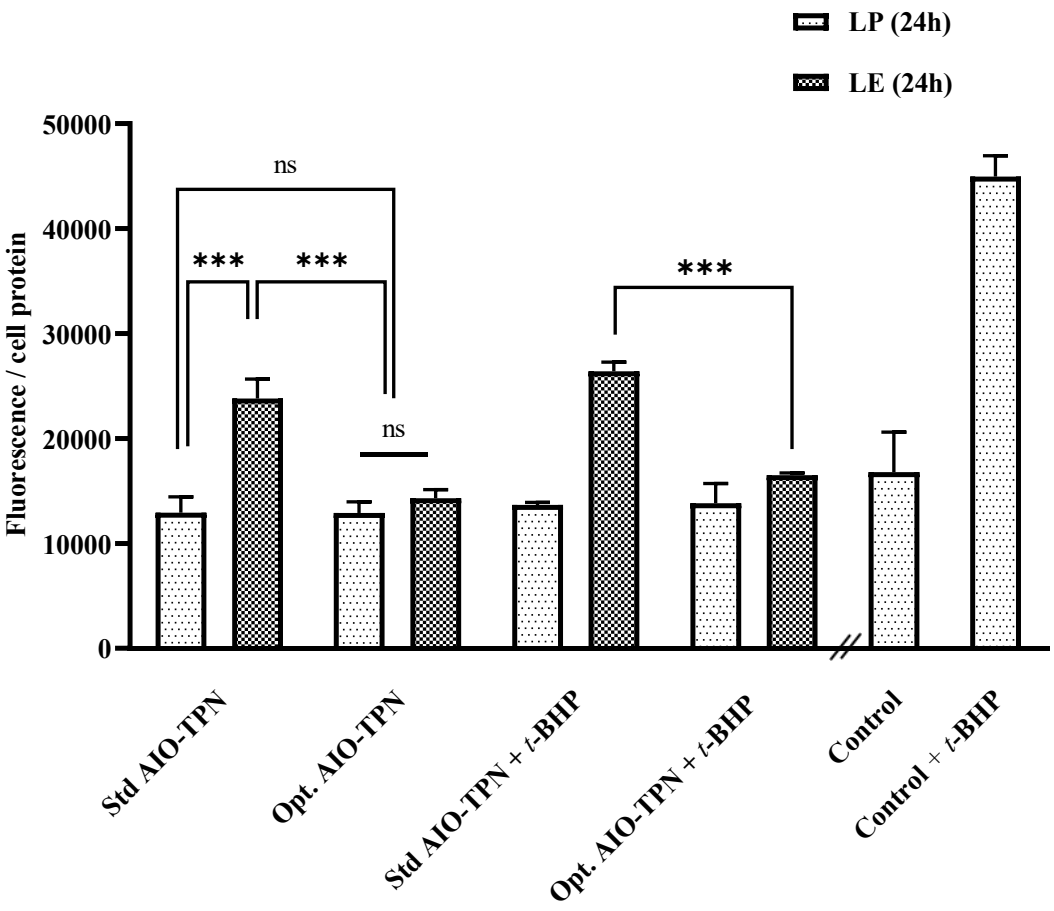

TBARS (μM)
2.0
1.5
1.0
0.5
0.0
a
a
ab
c
bc
C:E 0:5
C:E 5:5
C:E 10:10
C:E 20:20
C:E 40:40

Figure 3.22: ROS production of THP-1 cells treated with standard AIO-TPN and optimized TPN.

The blank well intensity was subtracted from each reading to obtain the corrected fluorescence intensity. The corrected fluorescence intensity is directly proportional to ROS production. The ROS production was normalized by the protein concentration of each well. Bars represent the mean ± SD (n=3); *$p < 0.05$, **$p < 0.01$, and ***$p < 0.001$. Significant differences were determined by two-way ANOVA followed by multiple comparisons with Tukey's HSD test. Two-way ANOVA: Light exposure on ROS, $F(1,16) = 194.5$, $p < 0.0001$; standard and optimized AIO-TPN treated THP-1 cells on ROS levels, $F(3, 16) = 34.88$, $p < 0.0001$; Light exposure × standard and optimized AIO-TPN treated THP-1 cells interaction, $F(3, 16) = 33.05$, $p < 0.0001$. Std. AIO-TPN: standard all-in-one TPN light protected (LP) or light exposed (LE) for 24 hours; Opt. AIO-TPN: optimized AIO-TPN. Std AIO-TPN or Opt. AIO-TPN + *t*-BHP: *tert*-butyl hydroperoxide added standard or optimized AIO-TPN at 100 μM; Control: untreated THP-1 cells with media; Control + *t*-BHP: THP-1 cells were added with 100 μM *t*-BHP.

Figure 3.22


LP (24h)
LE (24h)
Fluorescence / cell protein
50000
40000
30000
20000
10000
0
ns
***
***
ns
***
Std AIO-TPN
Opt. AIO-TPN
Std AIO-TPN + t-BHP
Opt. AIO-TPN + t-BHP
Control
Control + t-BHP

LE-optimized AIO-TPN treated THP-1 monocytes produced lower ROS (14312 (±803)) fluorescence reading of DCF than LE-standard AIO-TPN treated THP-1 cells (23813 (±1843)) fluorescence reading of DCF ($p < 0.05$). A similar pattern was noticed for AIO-TPN added with *t*-BHP ($p < 0.0001$).

3.15 Cellular antioxidant activity (CAA) of THP-1 cells

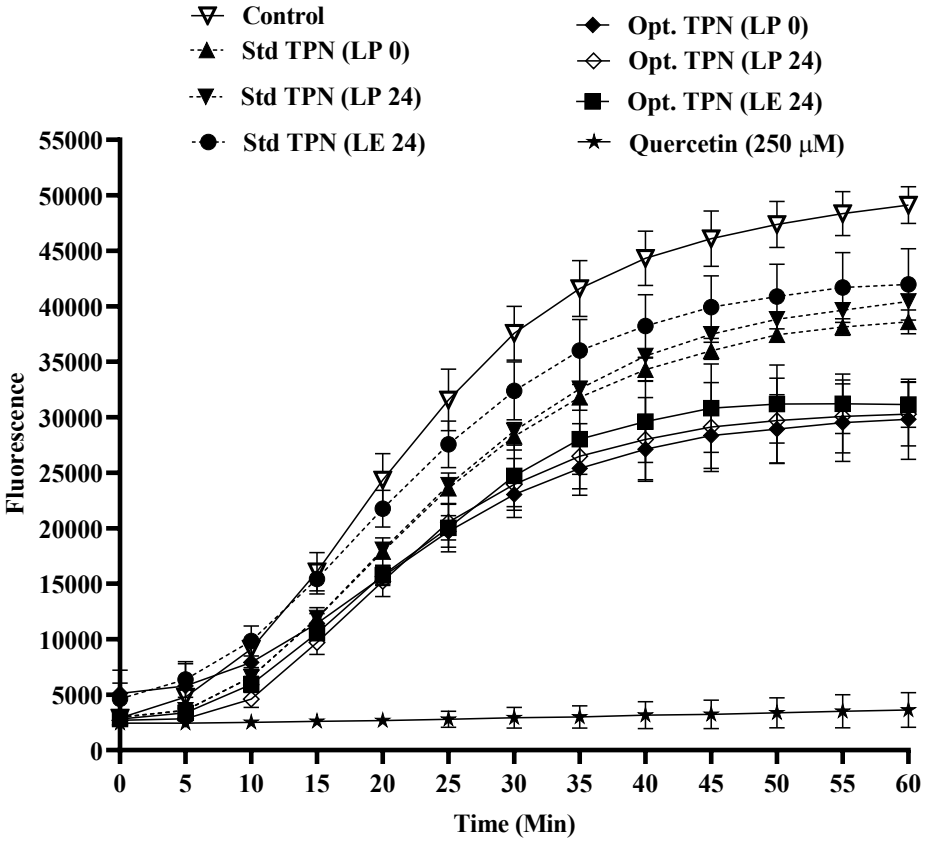
A CAA assay was carried out using THP-1 human monocytes with quercetin as a standard antioxidant. The index of antioxidant activity increased over time for 60 minutes (see *section 2.20*). The antioxidant activity of each sample showed a sigmoid pattern plotted for 60 minutes. As shown in **Figure 3.23 A**, the DCF fluorescence reading of THP-1 cells treated with AIO-TPN samples increased with time due to the generation of ROS by AAPH, which induces the generation of free radicals. ROS generation was suppressed by antioxidants in the cells. The cells with low antioxidants reflected the higher fluorescence reading at each time point. The finding showed that optimized AIO-TPNs of all forms had higher CAA values compared to those of standard AIO-TPNs. For instance, the optimized LP AIO-TPN for 24 hours had the highest CAA activity (36.97 (±9.63) %), followed by LP-optimized AIO-TPN at time zero (36.16 (±6.4) %) and LE-optimized AIO-TPN for 24 hours (33.93 (±9.1%)). The CAA value of LE-standard TPN had the lowest CAA value (13.37 (±5.069) %), which was significantly lower than the optimized AIO-TPNs (**Figure 3.23 B**).

Figure 3.23: CAA of THP-1 cells treated with various forms of AIO-TPNs.

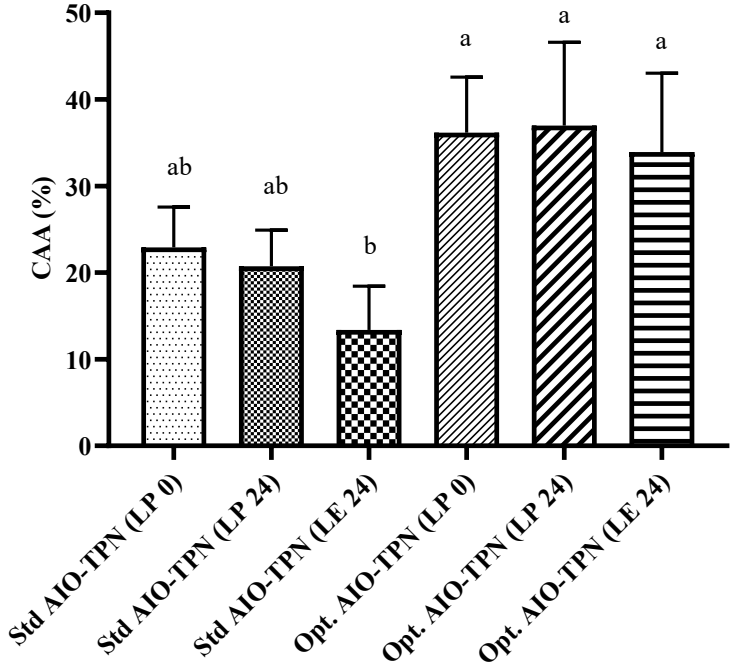
A: ROS generation by THP-1 cells in terms of 2',7'-dichlorofluorescein (DCH) measurements treated with antioxidants enriched AIO-TPNs (light protected (LP) or light exposed (LE)) and standard AIO-TPN (LP and LE). The fluorescence reading was obtained every 5 minutes over 60 minutes. Quercetin, a strong antioxidant at 250 μM was added to measure the CAA of the THP-1 cells as a positive control. **B**: The CAA of THP-1 cells in percentage based on the area under curve (AUC) compared to the control (phosphate-buffered saline treated cells). The AUC was estimated to determine the CAA based on the equation described in *section 2.20*. Bars represent the mean ± SD (n=3). Different lowercase letters indicate significant differences ($p < 0.05$) among the CAA levels generated by THP-1 cells. Significant differences were determined by one-way ANOVA followed by multiple comparison using Tukey's HSD test. Std TPN: standard AIO-TPN; opt. TPN: optimized AIO-TPN; LP (0): light protected at time zero; LP (24 h): light exposed for 24 hours; LE (24 h): light exposed for 24 hours.

Figure 3.23

(A)



(B)



3.16 Ferric reducing antioxidant power (FRAP) assay of optimized AIO-TPN treated to THP-1 cells

In the present study, THP-1 cells were treated with various forms of AIO-TPNs to assess the antioxidants in the cell supernatant. The presence of antioxidants in the AIO-TPNs reduced ferric cyanide (Fe^{3+}) to ferrous cyanide (Fe^{2+}) by donating an electron. The supernatant of standard AIO-TPN and optimized AIO-TPN treated THP-1 cells were subjected to a FRAP assay. In the results obtained, LP-optimized AIO-TPN whether LP at time zero (46.93 (±6.80) Trolox equivalents (TE) μg/mL) or 24 hours (49.5 (±7.73) TE μg/mL) exhibited a higher FRAP activity versus LE-optimized AIO-TPN for 24 hours (23.63 (±2.67) TE μg/mL; $p < 0.05$). The FRAP activity of LE-standard AIO-TPN and LE-optimized AIO-TPN for 24 hours were the same. *t*-BHP-added AIO-TPNs showed a similar trend with standard and optimized AIO-TPNs. However, the *t*-BHP-added LP-optimized TPN showed lower FRAP activity than LP-optimized AIO-TPN for 24 hours (**Figure 3.24**).

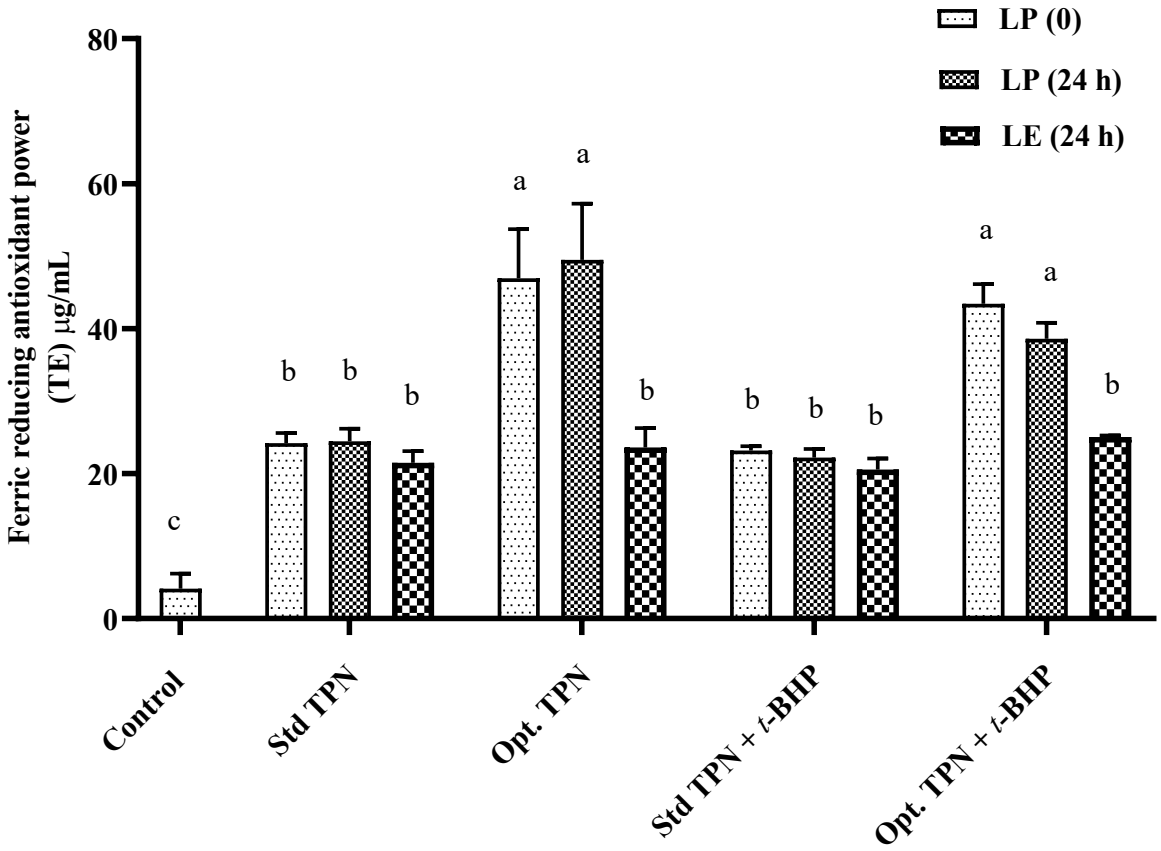
3.17 Endoplasmic reticular (ER) stress of THP-1 cells

THP-1 cells were treated with standard and optimized TPN of LP and LE samples and incubated for 16 hours. The cell lysate was prepared for Western blot analysis. It revealed that expression of GRP78 protein in LE-standard AIO-TPN appeared higher compared to LE-optimized TPN (**Figure 3.25 A**). However, the expression did not show a significant difference (**Figure 3.25 B**). However, tunicamycin at 0.5 μg/mL activated the ER stress by increasing the expression of GRP78 protein. Standard AIO-TPN LP for 24 hours and LE for 24 hours seemed to have had greater influence on ER stress compared to LP and LE-optimized TPN for 24 hours, but those were nonsignificant.

Figure 3.24: Ferric reducing power assay (FRAP) of THP-1 human monocytes treated with standard AIO-TPN and optimized AIO-TPN with positive control.

The results were given in Trolox equivalents (TE) (μg/mL). Bars represent the mean ± SD (n=3). Different lowercase letters indicate significant differences ($p < 0.05$) among the ROS levels generated by THP-1 cells. Significant differences were determined by one-way ANOVA followed by multiple comparisons using Tukey's HSD. Control: untreated THP-1 cells with media; Std TPN: standard AIO-TPN; Opt. TPN: optimized AIO-TPN; Std TPN/opt. TPN + *t*-BHP: *tert*-butyl hydroperoxide added standard or optimized AIO-TPNs at 100 μM.

Figure 3.24


LP (0)
LP (24 h)
LE (24 h)
Ferric reducing antioxidant power (TE) μg/mL
80
60
40
20
0
c
b
b
b
a
a
b
b
b
b
a
a
b
Control
Std TPN
Opt. TPN
Std TPN + t-BHP
Opt. TPN + t-BHP

3.18: Cellular GSH and GSSG levels

The redox status of the THP-1 cells was assessed by determining the GSH and GSSG levels. THP-1 human monocytic cells were treated with various forms of AIO-TPNs and incubated for 16 hours. The GSH and total glutathione (GSH + GSSG) were estimated in cell lysates (see *section 2.22*). It showed the GSH level of LP-standard AIO-TPN (36.37 (±4.27) nM), LP-optimized AIO-TPN at time zero (44.47 (±3.61) nM), and LP-standard AIO-TPN for 24 hours (50.72 (±12.72) nM) were higher than that of LP-optimized AIO-TPN for 24 hours (27.74 (±2.47) nM), LE-standard TPN for 24 hours (31.05 (± 9.31) nM), and LE-optimized AIO-TPN for 24 hours (23.93 (±4.48) nM) (**Figure 3.26 A**). The percentage GSH levels of the LP-standard AIO-TPNs at time zero (95.54 (±1.63) %) and LP-standard AIO-TPNs for 24 hours (95.54 (±1.63) %) did not change compared to untreated cells with AIO-TPN (97.06 (±0.45) %). Conversely, the percentage of GSH was significantly low in optimized TPNs, compared to control ($p > 0.05$).

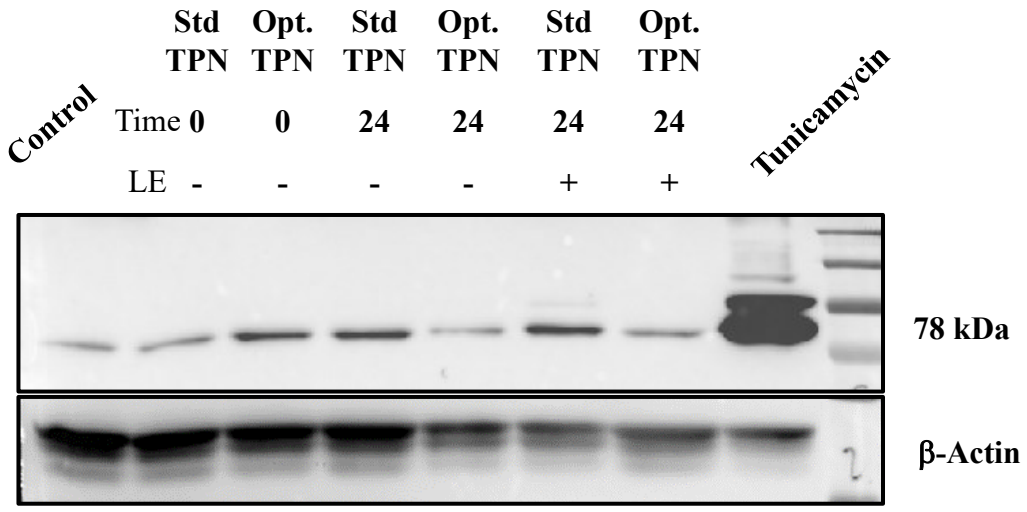
This study observed that the GSSG levels were high in THP-1 cells treated with optimized TPNs (**Figure 3.26 B**). Moreover, cell lysate of untreated THP-1 monocytes had the highest redox potential (the ratio of GSH/GSSG) (33.58 (±5.427)), compared to the THP-1 cells treated with AIO-TPNs. The redox potential of standard TPN was significantly higher than that of optimized TPN. However, the GSH/GSSG ratio was gradually decreased from LP-standard AIO-TPN to LE-standard AIO-TPN for 24 hours. For instance, the LP (at time zero) standard TPN had GSH/ GSSG ratio of 23.62 (±9.15), followed by redox potential of LP (24 hours) standard TPN (15.0 (±8.88)) and LE-standard TPN (6.14 (±3.91)).

Figure 3.25: Western blot analysis of GRP78 protein in THP-1 cells.

A. THP-1 cells were incubated for 16 hours with optimized AIO-TPN or standard AIO-TPN. After incubation, cell lysates were prepared for Western blot analysis. Expression of ER stress protein, GRP78 was viewed using BIORAD ChemiDoc imaging system. Tunicamycin at 0.5 μg/mL was added to the cells for positive control and incubated for 16 hours to induce ER stress (lane 8). **B.** Quantification of GRP78 was normalized with β-actin. Bars represent the mean ± SD (*n*=3). Significant differences were determined by one-way ANOVA followed by multiple comparisons using Tukey's HSD. Time 0: standard TPN (Std TPN) light protected (LP) for 0 time: Time 24: the AIO-TPN sampled exposed to light (+) or protected form light (-) for 24 hours.

Figure 3.25

(A)



(B)

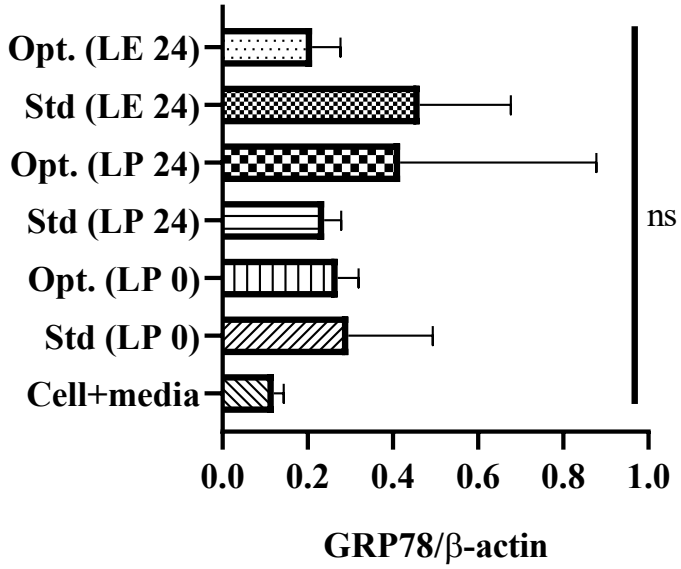
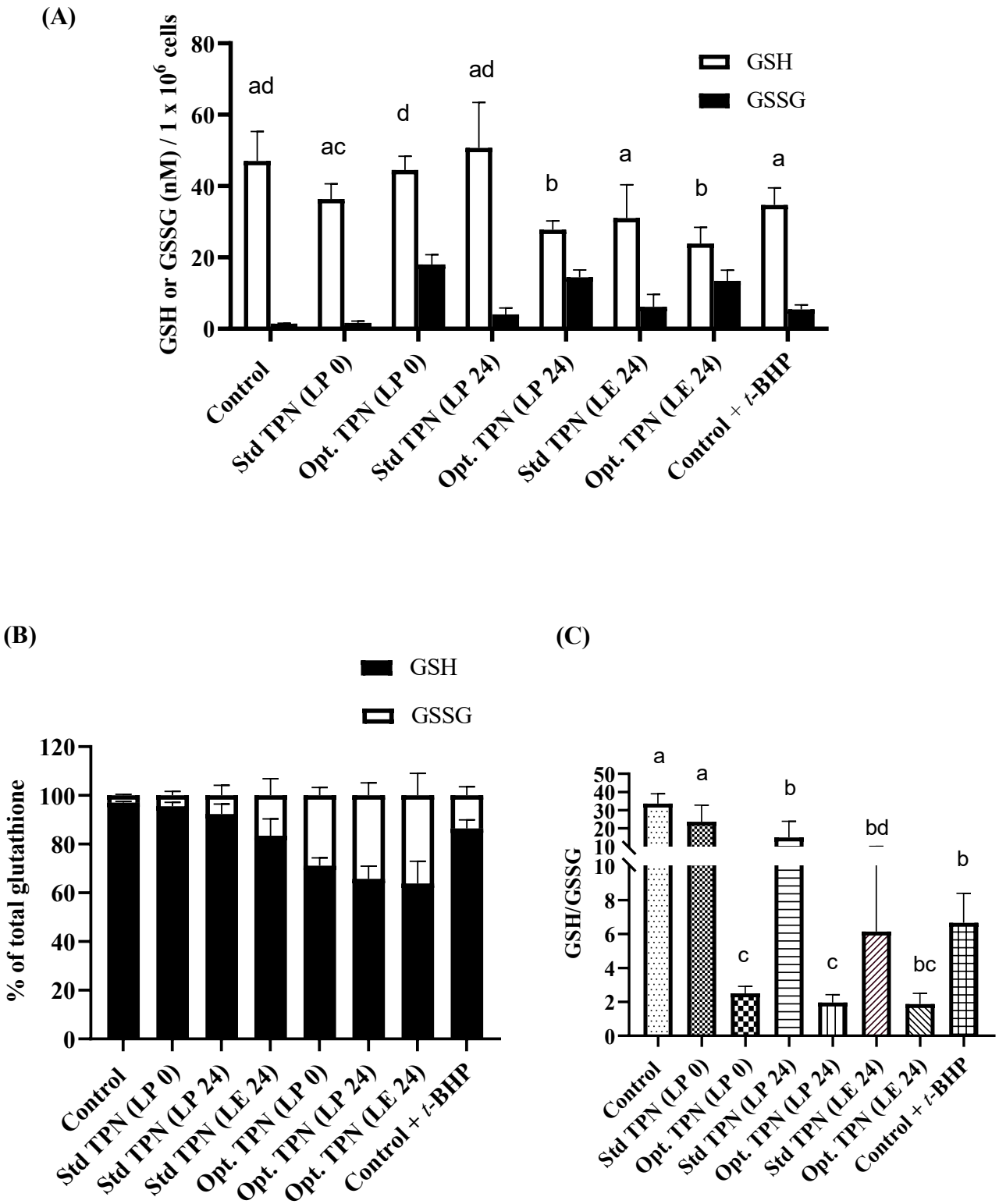


Figure 3.26: Reduced form of glutathione (GSH) and oxidized form of glutathione (GSSG) measurements on THP-1 human monocytes treated with or without various forms of AIO-TPNs.

A: GSH or GSSG levels within THP-1 cells treated with light protected (LP) or light exposed (LE) standard and optimized AIO-TPNs for 0 or 24 hours. A total of $1x10^6$ cell/mL were incubated with AIO-TPNs for 16 hours. Cell lysates were used to estimate the GSH and total glutathione levels. The levels are expressed in nM per 1 x 10^6 cells. **B:** Percentage of total glutathione with GSH and GSSG. The percentage of GSH was calculated by dividing it by total glutathione (GSH + GSSG). **C:** GSH to GSSG ratio in THP-1 cells treated with various TPNs. Bars represent the mean ± SD (n=3). Different lowercase letters indicate significant differences ($p < 0.05$). Control: untreated THP-1 cells incubated for 16 hours; Control+ *t*-BHP: the THP-1 cells were treated with 100 μM *tert*-butyl hydroperoxide; LP (0): light protected AIO-TPN at time zero; LP (24): light protected AIO-TPN for 24 hours; LE (24): light exposed AIO-TPN for 24 hours.

Figure 3.26


(A)
GSH or GSSG (nM) / 1 x 10^6 cells
GSH
GSSG
ad
ac
d
ad
b
a
b
a
Control
Std TPN (LP 0)
Opt. TPN (LP 0)
Std TPN (LP 24)
Opt. TPN (LP 24)
Std TPN (LE 24)
Opt. TPN (LE 24)
Control + t-BHP
(B)
% of total glutathione
GSH
GSSG
(C)
GSH/GSSG
a
a
b
c
c
bd
bc
b

Interestingly, all forms of optimized TPNs had low levels of redox potentials, ranging from 1.871 to 2.499 (**Figure 3.26 C**).

3.19 Determination of the composition of fatty acids, oxidized fatty acids, and F_2-isoprostanes in AIO-TPN using ESI-MS/MS

The fatty acid composition of AIO-TPN was determined using electrospray ionization (ESI)-MS. The composition of fatty acids was estimated under negative mode. The composition of fatty acids in AIO-TPN is given in **Table 3.4**. The predominant fatty acid found in the standard AIO-TPN was oleic acid (48.87 (±2.22) %), followed by palmitic acid (15.02 (±1.03) %). The ratio of omega-3 to omega-6 fatty acids in the standard AIO-TPN was 1:2.62. Figure 3.27 shows the mass spectrum of LP standard AIO-TPN at time zero under negative ESI. Similar patterns were observed for LP and LE optimized AIO-TPN (**Figures 3.1 and 3.2 of *Appendix III***). Oxidized fatty acids were calculated based on adding 16 mass units (oxygen atoms) to the *m/z* ratio. **Table 3.1 of *Appendix III*** shows the percentage of fatty acids oxidized in the AIO-TPN by damaging a single carbon-carbon double bond of the fatty acids. C18:2 fatty acid was oxidized at a higher level (1.26%). F_2-isoprostane (8-isoprostane $F_{2\alpha}$), a specific oxidative product of arachidonic acid, was analyzed from the TPNs. Mass spectrometer parameters for F_2-isoprostane with ESI-MS/MS were optimized. **Figure 3.3 of *Appendix III*** shows the fragmented pattern with the precursor ion at *m/z* 353 and the product ion at *m/z* 193. **Figure 3.4 of *Appendix III***) shows a representative mass spectrum of AIO-TPN for F_2-isoprostane. In the TPN samples, the F_2-isoprostane was not detected due to its low limit of detection (LOD) or low signal-to-noise (S/N) ratio.

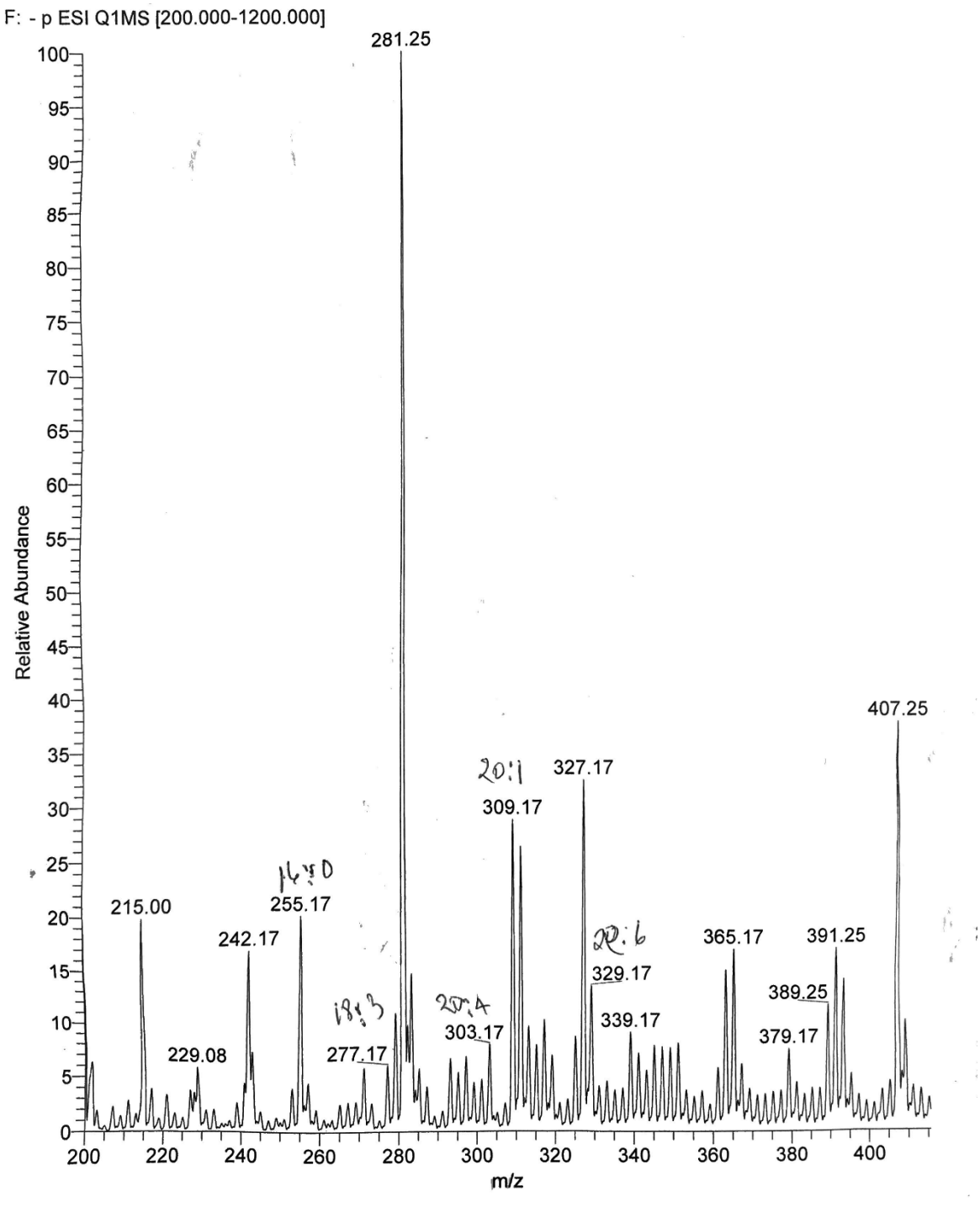
Table 3.4: The fatty acid composition of standard AIO-TPN (*n*=3).

	Name	Standard AIO-TPN Percentage (SD)
C6:0	Caproic acid	0.42 (0.16)
C8:0	Caprylic acid	3.92 (3.71)
C10:0	Capric acid	5.03 (2.41)
C12:0	Lauric acid	0.82 (0.47)
C12:1	Lauroleic acid	0.22 (0.17)
C16:0	Palmitic acid	15.02 (1.03)
C16:1	Palmitoleic acid	0.63 (0.25)
C18:0	Stearic acid	8.76 (0.29)
C18:1	Oleic acid (ω-9)	48.87 (2.22)
C18:2	Linoleic acid (ω-6)	8.52 (2.40)
C18:3	α-Linolenic acid (ALA) (ω-3)	0.35 (0.08)
C20:4	Arachidonic acid (ω-6)	3.29 (0.84)
C20:5	Eicosapentaenoic acid (EPA) (ω-3)	0.50 (0.36)
C22:6	Docosahexaenoic acid (DHA) (ω-3)	3.66 (1.02)
	ω3	**4.52**
	ω6	**11.81**
	ω3:ω6	**1:2.62**

Figure 3.27: Mass spectrum of light protected (LP) standard AIO-TPN at time zero.

A TSQ Quantis^{TM} mass spectrometer with electrospray ionization (ESI) in full scan negative ion mode generated the spectrum of free fatty acids in the LP standard AIO-TPN. The *m/z* ranged from 200 to 420 to visualize the spectra. The predominant fatty acid found in the AIO-TPN was oleic acid (C18:1), detected at *m/z* 281.25, followed by DHA (C22:6) at *m/z* 327.17, palmitic acid (C16:0) at *m/z* 255.17, and arachidonic acid (C20:4) at *m/z* 303.17.

Figure 3.27


F: - p ESI Q1MS [200.000-1200.000]
281.25
100
95
90
85
80
75
70
65
60
55
50
45
40
35
30
25
20
15
10
5
0
Relative Abundance
407.25
20:1
327.17
309.17
16:0
255.17
215.00
242.17
22:6
365.17
391.25
329.17
389.25
18:3
20:4
339.17
303.17
379.17
277.17
229.08
200
220
240
260
280
300
320
340
360
380
400
m/z

CHAPTER 4: DISCUSSION

4.1 Peroxide levels in TPN solutions induced by light exposure

The antioxidant systems of newborns are immature (Martini *et al.*, 2023). Even though they have potential enzymatic and non-enzymatic antioxidants in the body (Matyas and Zaharie, 2019), they are inefficient against oxidants. It is further weakened in babies born preterm, with gastrointestinal disorders or inborn errors of metabolism, particularly errors in glutathione metabolism (Ristoff and Larsson, 2007), coenzyme Q10 synthesis (Wongkittichote *et al.*, 2023), and superoxide dismutase 1 (SOD1) production (Park *et al.*, 2023), which make them more vulnerable against oxidants. Additionally, oxygen therapy for neonates with lung disorders or less oxygen saturation (SpO_2) triggers free radical generation in newborns (Ozsurekci and Aykac, 2016). Moreover, newborns with conditions of intestinal malrotation, intestinal atresia, pyloric stenosis, biliary atresia, Hirschsprung disease, short bowel syndrome, or preterm <32 weeks and/or <1500 g (Hair and Good, 2023) require an additional or exclusive intravenous nutritional support for their survival. Indeed, the Pediatric Intestinal Failure Consortium and the North American Society for Pediatric Gastroenterology, Hepatology and Nutrition (NASPGHAN) recommend providing parenteral nutrition for more than two months for aforementioned intestinal failure (Merritt *et al.*, 2017). Hence, intravenous nutrition plays a crucial role in the survival of such babies.

On the other hand, several studies have suggested that the administration of TPN causes several complications, such as PNALD, BPD, necrotizing enterocolitis, and gut atrophy (Kumar and Teckman, 2015; Nowak, 2020; Robinson *et al.*, 2021). One of the potential causes for these complications is oxidants generated in the TPN, which overwhelm the endogenous antioxidant

systems of newborns (Karthigesu *et al.*, 2021; Ozsurekci and Aykac, 2016) and eventually can cause complications. For example, the Pediatric Intestinal Failure Consortium Database suggests that 72% of neonates on TPN developed a conjugated bilirubin level of >2 mg/dL and half of them presented with conjugated bilirubin of >4 mg/dL, diagnostic markers of parenteral nutrition-associated cholestasis (Javid *et al.*, 2018).

The generation of oxidants in the TPN can occur through various processes. Indeed, TPN is prepared by mixing each fat-free component (see *section 2.1*), as described in *Appendix I*, at the pharmacy of the pediatric hospitals. Once the mixing is initiated, oxidant generation can begin due to the interaction between ingredients and/or external stimuli. After the preparation of the FF-TPN admixture, the commercially available ready-to-use lipid emulsion is either mixed directly with the FF-TPN or administered through a separate line that merges with the FF-TPN line at the Y-junction of the intravenous tubing, which connects to the neonate's vein. In routine clinical practice, it is often not feasible to shield the hanging bags containing FF-TPN, AIO-TPN, or lipid emulsions in bags or tubes from exposure to ambient, day, or phototherapy lighting. The stable light intensity in the NICU is challenging. For instance, the light intensity of the NICU units is approximately 3000 Lux, varying from 600 Lux to 5000 Lux (Dautovich *et al.*, 2019; Robinson *et al.*, 1990), depending on the conditions, including phototherapy for icterus neonates. However, light is essential for the circadian function of the newborns as well as staff who are on shift work in the NICU.

In this study, the newly opened light-protected SMOFlipid® had 1.8 mM H_2O_2 equivalent peroxides based on the FOX version II assay, which was validated for TPN studies elsewhere (Costello *et al.*, 1997; Laborie *et al.*, 2000b; Silvers *et al.*, 2001; Wolff and Woff, 1994). This assay is associated with a colorimetric method of the chromogenic Fe^{3+}-xylenol orange reaction.

In this method, the oxidation of ferrous ions in the presence of peroxides develops the purple color indicator due to the xylenol orange assay, which was described as a reference method for the determination of hydroperoxides by Wolff and Woff (1994). The main benefit of this method is that it does not need pretreatment, unlike chromatographic or spectroscopic methods (Dobarganes and Velasco, 2002). In addition, this method is not affected by environmental oxygen. Hence, the FOX version II assay would provide the results precisely and instantly.

Several researchers also found that newly opened parenteral nutrition solutions had significant amounts of peroxides (Brawley *et al.*, 1993; Laborie *et al*., 2000a; Mohamed *et al.*, 2023). Helbock *et al.* (1993) reported that lipid hydroperoxide in the Intralipid™-20% was 0.290 mM using high-performance liquid chromatography. The reasons for the peroxide contamination in the lipid emulsion, including SMOFlipid®, can be suggested by various factors: 1) light and air exposure at the TPN formulation area (Karthigesu *et al.*, 2021; Mosa and Nasef, 2015); 2) interaction of components in the SMOFlipid® solution (Leguina-Ruzzi and Ortiz, 2018); 3) material used for the packaging of the bag (Balet *et al.*, 2004); and 4) transport and storage (Hardy and Puzovic, 2009). Indeed, commercially available SMOFlipid® contains 28.9 % of polyunsaturated fatty acids (Xu *et al.*, 2021). This differed from my findings (16.32%). I also found that 3.9 % of fatty acids were oxidized. It could be suggested that a considerable amount of fatty acid in the SMOFlipid® for newborns might be oxidized well before reaching to the NICU due to the above-mentioned factors. In routine practice, the SMOFlipid® is sealed in a transparent bag without appropriate light protection covers or sheets and the bags are stored in a cardboard box and transported to pharmacy, where they are stored at room temperature.

Bag materials used to seal the SMOFlipid® is one of the crucial factors in increasing the lipid peroxidation. Though several bag materials have been developed for TPN storage, the currently available bag is made up of EVA. However, there is a lack of information on bag materials in the product monograph of SMOFlipid®. Balet *et al.* (2004) found that lipid emulsion stored in EVA bags generated higher peroxides compared to that in multilayer bags, which may prevent the light penetration efficiency. Indeed, lipid peroxidation can be induced by plastic materials (Hu and Palić, 2020; Kadac-Czapska *et al.*, 2024). Plastics, including micro or nano plastics, deteriorate the integrity of the polyunsaturated fatty acids and generate ROS (Smith *et al.*, 2024; Vercauteren *et al.*, 2024). Interestingly, the Omegaven® is sealed in a glass bottle, which may avoid the impact of plastics. The ASPEN position paper recommends protecting the TPN bag plus infusion sets for neonates (Robinson *et al.*, 2021). It was observed that the freshly prepared FF-TPN generated low levels of peroxides (0.09 mM H_2O_2 equivalents) compared to the AIO-TPN (1.04 mM H_2O_2 equivalents) or SMOFlipid®. Hence, this study suggests that the lipid emulsion is the major contributor to the oxidation in the TPN bag. Those peroxides were further aggravated when the TPN solutions were exposed to light.

Light exposure to AIO-TPN, lipid emulsions or during the preparation or transport significantly accelerates the peroxide formation (Karthigesu *et al.*, 2021). In this study, the light exposure at 3000 Lux resulted in approximately 40% elevation in peroxide levels in AIO-TPN and SMOFlipid® compared to light-protected counterparts. The elevation of lipid peroxidation could be explained due to various reasons. For instance, riboflavin is a photosensitive vitamin, and it turns to a radical form, which attracts the hydrogen from polyunsaturated fatty acids or other electron donors (see *section 1.7.2.2*) (Cardoso *et al.*, 2012; Karthigesu *et al.*, 2021; Silva *et al.*, 1998). In an *in vitro* study, Liu *et al*. (2015) found that DHA and arachidonic acids were

oxidized when retinal pigment epithelial cells were exposed to visible light. It also could be due to the lack of antioxidant vitamins due to their degradation with light, resulting in inefficient scavenging (Hoff and Michaelson, 2009). Interestingly, this study found that light exposure did not affect the peroxide formation of FF-TPN. However, there was a sudden increment of peroxide levels from 0 to 6 hours when the FF-TPN was exposed to 3000 Lux, then eventually dropped with time. This could be due to photosensitization of riboflavin at the initial stage and then the neutralization action of other antioxidants in the solution.

The findings on the increase in peroxides due to LE were consistent with others. Hoff and Michaelson (2009) reported that LE TPN generated 290-300 μM of peroxides, whereas the peroxide of LP TPN ranged from 60 to 130 μM. Kleiber *et al.* (2010) also described that LE TPN had higher peroxides (365 μM) compared to LP TPN (206 μM). In an *in vivo* human study, Neuzil *et al.*, (1995) showed that infusion of Intralipid^{TM} exposed to phototherapy lights to neonates affected with jaundice increased the formation of hydroperoxides by up to 60-fold higher after 24 hours compared to baseline (baseline: 9.6 μM vs. 24 hours: 633.9 μM). They also reported that syringes containing Intralipid^{TM} generated higher peroxides (59.2 μM) when exposed to light compared to baseline (7.5 μM). Another study found that completely protecting the TPN bag and infusion sets from light generated a significantly lower level of peroxides (146 μM *t*-BHT equivalents) compared to the light exposed counterparts (215 μM *t*-BHT equivalents) (Lavoie *et al.*, 1997).

A randomized prospective study comparing the effects of light exposure and light protection on TPN, which was conducted in the NICU at British Columbia's Children's Hospital, Vancouver between 2001 and 2004, found that infusion of LP TPN enhanced gut activity by

increasing the daily increments and cumulative volumes of enteral feeding compared to that of LE TPN infused neonates (Khashu *et al.*, 2006). LE syringe with Intralipid™ emulsion for 24 hours under phototherapy for hyperbilirubinemia generated three-fold higher hydroperoxides compared to LP syringes with Intralipid™ emulsion.

Light is one of the major contributors to peroxide formation in the TPN for neonates (Laborie *et al.*, 2000b; Lavoie *et al.*, 1997; Neuzil *et al.*, 1995). However, the impact of intensity or duration of light on peroxide generation is still unclear. Therefore, the influence of light intensity and duration of light exposure for TPN solutions on peroxidation was assessed. It was noticed that peroxide levels of AIO-TPN were not elevated up to the light intensity of 500 Lux, which is lower than the normal standard light intensity of a room in ambient light (800 Lux) (Bhandary *et al.*, 2021), whereas peroxide levels in SMOFlipid® were not increased with light intensity up to 250 Lux. In clinical practice, the amount of each ingredient for the TPN bag and the volume of infusion are calculated on a daily basis by the neonatologist or pediatrician. As a result, AIO-TPN infusions in the NICU are typically administered continuously over a 24-hour period to ensure that neonates receive their required nutrients throughout the day. Hence, the bag and infusion sets would likely remain exposed to light for an entire 24-hour during the infusion. In this study, 12-hour exposure of light on AIO-TPN and 6-hour exposure on SMOFlipid® did not increase the peroxide levels significantly compared to baseline. It is challenging to make comparisons with other studies because no research has been conducted specifically on the effects of light exposure duration on TPN samples. However, this study may recommend minimizing exposure to light in the NICU.

To assess the influence of air on peroxide levels, the parenteral solution was exposed to air with or without light exposure for 24 hours. The result shows that light and air-exposed AIO-TPN yielded a three-fold increase in peroxide levels compared to LP and $N_{2(g)}$-exposed AIO-TPN. Laborie *et al.* (2000b) reported that air or oxygen exposure to the TPN during preparation or infusion time generated peroxides profoundly. Air- and light-exposed FF-TPN produced 186 μM *t*-BHT equivalents within 24 hours compared to $N_{2(g)}$-exposed FF-TPN (50 μM *t*-BHT equivalents). Clinical studies revealed that infusion of LP TPN significantly ameliorates the harmful effects in neonates (Bassiouny *et al.*, 2009; Chessex *et al.*, 2007; Khashu *et al.*, 2006). Hence, this study suggests protecting the TPN from air or oxygen by keeping solutions under $N_{2(g)}$ in the entire procedure, such as during compounding TPN admixture at industry or in the pharmacy, as well as during infusion at NICU. However, such precautions may not always be possible in the clinical setting.

Taken together, the results regarding light, air exposure, and the duration of light exposure suggest that shielding TPN solutions from both air and light may decrease peroxides and could have substantial health benefits for neonates. However, simply lowering the light intensity in the NICU or keeping the room dim or dark may impede the detection of critical issues such as: monitoring air bubbles, color changes, or cloudiness in the TPN; detecting disruptions of TPN flow through the line connected to the neonate; clinical examination of skin; and intravenous cannulation as described previously. Therefore, rather than relying solely on reducing light exposure, a more effective strategy would be the enhancing antioxidant levels in TPN solutions to prevent peroxide formation while maintaining safety in the clinical setting. However, the optimization of antioxidants has been challenged due to the vast combinations of each antioxidant and their interactions. Hence, there should be a rapid mechanism to assess the

oxidants generated in the AIO-TPN. Among the different available assays, the FOX version II assay was chosen as a rapid and efficient method for detecting peroxides, using a straightforward bench-top *in vitro* approach.

4.2 Antioxidant vitamins C and E decreased the peroxide levels generated in the parenteral nutrition solutions

As described above, newborns possess an immature antioxidant system, and providing antioxidants, including vitamins, would help to improve their antioxidant capacity to overcome oxidative stress. Vitamin C is a well-proven aqueous antioxidant and cofactor for several enzymes (Berger, 2009). In contrast to these benefits, vitamin C can also intervene as a prooxidant at higher concentrations. However, the prooxidant level of vitamin C in neonates has still not been studied. Animal studies suggested that supplementation of vitamin C at 1000 mg/kg to rats resulted in prooxidant status (Seo and Lee, 2002). Another study revealed that intravenous administration of vitamin C at 500 mg/kg of rats elevated the ascorbate radical ($Asc^{\bullet-}$) concentrations nearly eight-fold in extracellular fluid compared to that in the blood (Chen *et al.*, 2007). However, human studies are limited. Podmore *et al.* (1998) reported that vitamin C at 500 mg/day oral supplementation for 6 weeks in healthy volunteers decreased the DNA damage in terms of decreasing 8-oxoguanine levels compared to placebo treatment. Another study revealed that vitamin C at 75 mg/kg/day (which can increase up to 200 mg/day without adverse effects) can be given (Yanase *et al.*, 2021). Hence, a dose of 200 mg/day is quite reasonable for TPN for newborns (Berger, 2009).

The data here show that increasing vitamin C from 24 to 72 mg/kg/day to the TPN that would be used for newborns at NICU did not decrease or increase the peroxide levels, which

remained high within the range of 1.4 to 1.5 mM H_2O_2 equivalents compared to LP AIO-TPN (1.0 mM H_2O_2 equivalents). The lack of effectiveness of the antioxidant property of vitamin C could be explained by reversible oxidation or prooxidant activity. For instance, vitamin C readily undergoes reversible oxidation to dehydroascorbic acid and further irreversible oxidation to a physiologically inactive form of 2,3-diketogluconic acid, which does not have antioxidant properties (Nimal *et al.*, 2022).

Vitamin E in parenteral nutrition solutions from the MVP and lipid emulsions is the major lipophilic antioxidant for newborns. It is a potent peroxyl radical scavenger that prevents the propagation of free radicals (Traber and Atkinson, 2007). Surprisingly, unlike other fat-solution vitamins, vitamin E does not produce toxic effects at higher doses as it is metabolized via initial omega-oxidation by the cytochrome P450 (Bell, 1989; Guthrie *et al.*, 2020). However, newborns, especially preterm babies, may be susceptible to hepatocellular damage from high doses. Indeed, parenteral infusion of 100 mg/day α-tocopherol acetate substantially increased plasma levels and eventually caused liver toxicity (Phelps, 1988). Vitamin E toxicity can occur if the plasma vitamin E concentration reaches to 80 μM (Biesalski, 2009). Brion *et al.* (2004) found that vitamin E intravenously provided at high doses of 7 mg/kg/day is safe for very-low birth weight babies.

In this study, increasing vitamin E from 4.6 to 10.9 mg/kg/day to the TPN did not decrease the peroxide levels in the *in vitro* assay. King *et al.* (2018) noticed that lipid peroxidation by assessing 4-hydroxy-2-nonenal was similar between Intralipid™ and SMOFlipid® regardless of vitamin E content (Intralipid™ and SMOFlipid® have 38 and 200 mg/L of vitamin E, respectively). Though DL-α-tocopherol acetate is a more stable and bioactive

form than α-tocopherol succinate for newborns (Jensen *et al.*, 1999), it did not decrease the peroxides efficiently upon adding alone.

The activity of vitamin E depends on other antioxidants, including vitamin C (Rizvi *et al.*, 2014). In this study, combinations of vitamin C at 2.27 mM (equivalent to 60 mg/kg/day) and E at 124.1 μM (equivalent to 8.8 mg/kg/day) for newborns at NICU decreased the peroxide levels from 1.8 to 1.2 mM H_2O_2 equivalents significantly (Karthigesu *et al.*, 2023). This could be due to the additive effect of vitamin C and E on peroxide levels (Karthigesu *et al.*, 2023). For instance, α-tocopherol reacts with peroxyl radicals and generates lipid hydroperoxide and the tocopheryl radical (Vit E-O•). The α-tocopheryl radical (Vit E-O•) accepts the proton from a donor, particularly from vitamin C to form reduced α-tocopherol, and then the latter turns into oxidized vitamin C (dehydroascorbic acid) (Traber and Stevens, 2011). If there is a lack of vitamin C or other antioxidants, Vit E-O• can re-initiate lipid peroxidation (Thomas and Stocker, 2000). My finding is consistent with other *in vitro* studies elsewhere (Chan, 1993; Porkkala-Sarataho *et al.*, 2000; Sato *et al.*, 1990).

Overall, supplementation of vitamin C (up to 60 mg/kg/day) and E (up to 8.8 mg/kg/day) for TPN under physiologically safe levels would decrease the peroxide levels rather than supplementing vitamin C or vitamin E separately with TPN.

4.3 Increasing selenium concentration did not decrease the peroxide levels in the TPN

Selenium is one of the vital microelements for newborns and is a constituent of selenoproteins, including thioredoxin reductases, glutathione peroxidases, and selenoprotein P, which are essential for antioxidant activity (Rayman, 2000). Although ASPEN recommends

administering 2 μg/kg/day of selenium to newborns (Vanek *et al.*, 2012), numerous clinical studies have reported that a substantial proportion of neonates on parenteral nutrition developed selenium deficiency, characterized by plasma levels falling below 50 μg/L (Chen *et al.*, 2016; Klinger *et al.*, 1999; Lee *et al.*, 2022; Pederson, 2011). This suggests that the current recommendations may be insufficient for preventing deficiency or benefit to the neonates, especially in vulnerable populations like preterm infants or those with prolonged dependence on parenteral nutrition. Indeed, Lee *et al.* (2022) reported that increasing selenium levels in parenteral nutrition proportionally increased the serum selenium concentration.

In this study, although increasing the selenium from 2 to 6 μg/kg/day in AIO-TPN did not significantly lower the peroxide levels, a slight decline in peroxide levels at a dose of 4 μg/kg/day or 0.24 μM concentration was observed. It could be due to the interaction with other trace elements, including copper (Hill, 1975). Indeed, *in vivo* selenium acts as a cofactor for GPx, which maintains the redox potential of GSH/GSSG (Bizerea-Moga *et al.*, 2024). The redox activity of selenium in the TPN bag is still unclear.

Lee *et al.* (2023) suggested increasing the intravenous selenium dose to 4.0 μg/kg/day to maintain the serum selenium concentration above the threshold of 50 μg/L. Additionally, Darlow *et al.* (2000) and Lee *et al.* (2023) mentioned that infusing selenium at the rate of 7 μg/kg/day could be beneficial for neonates. However, the maximum safe limit for intravenous selenium administration for neonates remains uncertain. ASPEN currently advises a daily selenium infusion at the dose of 100 μg/day for children weighing 10-40 kg (ASPEN, 2019b). Therefore, potential adjustments to selenium supplementation may be necessary to ensure adequate selenium status and enhance the antioxidant status in these newborns.

4.4 GSSG to the TPN decreased the peroxide levels

Glutathione, a tripeptide composed of glutamine, cysteine, and glycine, serves as a key antioxidant in the body. It helps protect cells from oxidative stress by neutralizing free radicals and ROS, playing a crucial role in maintaining cellular health and supporting the immune system (Lushchak, 2012). However, the endogenous synthesis of glutathione is limited in newborns due to the lack of such precursors, particularly cysteine. Moreover, enzymes involved in this synthetic pathway are immature in newborns (e.g., cystathionine synthase and cystathionase) (see *section 1.6*) (Mungala Lengo *et al.*, 2024). However, TPN formulations routinely used for neonatal infusion do not contain glutathione, primarily due to its instability in these mixtures. GSH degrades rapidly, with a loss of up to 60% within four hours after being added to the TPN admixture (Elremaly *et al.*, 2016). Therefore, GSSG has been used in animal studies because it remains more stable in TPN solutions (Hong *et al.*, 2007; Morin *et al.*, 2019; Raichani *et al.*, 2022). The infusion of GSSG increases its availability, which is then catabolized by γ-glutamyltransferase to release γ-glutamyl moiety. The remaining cysteinylglycine dipeptide is further catabolized by dipeptidase in the cells to release cysteine (Cotgreave and Schuppe-Koistinen, 1994; Deneke *et al.*, 1995), which may be used for GSH synthesis in the body. While the exact mechanism of GSH formation at cellular levels using the intravenous infused GSSG in the body remains unclear.

Morin *et al.* (2019) found that supplementing TPN with 10 μM GSSG in Hartley guinea pigs decreased the TPN-induced oxidative stress by lowering redox potential in the lungs. Mungala Lengo *et al.* (2024) demonstrated that TPN without GSSG induced hypermethylation of glutathione peroxidase (*GPX1*) and decreased the mRNA expression of *GPX1*, cysteine ligase (*GCLC*), and glutathione synthetase (*GSase*), whereas TPN with GSSG (12 μM; corresponding

to 1.3 mg/kg/day) fed to three-day-old guinea pigs did not show those expressions and had increased *SOD2* mRNA levels.

In this study, supplementation of GSSG at a concentration of 40 μM (equivalent to 4 mg/kg/day) resulted in a significant reduction in peroxide levels. Adding glutathione helps to reduce the H_2O_2 to H_2O, but this reaction is slow non-enzymatically. Gpx scavenges the H_2O_2 or free radicals more efficiently by interchanging the GSSG to 2GSH by donating or accepting protons (Ng *et al.*, 2007; Sies, 1999). However, despite this reduction, redox potential (GSH/GSSG) in THP-1 monocytes treated with GSSG-enriched AIO-TPN remained low, indicating an imbalance in the cellular redox state. Optimized TPN in this study was hypothesized to increase the GSH levels, but instead, we observed that it generated low levels of GSH and higher levels of GSSG. Hence, the ratio of GSH/GSSG was low. It could be due to the amount of GSSG levels in the optimized TPN, as a 40 μM level of GSSG to AIO-TPN was added. Moreover, when GSSG increases, the chance of oxidation of GSH is high (Giustarini *et al.*, 2016). Thus, the GSH to GSSG ratio was low in these experiments. This suggests that while GSSG may decrease oxidants in the TPN solution, it does not restore redox homeostasis at the cellular level. A longer duration of incubation may increase the GSH/GSSG ratio. For instance, *in vivo* animal studies showed that the administration of 10 to 12 μM GSSG increased the redox potential (Elremaly *et al.*, 2015; Lavoie *et al.*, 2022; Morin *et al.*, 2019; Mungala Lengo *et al.*, 2024). Under controlled mechanisms to prevent the factors that deteriorate GSH levels, adding GSH to the TPN may benefit neonates. For example, Hong *et al.* (2007) reported that TPN supplemented with GSH (20 mg/kg/day) in infant rabbits decreased TBARS levels and reduced cholestasis, compared to the rabbits receiving TPN without GSH.

To date, no clinical studies have examined TPN fortified with glutathione for neonates. Therefore, this study may provide valuable insights into the potential benefits of incorporating glutathione into TPN solutions. Though there is still no definitive consensus on using glutathione in TPN, several studies have suggested the potential benefits of adding glutathione, particularly for premature infants, to enhance antioxidant support and improve outcomes (Lavoie and Chessex, 2019). Hence, the antioxidant status of GSH or GSSG or its stability for TPN should be further examined and optimized by adding the suitable ratio of GSSG: GSH for TPN.

4.5 Decreasing concentrations of copper, zinc, or combinations of both did not lower the peroxide levels

Copper serves as an essential cofactor for enzymes involving redox reactions (Leung, 1998; Perks *et al.*, 2022). Moreover, copper can readily be interconverted between Cu^{+} and Cu^{2+}, which underlies its important role in redox reactions (Linder and Hazegh-Azam, 1996). Although ASPEN recommends providing copper at 20 μg/kg/day, there is no evidence for optimal copper levels for term and preterm neonates (Gupta *et al.*, 2018). The European Society of Pediatric Gastroenterology, Hepatology and Nutrition (ESPGHAN) and the European Society of Parenteral and Enteral Nutrition (ESPEN) 2018 guidelines recommend doubling the provision of copper in TPN to preterm infants from 20 to 40 μg/kg/day (Domellöf *et al.*, 2018). As intravenous administration of copper bypasses intestinal absorption, it may be toxic due to inefficient biliary excretion (Gupta *et al.*, 2018). Hence, deciding the optimal concentration of copper for TPN is challenging. On the other hand, copper at higher levels acts as a prooxidant due to its redox cycling between Cu^{2+}/ Cu^{+} (Falcone *et al.*, 2023; Mozuraityte *et al.*, 2016; Talbot, 2011) and high hepatotoxicity (Shike, 2009). In this study, decreasing copper levels for

AIO-TPN from 4.2 μM (corresponding to 40 μg/kg/day) to 1.05 μM (corresponding to 10 μg /kg/day) did not decrease the peroxide levels (which remained at 1.3 mM H_2O_2 equivalents compared to standard TPN (1.3 mM H_2O_2 equivalents)). Interestingly, this study observed that increasing copper above 4.2 μM (40 μg/kg/day) decreases the peroxide levels. This could be explained by the redox reaction of copper that scavenges the peroxides. However, the maximum dose of copper for TPN can be 300 μg/kg/day (Shike, 2009). Therefore, lowering copper levels may not be advantageous for TPN in terms of peroxidation. However, it may be better to ensure maintaining copper levels below toxic limits for neonates while still providing adequate amounts for their functional and developmental needs.

Zinc is an essential trace element required for the activity of more than 300 enzymes (Domellöf, 2014). Zinc at a dose of 200 μg/kg/day administered through TPN for neonates is adequate to maintain normal plasma zinc levels, and the dose is even considerably higher for infants with specific conditions, especially enterostomy (up to 300 μg/kg/day) (Suita *et al.*, 1984). Unlike copper, the adverse effect of zinc overdose is rarely reported (Cummings and Kovacic, 2009). However, one case report demonstrated that 1000-fold higher than the normal dose of zinc in TPN was given to a neonate accidentally, resulting in death (Grissinger, 2011). In adults, zinc toxicity is rare unless there is an accidental overdose of more than 50 mg/day in adults over a prolonged period (Jeejeebhoy, 2009; Osland *et al.*, 2014). In adults, Prasad *et al.* (1978) observed that zinc may cause adverse effects if consumed more than 150 mg/day for a longer period. Unlike copper or iron, zinc is a redox-inert metal that remains stable as a divalent cation (Zn^{2+}). As a result, it is not involved in redox reactions directly. However, zinc contributes to antioxidant defense through its role in the catalytic activity of copper/zinc superoxide dismutase (Cu/Zn-SOD) and by upregulating the production of metallothionein (Lee, 2018).

On the other hand, zinc can act as a prooxidant at higher concentrations (Lee, 2018). For instance, mitochondrial ROS production can be induced by excessive zinc. Zn^{2+}-ROS production can further trigger the mobilization of intracellular Zn^{2+} to the mitochondria and generate excessive ROS. In addition, excessive Zn in the endothelial cells may increase NADPH-oxidase 1 (Nox1) expression (Salazar *et al.*, 2017). Noh and Koh (2000) described that Zn^{2+} triggered the activation of protein kinase C (PKC), which led to the activation of Nox1 and generation of $O_2^{\bullet-}$, NO, and peroxynitrite (Pluth *et al.*, 2011). In this study, zinc from 10.19 μM (corresponding to 0.1 mg/kg/day) to 30.59 μM (corresponding to 0.3 mg/kg/day; the standard concentration) or increasing the concentration of Zn from 305.9 μM (corresponding to 3 mg/kg/day) to 30590.4 μM (corresponding to 300 mg/kg/day) did not change the peroxide levels in this *in vitro* experiment. Even the combinations of copper and zinc at various ratios also did not decrease the peroxide levels in this study. It is challenging to find the literature regarding zinc levels for TPN and oxidant assessment.

Determining the suitable zinc concentration in TPN for neonates, particularly to increase its antioxidant role, is a significant challenge in the clinical settings (Jin *et al.*, 2017). Balancing the requirement of adequate zinc levels or above minimum dietary reference intake to support antioxidant defenses while avoiding prooxidant activity at higher concentration or deficiency requires careful consideration of each individual needs and their medical conditions.

In this study, adding trace elements (i.e., zinc, copper, selenium, chromium, iodine and manganese at standard concentrations) did not significantly increase peroxide levels of LE AIO-TPN (from 1.17 to 1.23 mM H_2O_2 equivalents). The protective effect of trace elements could be due to the antioxidant activity of selenium and zinc at lower concentrations. This finding further

confirms the beneficial effect of adding trace elements to TPN. Bassiouny *et al.* (2009) revealed that urinary peroxides were not changed between newborn groups infused TPN with or without trace elements, regardless of whether light protected or not. Contrary to these findings, Steger and Mühlebach (2000) reported that adding trace elements to TPN increased the peroxide levels. They stored the TPN bag for 14 days. They also observed the pH changed over time. Hence, the increase of peroxides might be affected by various factors in this study.

Overall, incorporating trace elements into the TPN enhances the antioxidant activity. However, determining the suitable concentration is challenging for newborns. Hence, careful considerations, including interactions and safe levels, are required to be investigated.

4.6 Optimized AIO-TPN decreased peroxidation in *in vitro* bench-top experiment

Standard AIO-TPN for newborns contains 0.9 mM (equivalent to 24 mg/kg/day) of vitamin C, 64.8 μM (equivalent to 4.6 mg/kg/day) of vitamin E, 0.12 μM (equivalent to 2 μg/day) of selenium, 4.2 μM (equivalent to 4 μg/kg/day) of copper, 30.6 μM (equivalent to 300 μg/kg/day) of zinc. In this study, an optimized AIO-TPN with 2.27 mM (equivalent to 60 mg/kg/day) of vitamin C (2.5-fold increment), 124.1 μM (equivalent to 8.8 mg/kg/day) of vitamin E (two-fold increment), 0.24 μM (4 μg/kg/day) of selenium (two-fold increment), 40 μM (equivalent to 4 mg/kg/day) of glutathione (zero level at standard TPN), 4.2 μM (equivalent to 40 μg/kg/day) of copper (the same level), and 30.6 μM (equivalent to 300 μg/kg/day) of zinc (the same level) was prepared. All of the ingredients added to the optimized AIO-TPN were within the physiological range. This is the first study that optimized several antioxidants together, including glutathione, in *in vitro* experiments. Interestingly, peroxide levels were significantly lower in LE optimized AIO-TPN (1.16 mM H_2O_2 equivalents) compared to the peroxide levels

generated by LE standard AIO-TPN (1.44 mM H_2O_2 equivalents). The oxidants that are generated during exposure to light were effectively scavenged by a mixture of antioxidants added to the AIO-TPN.

While the optimized TPN showed a reduction in peroxide levels in bench-top experiments using the FOX version II assay, these results do not precisely reflect the complexity of a biological system. *In vitro* studies on cultured cells or *in vivo* studies in animal models are crucial for translating these findings into a meaningful understanding of how the TPN would decrease oxidative stress and enhance the antioxidants in neonates. These models provide a more realistic representation of biological responses, which cannot be depicted through bench-top assays alone.

4.7 *In vitro* cell work of TPN solutions improved the antioxidant status

4.7.1 Lipid peroxidation of THP-1 cells treated with optimized AIO-TPN was low compared to standard AIO-TPN

Loris *et al.* (1998) found that patients who received parenteral nutrition had higher concentrations of malondialdehyde, lower α-tocopherol and selenium status, compared to the control subjects. Among the various lipid emulsions available in the markets, Intralipid™, which contains high amounts of omega-6 fatty acids (ω6:ω3 = 7:1) and less amount of antioxidant vitamin E (38 μg/mL), produced profound lipid peroxidation compared to SMOFlipid®, which contains less omega-6 fatty acid (ω6:ω3 = 2.5:1) and higher vitamin E (200 μg/mL) (Calder *et al.*, 2010; Deshpande *et al.*, 2014; Raman *et al.*, 2017). Although SMOFlipid® has demonstrated benefits over other approved lipid emulsions in Canada, its effectiveness in preventing oxidation

or complications in newborns in clinical settings has been limited. For instance, Ozkan *et al.* (2019) demonstrated in a clinical trial that neonates infused with SMOFlipid® containing parenteral nutrition significantly reduced the incidence of severe BPD and had a shorter mean duration of non-invasive ventilation than the infants infused with soybean oil-based lipid emulsion. As SMOFlipid® has considerable phytosterols and less omega-3 fatty acids compared to Omegaven®, it may still cause adverse effects (Karthigesu *et al.*, 2021).

The THP-1 cells treated with LP 1% SMOFlipid® for 24 hours generated 1.65 μM TBARS compared to that of untreated THP-1 cells (1.15 μM). As previously mentioned, the SMOFlipid® contains a significant amount of polyunsaturated fatty acids and monounsaturated fatty acids (see *Appendix 1*), which are more susceptible to lipid peroxidation. Moreover, it had a considerable amount of phytosterols and lesser amounts of vitamin E than Omegaven®. When treated with 10% AIO-TPN to the THP-1 cells, it generated 1.7 μM TBARS. I also noticed that adding SMOFlipid® to the AIO-TPN slightly increased the TBARS levels. This suggests that antioxidants in the standard TPN are less effective in scavenging the lipid peroxides when adding SMOFlipid®. Moreover, this finding clearly suggested that lipid peroxidation was observed predominantly in SMOFlipid®. However, I noticed that LE (3000 Lux) standard AIO-TPN treated cells generated significantly higher lipid peroxidation (3.5 μM) compared to LP AIO-TPN treated cells (1.3 μM). This finding was consistent elsewhere. Picaud *et al.* (2004) reported that MDA levels in TPN admixtures after 24-hour light exposure significantly increased from 0.179 to 5.8 μM. They also found that the plasma MDA level of infants who received LE TPN was 13.88 μM, which was significantly higher than the control subjects (0.179 μM). AIO-TPN exposed for 24 hours to ambient light in the hospital pharmacy generated 10-fold higher MDA

compared to FF-TPN that was exposed to light for 24 hours (0.316 μM vs. 0.033 μM) (Picaud *et al.*, 2004).

As expected, the LE optimized AIO-TPN for 24 hours treated THP-1 monocytes generated 2.8-fold lower lipid peroxidation compared to that of LE standard AIO-TPN treated cells. These studies observed that lipid peroxidation of LP optimized AIO-TPN was lower than the LP standard AIO-TPN. This could be due to the interactions of antioxidants that were added to the TPN that could neutralize the peroxides.

4.7.2 Vitamins C and E added to the THP-1 cells with SMOFlipid® decreased TBARS

Zarban *et al*. (2015) reported that consumption of vitamin C at the dose of 500 mg and vitamin E at 100 IU for 30 days increased the free radical scavenging activity. Vitamin C and E supplementation in rats lowered the oxidative damage markers, such as 8-hydroxy-2'-deoxyguanosine, MDA, and 4-hydroxyalkenals (Ryan *et al.*, 2010). However, some *in vivo* studies reported that vitamin C and E infusion or supplementation had no effect on preventing oxidation or increasing antioxidant capacity (Huang *et al.*, 2002). Hence, human studies still found inconsistencies in synergistic or additive effective vitamins C and E on lipid peroxidation. To understand the cellular lipid peroxidation, fortification of the SMOFlipid® with vitamin C (from 5 to 40 μM), vitamin E (from 10 to 40 μM) or combinations of vitamins C + E were performed and treated in THP-1 cells. The results showed that adding vitamin C at increasing concentration to the cells did not reduce the lipid peroxidation, which is consistent with the *in vitro* bench-top assessment of peroxides using FOX version II assay. In this study, vitamin E alone or combinations of vitamins C + E at increasing concentrations decreased lipid peroxidation. In contrast to the *in vitro* bench-top finding of vitamin E activity, vitamin E added

to the THP-1 cells decreased lipid peroxidation. It could be due to its lipophilic properties and major antioxidant properties to prevent the oxidative damage of cell membrane phospholipids (Niki, 2014). As vitamin C is hydrophilic and is insoluble in fats, its effect on lipid peroxidation may not be as effective. Hence, I added the lipid-soluble form of vitamin C, 6-*O*-palmitoyl-L-ascorbic acid and examined lipid peroxidation. Ascorbic acid ester with the 16-carbon chain saturated fatty acid, palmitic acid, has amphipathic properties with both hydrophilic and lipophilic characters. It can dissolve in both aqueous and lipid solutions, with its lipophilic nature being more prominent than hydrophilic solubility (de Almeida Torres *et al.*, 2022). It has the property of high solubility in fats, facilitating the cellular delivery of vitamin C and antioxidants (Imran *et al.*, 2024). However, the antioxidant properties of 6-*O*-palmitoyl-L-ascorbic acid are still unclear. However, the results remained the same as water soluble form of vitamin C. There has been no previous study on the antioxidant properties of esterified vitamin C in parenteral nutrition.

In contrast, several *in vitro* studies demonstrated that vitamin C decreases oxidative stress. For instance, vitamin C added to human THP-1 cells exposed to 4-hydroxy-2-nonenal showed a significant reduction in protein carbonylation compared to THP-1 cells that were not pre-incubated with vitamin C (Miranda *et al.*, 2009). Vitamin C treatment at 1 μM for 5 days for the human keratinocytes decreased UVA irradiation-induced lipid peroxidation (Tebbe *et al.*, 1997). Some authors have reported that vitamin C promotes the formation of lipid peroxidation products from lipid hydroperoxides *in vitro*, even in the absence of catalytic iron (Lee *et al.*, 2001). An *in vivo* study reported that oral supplementation of vitamin C at 500 mg dose per day for two months without any other antioxidant did not prevent either *in vitro* lipoprotein oxidation resistance or *in vivo* lipid peroxidation in smoking men and increased TBARS levels (Nyyssönen

et al., 1997). Our data for vitamin E is consistent with the literature. Human immortalized lymphoblastoid cell lines (LCLs) treated with 100 μM vitamin E decreased lipid peroxidation (Ni and Eng, 2012). Another study revealed that L1210 murine leukemia cells treated with 20 μM concentration of vitamin E decreased the rate of free radical-mediated lipid peroxidation (Stehle *et al.*, 2016). Similarly, van Dam *et al.* (2003) reported that α-tocopherol added to human endothelial cells protected the H_2O_2-induced lipid peroxidation. They also reported that vitamin E supplementation decreased the endothelial dysfunction markers in the plasma. Though vitamins C and E have beneficial effects on protecting against oxidative stress, the adequate levels and combination of supplementation are crucial to avoid the adverse effects.

4.7.3 Vitamin C may act as a prooxidant at 40 μM concentration to the THP-1 cells

As previously mentioned in *section 4.2*, vitamin C can act as a prooxidant. Sae-Khow *et al.* (2022) reported that vitamin C in blood higher than 5 mM for 4 days acts as a prooxidant in the extracellular space. Human peripheral blood lymphocytes treated with 100-200 μM vitamin C induced DNA damage (Bhat *et al.*, 2006). Tessier *et al.* (2009) reported that increasing the dose of vitamin C supplementation (from 60 mg/day to 500 mg and 1000 mg/day) to type 2 diabetics did not decrease the LDL peroxidation in a randomized control study. The present study shows that adding vitamins C and E at increasing combinations from 5 μM of vitamin C and 5 μM of vitamin E, 10 μM of vitamin C and 10 μM of vitamin E, and 20 μM of vitamin C and 20 μM of vitamin E to the THP-1 cells decreased the peroxide levels gradually. Interestingly, this study noticed that there was a slight elevation in lipid peroxidation from 20 μM of vitamin C and 20 μM of vitamin E to 40 μM of vitamin C and 40 μM of vitamin E concentrations. Though increasing vitamin E from 5 to 40 μM gradually decreased the TBARS levels, increasing vitamin

C from 20 to 40 μM slightly increased the TBARS levels. This could be due to the prooxidant activity of vitamin C beyond 20 μM concentrations in the THP-1 monocytes. It could be confirmed by assessing the ascorbylperoxide levels or the biomolecular damage, especially DNA damage of the cells due to oxidants (Kaźmierczak-Barańska *et al.*, 2020). However, vitamin E at higher concentrations also acts as a prooxidant as previously mentioned (Bowry and Stocker, 1993). Bowry and Stocker (1993) suggested in an *in vivo* human study that > 400 IU supplementation of vitamin E might lead to a prooxidant status. However, there is no clear demarcation in the levels of vitamins C and E on prooxidant status in infants or adults.

4.7.4 Optimized AIO-TPN significantly decreased the ROS by THP-1 human monocyte cells

ROS are generated during the oxidation of metabolic intermediates in the mitochondria (de Almeida *et al.*, 2022; Dan Dunn *et al.*, 2015; Gruber *et al.*, 2008). The most common ROS are hydroxyl radical ($^{\bullet}OH$), superoxide ($O_2^{\bullet-}$) and H_2O_2 (see *section 1.2*), which are formed during the activity of Nox, radical exposure, or cells under stress (Liu *et al.*, 2018). Even though ROS at lower concentrations act as signalling molecules for various biochemical pathways, the overproduction of ROS can cause oxidative damage to the biomolecules, including lipids, proteins, and DNA, which has been associated with cardiovascular, cancer, neurological, and respiratory diseases (Juan *et al.*, 2021). Antioxidants play a major role in scavenging those ROS molecules. This study, for the first time, examined the ROS activity of THP-1 human monocytes, which were treated with LP or LE AIO-TPN of both optimized and standard TPN. It was found that LE standard AIO-TPN for 24 hours treated to THP-1 cells had high levels of ROS. Hamdan and Puckett (2023) mentioned that ROS was formed in high amounts when TPN was exposed to light. Optimized AIO-TPN to the THP-1 cells significantly decreased the peroxide levels. This

could be due to the antioxidant levels in the optimized AIO-TPN compared to the standard AIO-TPN. For instance, Lan *et al.* (2022) reported that α-tocopherol from 1 to 100 μM in treated bone marrow-derived mesenchymal stem cells (BMSCs) incubated for four hours decreased the ROS levels in a dose-dependent manner. Another study on antioxidant vitamin C revealed that the supplementation of vitamin C at 1000 mg to the healthy women following a single bout of exercise decreased the ROS levels (Yimcharoen *et al.*, 2019). ROS generation was elevated in THP-1 monocytes treated with a herbal medicinal extract from *Leonurus japonicus* Houttuyn, for 24 hours (Park *et al.*, 2022).

Therefore, antioxidant-fortified TPN may decrease ROS levels. It is important to assess the antioxidant status of cells to understand better their ability to counteract oxidants and enhance their protective activity.

4.7.5 Optimized AIO-TPN significantly increased the antioxidant levels

ROS generated during normal cellular metabolism, free radical reaction or exposure of exogenous oxidants can be scavenged by endogenous or exogenous antioxidants (Nwachukwu *et al.*, 2021). The scavenging capacity of antioxidants becomes ineffective when ROS levels exceed the antioxidant capacity of a cell, resulting in oxidative stress. Therefore, it is essential for adequate antioxidants to be present in cells to maintain their redox balance. Assessing the antioxidant status within cells reflects their overall antioxidant capacity and the collective ability of these antioxidants to neutralize harmful ROS. Benzie and Strain (1996) developed the method to measure the antioxidant status of plasma using FRAP assay as a novel method to assess the antioxidant power. It is now widely used to assess the antioxidant status of various biological fluids or plant extracts, including cell lysate and cell supernatants (Belmans *et al.*, 2019; Luo *et*

al., 2019; Madhyastha *et al.*, 2023; Nwachukwu *et al.*, 2021). The FRAP assay is based on the ability of antioxidants to reduce the ferric iron in the form of a colorless Fe^{3+}-2,4,6-tripyridyl-S-triazine (TPTZ) complex to the stable, intense blue color divalent form of Fe^{2+}-TPTZ complex at low pH (Benzie and Strain, 1996; Pulido *et al.*, 2000).

It was found that LP-optimized AIO-TPN showed higher antioxidant activity compared to standard AIO-TPN. This study showed that the antioxidant capacity of THP-1 cells treated with LE-optimized AIO-TPN was similar to the LE standard AIO-TPN. This may be due to the reduction reaction of ferric ions, which are affected by light exposure. Light exposure produces profound hydrogen peroxides in AIO-TPN, as described in *section 3.2*, which converts ferrous to ferric ion via the Fenton reaction (see *section 1.7.1*). This assay could be assessed by adding strong antioxidants like quercetin, which is three-fold higher in activity than Trolox (Firuzi *et al.,* 2005), to enhance the antioxidant status. This study, for the first time, reported the antioxidant capacity of THP-1 cells with parenteral nutrition based on FRAP assay. As the optimized TPN did not show the efficiency of antioxidant activity with the FRAP assay, it could show high expression of antioxidative-related proteins or genes (e.g., superoxide dismutase 2 (*SOD2)*, glutathione peroxidase (*GPX1)*, catalase (*CAT),* NAD(P)H dehydrogenase quinone 1 *(NQO1)*). Assessing such proteins or gene expressions could provide significant results for LE AIO-TPN treated THP-1 cells (Gelain *et al.*, 2009). Hence, assessing one or more of those proteins may provide some insights into these findings.

Assessing antioxidant capacity based on the chemical methods, such as oxygen radical absorption capacity (ORAC), 2,2-diphenyl-1-picrylhydrazyl (DPPH) scavenging, Trolox equivalent antioxidant capacity (TEAC), and FRAP assays, is cost-effective, reliable, and highly reproducible (Shahidi and Zhong, 2015). However, those *in vitro* chemical methods do not

represent the real scenario of cellular activity on scavenging the ROS. Indeed, the findings from those chemical methods may fail to provide sufficient evidence to add antioxidants for TPN in clinical practice (Granato, 2023). Moreover, CAA advances chemical assays by considering cellular absorption, metabolism, distribution, and action of antioxidants within cells, providing a more precise prediction of antioxidant activity in biological systems (Martinelli *et al.*, 2021).

In this study, the CAA method has been employed using THP-1 human monocytes to translate the antioxidant ability in biological systems due to its redox activity (Treml *et al.*, 2021). In the CAA method, the preincubated cells with antioxidants are challenged with the ROS inducer, AAPH, to assess the scavenging action of antioxidants. Overexpressed ROS beyond the capacity of antioxidants, convert DCFH-DA to 2',7'-dichloroflurescein (DCF), which is disproportional to the CAA. The CAA of standard and optimized AIO-TPN of LP or LE were examined. The results showed that optimized AIO-TPN had higher CAA activity compared to standard AIO-TPN. Hence, the findings of this study clearly show that adding vitamins C and E, selenium, and glutathione to TPN scavenges the ROS generated in the TPN effectively. Treml *et al.* (2021) demonstrated that THP-1 human monocytes treated with acteoside (caffeoyl phenylethanoid glycoside obtained from *Paulownia tomentosa*) and diplacone (geranylated flavanone) exhibited higher CAA levels compared to other phenolics, such as morusin (the prenylated flavones obtained from *Morus alba*) and pomiferin (prenylated isoflavone, comes from *Maclura pomifera*). Wolfe *et al.* (2008) found the vitamin C-rich fruits (blueberry and blackberry extracts treated HepG2 cells) had high CAA activity (232-292 QE/100 g of fruit). Substantial studies have been reported on plant extracts, including fruits and vegetables. Hence, comparing my findings with other studies is challenging as this study tried the THP-1 monocytes for parenteral nutrition. The CAA activity was high in HepG2 cells treated with glycated tilapia

hydrolysate (Zhang *et al.*, 2024). The alkaline and acid phenolic extracts of mango peels showed the highest antioxidant potential (60 and 51%) at 125 μg/mL treated to Caco2 cells (Pacheco-Ordaz *et al.*, 2018).

Encapsulated antioxidant β-carotene after an *in vitro*-permeability test using Caco-2 cells exhibited enhanced CAA compared with free β-carotene (Du *et al.*, 2019). Xia *et al.* (2022) stated that selenium-enriched oyster hydrolysate peptides at 50 μg/mL to the HepG2 cells enhanced the CAA. Remarkably, this study revealed that optimized TPN enhanced the CAA activity of THP-1 cells.

4.8 TPN-treated cells showed considerable endoplasmic reticular stress

The ER, a cardinal membrane-bound organelle, is responsible for the biosynthesis of lipids, sterols, membrane-bound and secreted proteins, and glycoproteins. Newly synthesized proteins in the ER require folding and/or assembly prior to trafficking to specific locations to perform their functions (Zhang and Kaufman, 2008). The ER is very sensitive to alterations in intracellular homeostasis and undergoes stress due to endogenous or exogenous insults, including ROS (Fedoroff, 2006), hypoxia (Feldman *et al.*, 2005), hyperglycemia, and hyperlipidemia (Back *et al.*, 2012; Fonseca *et al.*, 2011). ER stress is a potential risk factor for many diseases, such as neurodegenerative disorders, type 2 diabetes, atherosclerosis, liver disease, and cancer (Ozcan and Tabas, 2012; Wu *et al.*, 2018). Moreover, cellular iron homeostasis can be disturbed under ER stress (Oliveira *et al.*, 2011). In response to the aforementioned insults, the ER prompts a protective or adaptive response, to restore the ER homeostasis. If the stress signal is severe, the ER initiates apoptotic pathways (Lin *et al.*, 2008).

GRP78 or BiP, also known as 78 kDa glucose-regulated protein, is a representative ER stress marker that belongs to the ER chaperone heat shock protein (HSP). The stress inducible protein GRP78, one of the 13 highly related proteins of HSPA/ HSP70 found in the ER, is induced upon cell stress (Stetler *et al.*, 2010). In healthy cells, GRP78 regulates protein folding to prevent the accumulation of misfolded proteins and maintains ER homeostasis and cell protection (Lee, 2005; Pfaffenbach and Lee, 2011). This 654 amino acid protein corrects the folding and assembly and prevents the incorrect folding (Hendershot *et al.*, 1994). In the healthy and balanced cell (under homeostasis), GRP78 is bound in an inactive form to activating transcription factor 6 (ATF6), protein kinase RNA-like endoplasmic reticulum kinase (PERK), and inositol-requiring enzyme 1 (IRE1), which are UPR transmembrane stress sensors. When the cell is exposed to unfolded proteins that are accumulated in the ER, the ER responds to the stress and increases the expression of GRP78, released from the UPR sensors (Pfaffenbach and Lee, 2011).

In this study, GRP78 expression was higher in the LE standard TPN than optimized AIO-TPN. However, Li *et al.* (2021) demonstrated that Hantaan virus inoculation to differentiated THP-1 cells and incubated for two hours induced GRP78 expression. THP-1 monocytes treated with lipopolysaccharide, palmitic acid, or oleic acid promoted ER stress by increasing the expression of CCAAT-enhancer-binding protein homologous protein (*CHOP*), activating transcription factor 6 (*ATF6*), *SOD2*, and nuclear factor erythroid 2-related factor 2 (*NRF2*) expression (Akhter *et al.*, 2023). However, they did not study the expression of GRP78. In another study on THP-1 macrophages, UPR markers were increased in individuals with type 2 diabetes when compared with non-diabetic individuals.

Liu *et al.* (2023) demonstrated that lung cancer cells under stress responded to transcriptional regulation that was mediated by expression of GRP78. Tunicamucin at 0.5 μg/mL induced the ER reticular stress in this study. It is consistent with studies elsewhere (Guha *et al.*, 2017; Serrano-Negrón *et al.*, 2018). Exposure of THP-1 macrophages to acetylated low-density lipoprotein (AcLDL) (100 mg/mL) for 24 hours resulted in a significant increase in transcription factor C/EBP homologous protein (CHOP) reflecting ER stress (Tao *et al.*, 2009). Another study revealed that THP-1 monocytes exposed to plasma from type 2 diabetic patients resulted in increasing expression of *GRP78* and *CHOP* mRNA levels compared to that of aged-matched healthy controls (Restaino *et al.*, 2017). There is limited study on TPN and ER stress. This study shows that oxidant-contaminated TPN may induce ER stress.

4.9 Oxidized fatty acids and F_2-isoprostane levels in the TPN

As described before, the SMOFlipid® or AIO-TPN had a high content of unsaturated fatty acids. The omega-6 to omega-3 fatty acid ratio of the AIO-TPN sample was 2.6:1 in this study. The predominant fatty acids found in the AIO-TPN were monounsaturated fatty acid (oleic acid = 48.87%), followed by a considerable percentage of long-chain polyunsaturated fatty acids (16.33%). The finding was consistent with other studies (Chen *et al.*, 2021; Sadu Singh *et al.*, 2020). The polyunsaturated fatty acids are more prone to oxidation. In this study, a total of 3.87% fatty acids was oxidized. Assessing the specific oxidative products formed form polyunsaturated fatty acids, including F_2-isoprostane are good markers to ensure oxidation.

F_2-isoprostanes are prostaglandin-like compounds that are non-enzymatically formed by free radical-induced peroxidation reaction of the omega-6 fatty acid arachidonic acid (C20:4; ω-6 FA). It is one of the predominant and reliable specific lipid biomarkers for oxidative stress in

humans (Ma *et al.*, 2017; Milne *et al.*, 2011). Quantification of F_2-isoprostanes is widely considered a "gold standard" method to assess oxidative status in neonates (Casetta *et al.*, 2012). Various analytical methods have been proposed for measuring F_2-isoprostanes in tissues and fluids. Some of these methods rely on enzyme immunoassays and radioimmunoassays, which are sensitive but require complex clean-up procedures involving solid-phase extraction (SPE) to eliminate interfering compounds from the biological matrix. Additionally, these methods demand large volumes of blood or tissue and are specific only to F_2-isoprostanes, making them unsuitable for newborns. Gas chromatography coupled with mass spectrometry (GC-MS) and tandem mass spectrometry (GC-MS/MS) have also been used to quantify these analytes. Although these methods offer high sensitivity and specificity and are relatively quick processes, they involve labor-intensive and time-consuming derivatization steps, making them impractical for routine analysis. High-performance liquid chromatography coupled with mass spectrometry (LC-MS/MS) offers more benefits than GS-MS/MS in terms of sample preparation and processing time (Casetta *et al.*, 2012; Javid *et al.*, 2018).

Infusion of TPN contaminated with ROS enhances the lipid peroxidation, particularly of arachidonic acid in *in vivo* experiments (with preterm human infants and rats) (Morrow *et al.*, 1992; Roggero *et al.*, 2010) as well as *in vitro* cellular experiments (with liver microsomes from Harlan Spraque-Dawley rats) (Longmire *et al.*, 1994). Deshpande *et al.* (2014) demonstrated that infusing SMOFlipid® (high omega-3 and vitamin E levels) to preterm neonates (23-30 weeks) for seven days decreased the plasma F_2-isoprostane levels compared to baseline. They also noticed the F_2-iosprostane level was high in infants who received Clinoleic (high omega-6 and low vitamin E levels) compared to SMOFlipid® at day eight. Roggero *et al.* (2010) reported no significant difference in the generation of F_2-isoprostane in infants who received parenteral

nutrition for seven days between different types of lipid emulsion to newborns (Intralipid^{TM}, Clinoleic, Lipofundin).

F_2-isoprostane levels are high in preterm neonates (at approximately 4250 pmol/L), indicating extreme oxidative stress in the immediate postnatal period (Deshpande *et al.*, 2009). Although the levels were decreased with supplementing lipid emulsions for six days, the infants who received the soybean oil-based lipid emulsion (low vitamin E content = 14.5 µg/mL) had slightly higher isoprostane levels compared to infants who received olive oil-based lipid emulsion (higher vitamin E content = 30.3 µg/mL) (Deshpande *et al.*, 2009). As a significant peak was not noticed in standard TPN for isoprostane using ESI mode, it would be better to detect isoprostane from the cell lysates or plasma. Vitamin C at 500 mg/day with parenteral nutrition to patients after gastrointestinal surgery significantly decreased 8-isoprostane in the urine compared to patients who received 100 mg/day (Yamazaki *et al.*, 2011).

The ESI method was employed to detect the F_2-isoprostanes in the TPN samples. The reference F_2-isoprostane (8-isoprostane $F_{2\alpha}$) at the concentration of 100 ng/mL showed a significant peak and fragmented ion pattern at MS-MS mode. Literature studies showed the normal range of F_2-isoprostanes from the plasma sample, which ranges from 3000-4000 pM. The LE TPN may produce a low level of F_2-isoprostanes beyond the detection limit.

Overall, antioxidants to the AIO-TPN play a major role in decreasing *in vitro* oxidants and increasing the antioxidant capacity of the cells those treated with optimized TPN. TPN feeding induce ER stress, which may be harmful to the growing children who may be affected by diseases associated with ER stress, as previously described. Moreover, oxidant-overwhelmed TPN administration may alter their metabolic conditions in early childhood and resulting

metabolic syndrome or oxidants-associated diseases in adulthood life. Indeed, excessive oxidative stress can cause several pathological conditions, including cancer, neurodegeneration, cardiovascular diseases, diabetes, and kidney diseases, as discussed elsewhere (Oteiza *et al.*, 2004; Pisoschi and Pop, 2015; Poljsak *et al.*, 2013). Hence, the findings of these studies recommend protecting TPN bags and tubes from light or air during preparation, transport, and infusion at the NICU. Additionally, the study suggests adding additional amounts of vitamins C and E, selenium, and glutathione to the TPN within the physiologically safe levels to minimize *in vivo* oxidation and reduce complications associated with these oxidants.

4.10 Strengths, study limitations, and future directions

For the first time, the antioxidant status of TPN by combining various ingredients, such as vitamins C and E, selenium, GSSG, zinc, and copper, at numerous levels and corresponding combinations was evaluated. Previous studies have generally performed the antioxidant status experiments *in vitro* or *in vivo*, using one or two ingredients without mimicking the clinical scenario, including light exposure. Moreover, this study was driven by utilizing THP-1 human monocytes to assess ROS, TBARS, FRAP, CAA, and ER stress. However, there are some limitations.

This study was performed only using one source of light (LED bright white light) to fix the Lux. Since this study was conducted with a wide range of ingredient combinations, introducing additional light sources used in NICUs, such as amber, soft white, cool white, and daylight, would further intensify the results. Moreover, different bag materials (EVA, PVC, di(2-ethylhexyl) phthalate (DEPH) or polypropylene-based film), thickness, or use of multi-layered bags, may impact light penetration and peroxide generation. Riboflavin and manganese also

impact the peroxide formation and interact with other ingredients. Moreover, aluminum toxicity and its associated oxidative stress could be investigated.

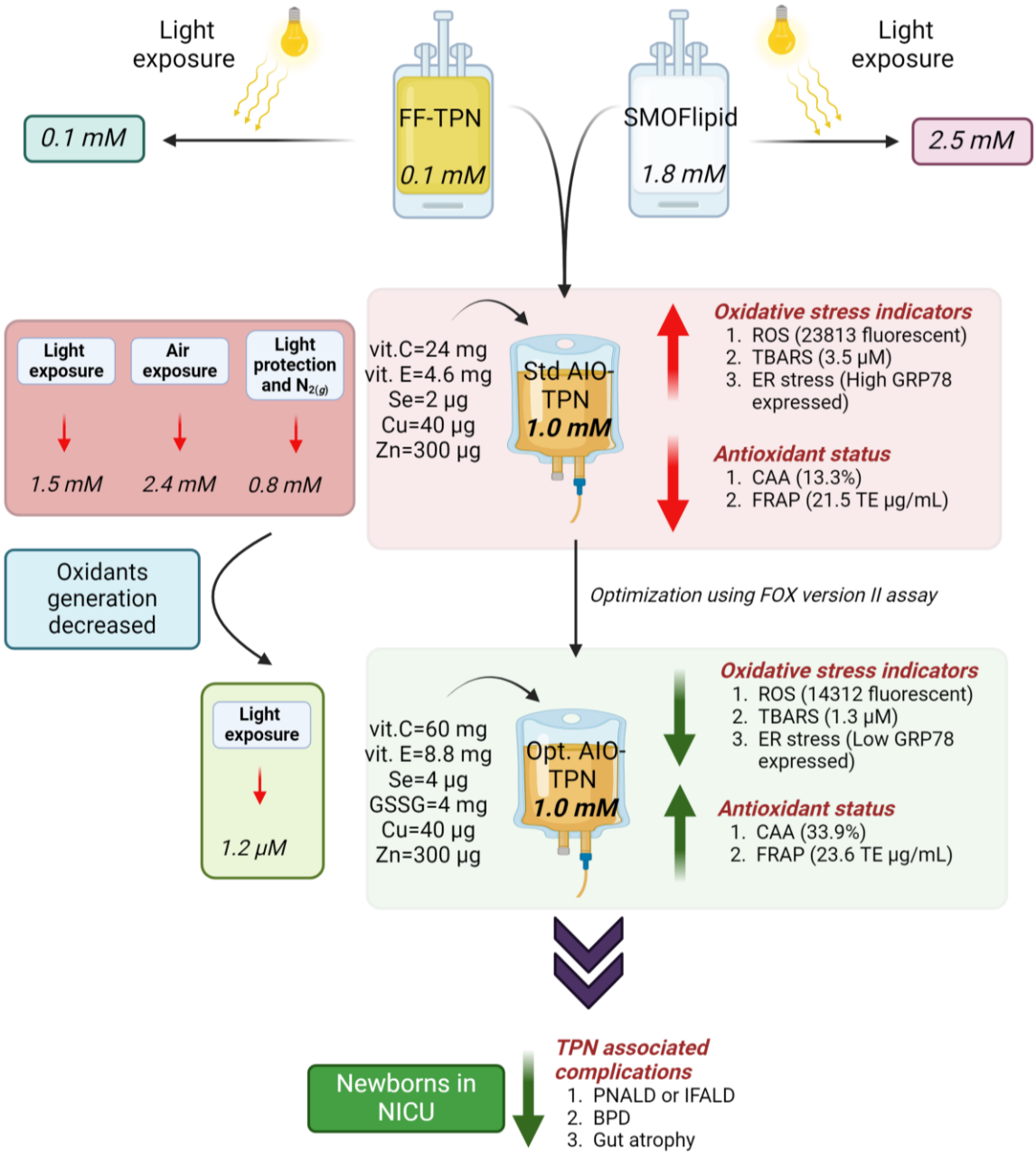
Additionally, specific oxidative products, such as 4-hydroxy-2-nonenal and 4-hydroxy-2-hexanal measurement using LC-MS/MS could provide further comprehension of oxidation in TPN. Separation of F_2-isoprostanes, including all four regioisomers (5-isoprostane, 8-isoprostane, 12-isoprostane, and 15-isoprostane) from other interference by using UHPLC before subjecting the sample to MS/MS would improve the sensitivity of the experiment. In this study, fatty acid analysis was performed using the direct injection method with ESI. Greater sensitivity in quantifying fatty acids and oxidized fatty acids in TPN samples could be achieved by employing chromatographic separation with internal and reference standards. The method should be validated to detect F_2-isoprostanes, including, quality control (QC), recovery, low of detection (LOD), and the limit of quantification (LOQ). The progress of this work has been significantly affected by the COVID-19 pandemic and the relocation of the laboratory, leading to a substantial delay in the re-calibration of the LC-MS/MS and the research work.

Though THP-1 monocytes generated strong results in this study, repeating the experiments with endothelial cells (which are in direct contact with nutrients), or hepatocytes (involved in nutrient metabolism and storage) could yield even more comprehensive outcomes. It was found that the expression of ER stress protein was inefficient in THP-1 monocytes. qPCR analyses or examining different ER stress proteins, such as ATF6, PERK, and IRE1, would be the best approach for detecting ER stress caused by TPN.

Figure 4.1: Schematic representation of the summary of outcomes for *in vitro* optimization of antioxidants of AIO-TPN for neonates

FF-TPN: fat free total parenteral nutrition; SMOFlipid®: the lipid emulsion used to prepare all-in-one (AIO)-TPN; ROS: reactive oxygen species; TBARS: thiobarbituric acid reactive substances for lipid peroxidation; ER stress: endoplasmic reticular stress; CAA: cellular antioxidant activity; FRAP: ferric reducing antioxidant power; NICU: neonatal intensive care unit; PNALD: parenteral nutrition associated liver disease; BPD: bronchopulmonary dysplasia.

Schematic representation of the summary of outcomes



Created with BioRender.com

Oxidative stress is closely linked to inflammation, and THP-1 cells are used to study the interplay between oxidative stress and inflammatory signaling pathways. Examining the expression and secretion of pro-inflammatory cytokines (e.g. interleukin-6, tumor necrosis factor-alpha) and chemokines in response to oxidative stress induction would provide better insight. THP-1 cells serve as a valuable model for elucidating the molecular mechanisms underlying oxidative stress-induced cellular damage. Investigating signaling pathways involved in oxidative stress response, DNA damage repair mechanisms, mitochondrial dysfunction, and redox-sensitive transcription factors such as nuclear factor kappa B (NF-κB) and nuclear factor erythroid 2-related factor 2 (Nrf2). The levels of antioxidative-related markers (*Nrf2*, *CAT*, *SOD-1*, and *SOD-2*) can also be assessed.

The stability of vitamins C and E and glutathione in the TPN requires further investigation. For instance, vitamin C can be easily oxidized in solution. Compounding vitamin C with other micronutrients may cause precipitation and degradation of vitamin C. Moreover, vitamin E degradation was substantial if mixtures were prepared in TPN. Despite considering the optimum antioxidant levels by increasing the concentrations, it is crucial to conduct toxicity studies to establish the maximum safe dosage for neonates.

Infusion of antioxidant-optimized TPN through several mechanisms, as described above, to the preterm babies and assessing the *in vivo* oxidant and antioxidant parameters would lead to developing a novel TPN formula to ameliorate the complications associated with oxidants.

4.11 Conclusions

For the first time the optimization of TPN for neonates by supplementing antioxidants, minimizing the prooxidant levels, and then examining such optimized TPN on lipid peroxidation,

ROS formation, antioxidant capacity, and ER stress using THP-1 human monocytes was established. This study revealed that external factors such as light and air exposure accounted for high peroxide generations in the TPN. Therefore, this study suggests minimizing the light exposure at NICUs and formulating the TPN admixture under nitrogen exposure or to developing strategies targeting the cautious use of oxygen in newborns. Both vitamins C and E have a significant additive effect on lowering the peroxides in the TPN. This study also implies that increasing selenium levels for TPN influences peroxide levels. My study strengthens the need to add glutathione to TPN for improving the antioxidant status. However, prooxidant levels of zinc and copper were not detected in the study. This study supported, for the first time, the assessment of CAA and ER stress using THP-1 cells after treatment with various TPN solutions. Optimizing TPN using antioxidants demonstrated lowering lipid peroxidation, better antioxidant capacity, and improving ER stress. Overall, this study gives novel insight into a promising strategy to develop a new TPN formula for newborns, particularly preterm babies, on decreasing *in vitro* oxidants and eventually minimizing the complications associated with oxidative stress in neonates.

REFERENCES

Abdul-Razzak, K.K., Nusier, M.K., Obediat, A.D. and Salim, A.M. (2007), "Antioxidant vitamins and hyperbilirubinemia in neonates", *GMS German Medical Science*, German Medical Science, Vol. 5, pp 1-5.

Van Aerde, J.E., Duerksen, D.R., Gramlich, L., Meddings, J.B., Chan, G., Thomson, A.B.R. and Clandinin, M.T. (1999), "Intravenous fish oil emulsion attenuates total parenteral nutrition-induced cholestasis in newborn piglets", *Pediatric Research*, Nature Publishing Group, Vol. 45 No. 2, pp. 202–208.

Aitken, J. and Williams, F.L.R. (2014), "A systematic review of thyroid dysfunction in preterm neonates exposed to topical iodine", *Archives of Disease in Childhood-Fetal and Neonatal Edition*, BMJ Publishing Group, Vol. 99 No. 1, pp. F21–F28.

Akhter, N., Wilson, A., Arefanian, H., Thomas, R., Kochumon, S., Al-Rashed, F., Abu-Farha, M., *et al.* (2023), "Endoplasmic reticulum stress promotes the expression of TNF-α in THP-1 cells by mechanisms involving ROS/CHOP/HIF-1α and MAPK/NF-κB pathways", *International Journal of Molecular Sciences*, MDPI, Vol. 24 No. 20, p. 15186.

de Almeida, A.J.P.O., de Oliveira, J.C.P.L., da Silva Pontes, L.V., de Souza Júnior, J.F., Gonçalves, T.A.F., Dantas, S.H., de Almeida Feitosa, M.S., *et al.* (2022), "ROS: basic concepts, sources, cellular signaling, and its implications in aging pathways", *Oxidative Medicine and Cellular Longevity*, Wiley Online Library, Vol. 2022 No. 1, p. 1225578.

de Almeida Torres, R.J., dos Anjos Ferreira, A.L., Luchini, A., de Almeida Torres, R.J. and Correa, C.R. (2022), "The role of non-enzymatic antioxidants on age-related macular degeneration", *Frontiers in Drug, Chemistry and Clinical Research*, Romford, Vol. 5, pp. 1–19.

Andrés, C.M.C., Pérez de la Lastra, J.M., Juan, C.A., Plou, F.J. and Pérez-Lebeña, E. (2022), "Chemistry of hydrogen peroxide formation and elimination in mammalian cells, and its role in various pathologies", *Stresses*, MDPI, Vol. 2 No. 3, pp. 256–274.

Anne, P., Frank, L. and Roberts, R.J. (1976), "Developmental characteristics of pulmonary superoxide dismutase: relationship to idiopathic respiratory distress syndrome", *Pediatric Research*, Nature Publishing Group, Vol. 10 No. 3, pp. 154–158.

ASPEN. (2019a), "Important Facts About Parenteral Selenium (Selenius Acid)". *American Society for Parenteral and Enteral Nutrition,* Available at: https://www.nutritioncare.org/uploadedFiles/Documents/Guidelines_and_Clinical_Resources/Selenium%201-sheet.pdf (Accessed 19 June 2024).

ASPEN. (2019b), "Parenteral Nutrition Resources: Appropriate Dosing for Parenteral Nutrition", *American Society for Parenteral and Enteral Nutrition*, Available at: https://www.nutritioncare.org/pnresources/ (Accessed 24 September 2024).

Ayala, A., Muñoz, M.F. and Argüelles, S. (2014), "Lipid peroxidation: production, metabolism, and signaling mechanisms of malondialdehyde and 4-hydroxy-2-nonenal", *Oxidative Medicine and Cellular Longevity*, Wiley Online Library, Vol. 2014 No. 1, p. 360438.

Ayesh, B.M., Abed, A.A. and Faris, D.M. (2014), "In vitro inhibition of human leukemia THP-1 cells by Origanum syriacum L. and Thymus vulgaris L. extracts", *BMC Research Notes*, Springer, Vol. 7, pp. 1–6.

Back, S.H., Kang, S.-W., Han, J. and Chung, H.-T. (2012), "Endoplasmic reticulum stress in the β-cell pathogenesis of type 2 diabetes", *Journal of Diabetes Research*, Wiley Online Library, Vol. 2012 No. 1, p. 618396.

Balet, A., Cardona, D., Jané, S., Molins-Pujol, A.M., Sanchez Quesada, J.L., Gich, I. and Mangues, M.A. (2004), "Effects of multilayered bags vs ethylvinyl-acetate bags on oxidation of parenteral nutrition", *Journal of Parenteral and Enteral Nutrition*, Wiley Online Library, Vol. 28 No. 2, pp. 85–91.

Bassiouny, M.R., Almarsafawy, H., Abdel-Hady, H., Nasef, N., Hammad, T.A. and Aly, H. (2009), "A randomized controlled trial on parenteral nutrition, oxidative stress, and chronic lung diseases in preterm infants", *Journal of Pediatric Gastroenterology and Nutrition*, LWW, Vol. 48 No. 3, pp. 363–369.

Behrman, R.E., Saldanha, R.L., Cepeda, E.E. and Poland, R.L. (1982), "The effect of vitamin E prophylaxis on the incidence and severity of bronchopulmonary dysplasia", *The Journal of Pediatrics*, Elsevier, Vol. 101 No. 1, pp. 89–93.

Bekeschus, S., Schmidt, A., Bethge, L., Masur, K., von Woedtke, T., Hasse, S. and Wende, K. (2016), "Redox stimulation of human THP-1 monocytes in response to cold physical plasma", *Oxidative Medicine and Cellular Longevity*, Wiley Online Library, Vol. 2016 No. 1, p. 5910695.

Bell, E.F. (1989), "Upper limit of vitamin E in infant formulas", *The Journal of Nutrition*, Elsevier, Vol. 119, pp. 1829–1831.

Belmans, N., Gilles, L., Virag, P., Hedesiu, M., Salmon, B., Baatout, S., Lucas, S., *et al.* (2019), "Method validation to assess in vivo cellular and subcellular changes in buccal mucosa cells and saliva following CBCT examinations", *Dentomaxillofacial Radiology*, Oxford University Press, Vol. 48 No. 6, p. 20180428.

Benzie, I.F.F. and Strain, J.J. (1996), "The ferric reducing ability of plasma (FRAP) as a measure of 'antioxidant power': the FRAP assay", *Analytical Biochemistry*, Elsevier, Vol. 239 No. 1, pp. 70–76.

Berger, M.M. (2009), "Vitamin C requirements in parenteral nutrition", *Gastroenterology*, Elsevier, Vol. 137 No. 5, pp. S70–S78.

Bertolo, R.F., Pencharz, P.B. and Ball, R.O. (2014), "Tissue mineral concentrations are profoundly altered in neonatal piglets fed identical diets via gastric, central venous, or portal venous routes", *Journal of Parenteral and Enteral Nutrition*, Wiley Online Library, Vol. 38 No. 2, pp. 227–235.

Bertolo, R.F.P., Brunton, J.A., Pencharz, P.B. and Ball, R.O. (2003), "Arginine, ornithine, and proline interconversion is dependent on small intestinal metabolism in neonatal pigs", *American Journal of Physiology-Endocrinology and Metabolism*, American Physiological Society Bethesda, MD, Vol. 284 No. 5, pp. E915–E922.

Bhandari, V., Maulik, N. and Kresch, M. (2000), “Hyperoxia causes an increase in antioxidant enzyme activity in adult and fetal rat type II pneumocytes”, *Lung*, Springer, Vol. 178, pp. 53–60.

Bhat, S.H., Azmi, A.S., Hanif, S. and Hadi, S.M. (2006), “Ascorbic acid mobilizes endogenous copper in human peripheral lymphocytes leading to oxidative DNA breakage: a putative mechanism for anticancer properties”, *The International Journal of Biochemistry & Cell Biology*, Elsevier, Vol. 38 No. 12, pp. 2074–2081.

Bhatia, J., Mims, L.C. and Roesel, R.A. (1981), “The effect of phototherapy on amino acid solutions containing multivitamins.”, *The Journal of Pediatrics*, Elsevier, Vol. 96 No.2, pp. 284–286.

Bhatia, J., Moslen, M.T., Haque, A.K., McCleery, R. and Rassin, D.K. (1993), “Total parenteral nutrition-associated alterations in hepatobiliary function and histology in rats: is light exposure a clue?”, *Pediatric Research*, Nature Publishing Group, Vol. 33 No. 5, pp. 487–492.

Bhattarai, K.R., Riaz, T.A., Kim, H.-R. and Chae, H.-J. (2021), “The aftermath of the interplay between the endoplasmic reticulum stress response and redox signaling”, *Experimental & Molecular Medicine*, Nature Publishing Group UK London, Vol. 53 No. 2, pp. 151–167.

Biesalski, H.K. (2009), “Vitamin E requirements in parenteral nutrition”, *Gastroenterology*, Elsevier, Vol. 137 No. 5, pp. S92–S104.

Bizerea-Moga, T.O., Pitulice, L., Bizerea-Spiridon, O. and Moga, T.V. (2024), “Exploring the Link between Oxidative Stress, Selenium Levels, and Obesity in Youth”, *International Journal of Molecular Sciences*, MDPI, Vol. 25 No. 13, p. 7276.

Bligh, E.G. and Dyer, W.J. (1959), “A rapid method of total lipid extraction and purification”, *Canadian Journal of Biochemistry and Physiology*, NRC Research Press Ottawa, Canada, Vol. 37 No. 8, pp. 911–917.

Boullata, J.I., Gilbert, K., Sacks, G., Labossiere, R.J., Crill, C., Goday, P., Kumpf, V.J., *et al.* (2014), “ASPEN clinical guidelines: parenteral nutrition ordering, order review, compounding, labeling, and dispensing”, *Journal of Parenteral and Enteral Nutrition*, Wiley Online Library, Vol. 38 No. 3, pp. 334–377.

Bowry, V.W., Mohr, D., Cleary, J. and Stocker, R. (1995), “Prevention of tocopherol-mediated peroxidation in ubiquinol-10-free human low density lipoprotein (∗)”, *Journal of Biological Chemistry*, ASBMB, Vol. 270 No. 11, pp. 5756–5763.

Bowry, V.W. and Stocker, R. (1993), “Tocopherol-mediated peroxidation. The prooxidant effect of vitamin E on the radical-initiated oxidation of human low-density lipoprotein”, *Journal of the American Chemical Society*, ACS Publications, Vol. 115 No. 14, pp. 6029–6044.

te Braake, F.W.J., Schierbeek, H., Vermes, A., Huijmans, J.G.M. and van Goudoever, J.B. (2009), “High-dose cysteine administration does not increase synthesis of the antioxidant glutathione preterm infants”, *Pediatrics*, American Academy of Pediatrics, Vol. 124 No. 5, pp. e978–e984.

Brawley, V., Bhatia, J. and Karp, W.B. (1993), "Hydrogen peroxide generation in a model paediatric parenteral amino acid solution", *Clinical Science*, Portland Press Ltd., Vol. 85 No. 6, pp. 709–712.

Bray, T.M. and Bettger, W.J. (1990), "The physiological role of zinc as an antioxidant", *Free Radical Biology and Medicine*, Elsevier, Vol. 8 No. 3, pp. 281–291.

Brion, L.P., Bell, E.F., Raghuveer, T.S. and Soghier, L. (2004), "What is the appropriate intravenous dose of vitamin E for very-low-birth-weight infants?", *Journal of Perinatology*, Nature Publishing Group, Vol. 24 No. 4, pp. 205–207.

Buettner, G.R. (1993), "The pecking order of free radicals and antioxidants: lipid peroxidation, α-tocopherol, and ascorbate", *Archives of Biochemistry and Biophysics*, Elsevier, Vol. 300 No. 2, pp. 535–543.

Buettner, G.R. and Jurkiewicz, B.A. (1996), "Catalytic metals, ascorbate and free radicals: combinations to avoid", *Radiation Research*, Radiation Research Society, Vol. 145 No. 5, pp. 532–541.

Bullough, J. and Rea, M.S. (1996), "Lighting for neonatal intensive care units: some critical information for design", *International Journal of Lighting Research and Technology*, SAGE Publications Sage UK: London, England, Vol. 28 No. 4, pp. 189–198.

Buonocore, G., Perrone, S. and Tataranno, M.L. (2010), "Oxygen toxicity: chemistry and biology of reactive oxygen species", *Seminars in Fetal and Neonatal Medicine*, Vol. 15, Elsevier, pp. 186–190.

Burge, J.C., Flancbaum, L. and Holcombe, B. (1994), "Copper decreases ascorbic acid stability in total parenteral nutrition solutions", *Journal of the American Dietetic Association*, Elsevier Science Publishers, Vol. 94 No. 7, pp. 777–780.

Burrin, D., Stoll, B. and Moore, D. (2013), "Digestive physiology of the pig symposium: intestinal bile acid sensing is linked to key endocrine and metabolic signaling pathways", *Journal of Animal Science*, Oxford University Press, Vol. 91 No. 5, pp. 1991–2000.

Burrin, D.G., Ng, K., Stoll, B. and De Pipaón, M.S. (2014), "Impact of new-generation lipid emulsions on cellular mechanisms of parenteral nutrition–associated liver disease", *Advances in Nutrition*, Elsevier, Vol. 5 No. 1, pp. 82–91.

Buys, M., Scheepers, P.A. and Levin, A.I. (2015), "Lipid emulsion therapy: non-nutritive uses of lipid emulsions in anaesthesia and intensive care", *Southern African Journal of Anaesthesia and Analgesia*, Vol. 21 No. 5, pp. 5–11.

Calder, P.C., Jensen, G.L., Koletzko, B. V, Singer, P. and Wanten, G.J.A. (2010), "Lipid emulsions in parenteral nutrition of intensive care patients: current thinking and future directions", *Intensive Care Medicine*, Springer, Vol. 36, pp. 735–749.

Calkins, K.L., Venick, R.S. and Devaskar, S.U. (2014), "Complications associated with parenteral nutrition in the neonate", *Clinics in Perinatology*, Elsevier, Vol. 41 No. 2, pp. 331–345.

Cao, S.S. and Kaufman, R.J. (2014), "Endoplasmic reticulum stress and oxidative stress in cell

fate decision and human disease", *Antioxidants & Redox Signaling*, Mary Ann Liebert, Inc. 140 Huguenot Street, 3rd Floor New Rochelle, NY 10801 USA, Vol. 21 No. 3, pp. 396–413.

Cardoso, D.R., Libardi, S.H. and Skibsted, L.H. (2012), "Riboflavin as a photosensitizer. Effects on human health and food quality", *Food & Function*, Royal Society of Chemistry, Vol. 3 No. 5, pp. 487–502.

Carr, A. and Frei, B. (1999), "Does vitamin C act as a pro-oxidant under physiological conditions?", *The FASEB Journal*, Wiley Online Library, Vol. 13 No. 9, pp. 1007–1024.

Carr, A.C., Zhu, B.-Z. and Frei, B. (2000), "Potential antiatherogenic mechanisms of ascorbate (vitamin C) and α-tocopherol (vitamin E)", *Circulation Research*, American Heart Association, Vol. 87 No. 5, pp. 349–354.

Casetta, B., Longini, M., Proietti, F., Perrone, S. and Buonocore, G. (2012), "Development of a fast and simple LC-MS/MS method for measuring the F2-isoprostanes in newborns", *The Journal of Maternal-Fetal & Neonatal Medicine*, Taylor & Francis, Vol. 25 No. sup1, pp. 114–118.

Chan, A.C. (1993), "Partners in defense, vitamin E and vitamin C", *Canadian Journal of Physiology and Pharmacology*, NRC Research Press Ottawa, Canada, Vol. 71 No. 9, pp. 725–731.

Chen, C.H., Harris, M.B., Partipilo, M.L., Welch, K.B., Teitelbaum, D.H. and Blackmer, A.B. (2016), "Impact of the nationwide intravenous selenium product shortage on the development of selenium deficiency in infants dependent on long-term parenteral nutrition", *Journal of Parenteral and Enteral Nutrition*, Wiley Online Library, Vol. 40 No. 6, pp. 851–859.

Chen, I.-L., Hung, C.-H. and Huang, H.-C. (2021), "Smoflipid is better than lipofundin for long-term neurodevelopmental outcomes in preterm infants", *Nutrients*, MDPI, Vol. 13 No. 8, p. 2548.

Chen, Q., Espey, M.G., Sun, A.Y., Lee, J.-H., Krishna, M.C., Shacter, E., Choyke, P.L., *et al.* (2007), "Ascorbate in pharmacologic concentrations selectively generates ascorbate radical and hydrogen peroxide in extracellular fluid in vivo", *Proceedings of the National Academy of Sciences*, National Acad Sciences, Vol. 104 No. 21, pp. 8749–8754.

Chessex, P., Friel, J., Harrison, A., Rouleau, T. and Lavoie, J.-C. (2005), "The mode of delivery of parenteral multivitamins influences nutrient handling in an animal model of total parenteral nutrition", *Clinical Nutrition*, Elsevier, Vol. 24 No. 2, pp. 281–287.

Chessex, P., Harrison, A., Khashu, M. and Lavoie, J.-C. (2007), "In preterm neonates, is the risk of developing bronchopulmonary dysplasia influenced by the failure to protect total parenteral nutrition from exposure to ambient light?", *The Journal of Pediatrics*, Elsevier, Vol. 151 No. 2, pp. 213–214.

Chessex, P., Laborie, S., Nasef, N., Masse, B. and Lavoie, J. (2017), "Shielding parenteral nutrition from light improves survival rate in premature infants: A meta-analysis", *Journal of Parenteral and Enteral Nutrition*, Wiley Online Library, Vol. 41 No. 3, pp. 378–383.

Chessex, P., Lavoie, J.-C., Rouleau, T., Brochu, P., St-Louis, P., Lévy, E. and Alvarez, F. (2002), "Photooxidation of parenteral multivitamins induces hepatic steatosis in a neonatal guinea pig model of intravenous nutrition", *Pediatric Research*, Nature Publishing Group, Vol. 52 No. 6, pp. 958–963.

Chong, W.C., Shastri, M.D. and Eri, R. (2017), "Endoplasmic reticulum stress and oxidative stress: a vicious nexus implicated in bowel disease pathophysiology", *International Journal of Molecular Sciences*, MDPI, Vol. 18 No. 4, p. 771.

Chung, H.R., Shin, C.H., Yang, S.W., Choi, C.W. and Kim, B. Il. (2009), "Subclinical hypothyroidism in Korean preterm infants associated with high levels of iodine in breast milk", *The Journal of Clinical Endocrinology & Metabolism*, Oxford University Press, Vol. 94 No. 11, pp. 4444–4447.

Clayton, P.T., Bowron, A., Mills, K.A., Massoud, A., Casteels, M. and Milla, P.J. (1993), "Phytosterolemia in children with parenteral nutrition—Associated cholestatic liver disease", *Gastroenterology*, Elsevier, Vol. 105 No. 6, pp. 1806–1813.

Costello, I., Evans, P., Kovar, I.Z. and Halliwell, B. (1997), "Reactive Oxygen Species & Lipid Hydroperoxides in Neonatal Parenteral Nutrition Solutions. 63", *Pediatric Research*, Nature Publishing Group, Vol. 42 No. 3, p. 395.

Cotgreave, I.A. and Schuppe-Koistinen, I. (1994), "A role for γ-glutamyl transpeptidase in the transport of cystine into human endothelial cells: relationship to intracellular glutathione", *Biochimica et Biophysica Acta (BBA)-Molecular Cell Research*, Elsevier, Vol. 1222 No. 3, pp. 375–382.

Cummings, J.E. and Kovacic, J.P. (2009), "The ubiquitous role of zinc in health and disease", *Journal of Veterinary Emergency and Critical Care*, Wiley Online Library, Vol. 19 No. 3, pp. 215–240.

Currò, M., Saija, C., Trainito, A., Trovato-Salinaro, A., Bertuccio, M.P., Visalli, G., Caccamo, D., *et al.* (2023), "Rotenone-induced oxidative stress in THP-1 cells: Biphasic effects of baicalin", *Molecular Biology Reports*, Springer, Vol. 50 No. 2, pp. 1241–1252.

Dąbrowska, K., Zaczek, Z., Panczyk, M., Osowska, S., Kowalczyk, P., Kramkowski, K. and Sobocki, J. (2023), "Molecular Oxygen Levels and Percentages of DNA Damage in TPN Patients", *Nutrients*, MDPI, Vol. 15 No. 9, p. 2206.

van Dam, B., van Hinsbergh, V.W.M., Stehouwer, C.D.A., Versteilen, A., Dekker, H., Buytenhek, R., Princen, H.M., *et al.* (2003), "Vitamin E inhibits lipid peroxidation-induced adhesion molecule expression in endothelial cells and decreases soluble cell adhesion molecules in healthy subjects", *Cardiovascular Research*, Elsevier Science, Vol. 57 No. 2, pp. 563–571.

Dan Dunn, J., Alvarez, L.A.J., Zhang, X. and Soldati, T. (2015), "Reactive oxygen species and mitochondria: A nexus of cellular homeostasis", *Redox Biology*, Elsevier B.V., Vol. 6, p. 472, doi: 10.1016/J.REDOX.2015.09.005.

Darlow, B.A., Winterbourn, C.C., Inder, T.E., Graham, P.J., Harding, J.E., Weston, P.J., Austin, N.C., *et al.* (2000), "The effect of selenium supplementation on outcome in very low birth

weight infants: a randomized controlled trial", *The Journal of Pediatrics*, Elsevier, Vol. 136 No. 4, pp. 473–480.

Dautovich, N.D., Schreiber, D.R., Imel, J.L., Tighe, C.A., Shoji, K.D., Cyrus, J., Bryant, N., *et al.* (2019), "A systematic review of the amount and timing of light in association with objective and subjective sleep outcomes in community-dwelling adults", *Sleep Health*, Elsevier, Vol. 5 No. 1, pp. 31–48.

Deneke, S.M., Susanto, I., Vogel, K.A., Williams, C.E. and Lawrence, R.A. (1995), "Mechanisms of use of extracellular glutathione by lung epithelial cells and pulmonary artery endothelial cells.", *American Journal of Respiratory Cell and Molecular Biology*, American Public Health Association, Vol. 12 No. 6, pp. 662–668.

Dennis, J.M. and Witting, P.K. (2017), "Protective role for antioxidants in acute kidney disease", *Nutrients*, MDPI, Vol. 9 No. 7, p. 718.

Deshpande, G., Simmer, K., Deshmukh, M., Mori, T.A., Croft, K.D. and Kristensen, J. (2014), "Fish Oil (SMOFlipid) and olive oil lipid (Clinoleic) in very preterm neonates", *Journal of Pediatric Gastroenterology and Nutrition*, LWW, Vol. 58 No. 2, pp. 177–182.

Deshpande, G.C. and Cai, W. (2020), "Use of lipids in neonates requiring parenteral nutrition", *Journal of Parenteral and Enteral Nutrition*, Wiley Online Library, Vol. 44, pp. S45–S54.

Deshpande, G.C., Simmer, K., Mori, T. and Croft, K. (2009), "Parenteral lipid emulsions based on olive oil compared with soybean oil in preterm (< 28 weeks' gestation) neonates: a randomised controlled trial", *Journal of Pediatric Gastroenterology and Nutrition*, LWW, Vol. 49 No. 5, pp. 619–625.

Deutsch, J.C. (1998), "Ascorbic acid oxidation by hydrogen peroxide", *Analytical Biochemistry*, Elsevier, Vol. 255 No. 1, pp. 1–7.

Diamond, I.R., Grant, R.C., Pencharz, P.B., de Silva, N., Feldman, B.M., Fitzgerald, P., Sigalet, D., *et al.* (2017), "Preventing the progression of intestinal failure–associated liver disease in infants using a composite lipid emulsion: a pilot randomized controlled trial of SMOFlipid", *Journal of Parenteral and Enteral Nutrition*, Wiley Online Library, Vol. 41 No. 5, pp. 866–877.

Diamond, I.R., de Silva, N.T., Tomlinson, G.A., Pencharz, P.B., Feldman, B.M., Moore, A.M., Ling, S.C., *et al.* (2011), "The role of parenteral lipids in the development of advanced intestinal failure–associated liver disease in infants: a multiple-variable analysis", *Journal of Parenteral and Enteral Nutrition*, Wiley Online Library, Vol. 35 No. 5, pp. 596–602.

Dinesh, O.C., Dodge, M.E., Baldwin, M.P., Bertolo, R.F. and Brunton, J.A. (2014), "Enteral arginine partially ameliorates parenteral nutrition–induced small intestinal atrophy and stimulates hepatic protein synthesis in neonatal piglets", *Journal of Parenteral and Enteral Nutrition*, Wiley Online Library, Vol. 38 No. 8, pp. 973–981.

Dobarganes, M.C. and Velasco, J. (2002), "Analysis of lipid hydroperoxides." *European Journal of Lipid Science and Technology,* Wiley Online Library,Vol. 104 No. 7, pp. 420-428.

Domellöf, M. (2014), "Nutritional care of premature infants: microminerals", *World Review of Nutrition and Dietetics,* Karger, Vol. 110, pp. 121–139.

Domellöf, M., Szitanyi, P., Simchowitz, V., Franz, A., Mimouni, F., Braegger, C., Bronsky, J., *et al.* (2018), "ESPGHAN/ESPEN/ESPR/CSPEN guidelines on pediatric parenteral nutrition: Iron and trace minerals", *Clinical Nutrition*, Elsevier, Vol. 37 No. 6, pp. 2354–2359, doi: 10.1016/J.CLNU.2018.06.949.

Du, Y., Bao, C., Huang, J., Jiang, P., Jiao, L., Ren, F. and Li, Y. (2019), "Improved stability, epithelial permeability and cellular antioxidant activity of β-carotene via encapsulation by self-assembled α-lactalbumin micelles", *Food Chemistry*, Elsevier, Vol. 271, pp. 707–714.

Dudrick, S.J. (2009), "History of parenteral nutrition", *Journal of the American College of Nutrition*, Taylor & Francis, Vol. 28 No. 3, pp. 243–251.

Dudrick, S.J. and Palesty, J.A. (2011), "Historical highlights of the development of total parenteral nutrition", *Surgical Clinics*, Elsevier, Vol. 91 No. 3, pp. 693–717.

Elremaly, W., Mohamed, I., Mialet-Marty, T., Rouleau, T. and Lavoie, J.-C. (2014), "Ascorbylperoxide from parenteral nutrition induces an increase of redox potential of glutathione and loss of alveoli in newborn guinea pig lungs", *Redox Biology*, Elsevier, Vol. 2, pp. 725–731.

Elremaly, W., Mohamed, I., Rouleau, T. and Lavoie, J.-C. (2015), "Adding glutathione to parenteral nutrition prevents alveolar loss in newborn Guinea pig", *Free Radical Biology and Medicine*, Elsevier, Vol. 87, pp. 274–281.

Elremaly, W., Mohamed, I., Rouleau, T. and Lavoie, J.-C. (2016), "Impact of glutathione supplementation of parenteral nutrition on hepatic methionine adenosyltransferase activity", *Redox Biology*, Elsevier, Vol. 8, pp. 18–23.

Esmaeilizadeh, M., Hosseini, M., Beheshti, F., Alikhani, V., Keshavarzi, Z., Shoja, M., Mansoorian, M., *et al.* (2019), "Vitamin C improves liver and renal functions in hypothyroid rats by reducing tissue oxidative injury", *International Journal for Vitamin and Nutrition Research*, Hogrefe Verlag.

Esterbauer, H. (1996), "Estimation of peroxidative damage. A critical review.", *Pathologie-Biologie*, Vol. 44 No. 1, pp. 25–28.

Esterbauer, H., Schaur, R.J. and Zollner, H. (1991), "Chemistry and biochemistry of 4-hydroxynonenal, malonaldehyde and related aldehydes", *Free Radical Biology and Medicine*, Elsevier, Vol. 11 No. 1, pp. 81–128.

Falcone, E., Stellato, F., Vileno, B., Bouraguba, M., Lebrun, V., Ilbert, M., Morante, S., *et al.* (2023), "Revisiting the pro-oxidant activity of copper: interplay of ascorbate, cysteine, and glutathione", *Metallomics*, Oxford University Press, Vol. 15 No. 7, p. mfad040.

Fang, S., Kempley, S.T. and Gamsu, H.R. (2001), "Prediction of early tolerance to enteral feeding in preterm infants by measurement of superior mesenteric artery blood flow velocity", *Archives of Disease in Childhood-Fetal and Neonatal Edition*, BMJ Publishing Group, Vol. 85 No. 1, pp. F42–F45.

Fedoroff, N. (2006), "Redox regulatory mechanisms in cellular stress responses", *Annals of Botany*, Oxford University Press, Vol. 98 No. 2, pp. 289–300.

Feldman, D.E., Chauhan, V. and Koong, A.C. (2005), "The unfolded protein response: a novel component of the hypoxic stress response in tumors", *Molecular Cancer Research*, AACR, Vol. 3 No. 11, pp. 597–605.

Fell, G.L., Nandivada, P., Gura, K.M. and Puder, M. (2015), "Intravenous lipid emulsions in parenteral nutrition", *Advances in Nutrition*, Elsevier, Vol. 6 No. 5, pp. 600–610.

Fessel, J.P., Porter, N.A., Moore, K.P., Sheller, J.R. and Roberts, L.J. (2002), "Discovery of lipid peroxidation products formed in vivo with a substituted tetrahydrofuran ring (isofurans) that are favored by increased oxygen tension", *Proceedings of the National Academy of Sciences*, National Acad Sciences, Vol. 99 No. 26, pp. 16713–16718.

Firuzi, O., Lacanna, A., Petrucci, R., Marrosu, G. and Saso, L. (2005), "Evaluation of the antioxidant activity of flavonoids by 'ferric reducing antioxidant power' assay and cyclic voltammetry", *Biochimica et Biophysica Acta (BBA)-General Subjects*, Elsevier, Vol. 1721 No. 1–3, pp. 174–184.

Foinard, A., Perez, M., Décaudin, B., Barthélémy, C., Tournoys, A., Storme, L. and Odou, P. (2014), "Heparin stability in parenteral nutrition bags prepared in a neonatal ICU", *Critical Care*, Springer, Vol. 18, pp. 1–182.

Fonseca, S.G., Gromada, J. and Urano, F. (2011), "Endoplasmic reticulum stress and pancreatic β-cell death", *Trends in Endocrinology and Metabolism: TEM*, Trends Endocrinol Metab, Vol. 22 No. 7, pp. 266–274.

Foucaud, L., Goulaouic, S., Bennasroune, A., Laval-Gilly, P., Brown, D., Stone, V. and Falla, J. (2010), "Oxidative stress induction by nanoparticles in THP-1 cells with 4-HNE production: stress biomarker or oxidative stress signalling molecule?", *Toxicology in Vitro*, Elsevier, Vol. 24 No. 6, pp. 1512–1520.

Frankel, D.A. (1993), "Supplementation of trace elements in parenteral nutrition: rationale and recommendations", *Nutrition Research*, Elsevier, Vol. 13 No. 5, pp. 583–596.

Gardner, H.W. (1989), "Oxygen radical chemistry of polyunsaturated fatty acids", *Free Radical Biology and Medicine*, Elsevier, Vol. 7 No. 1, pp. 65–86.

Gatto, F., Moglianetti, M., Pompa, P.P. and Bardi, G. (2018), "Platinum nanoparticles decrease reactive oxygen species and modulate gene expression without alteration of immune responses in THP-1 monocytes", *Nanomaterials*, MDPI, Vol. 8 No. 6, p. 392.

Gelain, D.P., Dalmolin, R.J.S., Belau, V.L., Moreira, J.C.F., Klamt, F. and Castro, M.A. (2009), "A systematic review of human antioxidant genes", *Frontiers in Bioscience*, Vol. 14 No. 12, pp. 4457–4463.

Ghasemi, M., Turnbull, T., Sebastian, S. and Kempson, I. (2021), "The MTT assay: utility, limitations, pitfalls, and interpretation in bulk and single-cell analysis", *International Journal of Molecular Sciences*, MDPI, Vol. 22 No. 23, p. 12827.

Giera, M., Lingeman, H. and Niessen, W.M.A. (2012), "Recent advancements in the LC-and GC-based analysis of malondialdehyde (MDA): a brief overview", *Chromatographia*, Springer, Vol. 75, pp. 433–440.

Giustarini, D., Tsikas, D., Colombo, G., Milzani, A., Dalle-Donne, I., Fanti, P. and Rossi, R. (2016), "Pitfalls in the analysis of the physiological antioxidant glutathione (GSH) and its disulfide (GSSG) in biological samples: An elephant in the room", *Journal of Chromatography B*, Elsevier, Vol. 1019, pp. 21–28.

Graham, E.M., Mishra, O.P. and Delivoria-Papadopoulos, M. (1998), "Anti-oxidants and oxidative stress injuries to the brain in the perinatal period", *Seminars in Neonatology*, Vol. 3, Elsevier, pp. 75–85.

Granato, D. (2023), "Cellular antioxidant activity measurement: An alternative to chemical antioxidant methods?", *Food Safety and Health*, Wiley Online Library, Vol. 1 No. 1, pp. 9–12.

Grand, A., Jalabert, A., Mercier, G., Florent, M., Hansel-Esteller, S., Cambonie, G., Steghens, J., *et al.* (2011), "Influence of vitamins, trace elements, and iron on lipid peroxidation reactions in all-in-one admixtures for neonatal parenteral nutrition", *Journal of Parenteral and Enteral Nutrition*, Wiley Online Library, Vol. 35 No. 4, pp. 505–510.

Grissinger, M. (2011), "A fatal zinc overdose in a neonate: confusion of micrograms with milligrams", *Pharmacy and Therapeutics*, MediMedia, USA, Vol. 36 No. 7, p. 393.

Grootjans, J., Kaser, A., Kaufman, R.J. and Blumberg, R.S. (2016), "The unfolded protein response in immunity and inflammation", *Nature Reviews Immunology*, Nature Publishing Group UK London, Vol. 16 No. 8, pp. 469–484.

Gruber, J., Schaffer, S. and Halliwell, B. (2008), "The mitochondrial free radical theory of ageing—where do we stand", *Front Biosci*, Vol. 13 No. 17, pp. 6554–6579.

Guertin, F., Roy, C.C., Lepage, G., Perea, A., Giguère, R., Yousef, I. and Tuchweber, B. (1991), "Effect of taurine on total parenteral nutrition-associated cholestasis", *Journal of Parenteral and Enteral Nutrition*, Wiley Online Library, Vol. 15 No. 3, pp. 247–251.

Guha, P., Kaptan, E., Gade, P., Kalvakolanu, D. V and Ahmed, H. (2017), "Tunicamycin induced endoplasmic reticulum stress promotes apoptosis of prostate cancer cells by activating mTORC1", *Oncotarget*, Impact Journals, LLC, Vol. 8 No. 40, p. 68191.

Gupta, K., Wang, H. and Amin, S.B. (2018), "Copper Supplementation in Premature Infants With Parenteral Nutrition–Associated Cholestasis", *Nutrition in Clinical Practice*, Wiley Online Library, Vol. 33 No. 5, pp. 718–724.

Guthrie, G., Stoll, B., Chacko, S., Lauridsen, C., Plat, J. and Burrin, D. (2020), "Rifampicin, not vitamin E, suppresses parenteral nutrition-associated liver disease development through the pregnane X receptor pathway in piglets", *American Journal of Physiology-Gastrointestinal and Liver Physiology*, American Physiological Society Bethesda, MD, Vol. 318 No. 1, pp. G41–G52.

Guzman, M., Manithody, C., Krebs, J., Denton, C., Besmer, S., Rajalakshmi, P., Jain, S., *et al.* (2020), "Impaired gut–systemic signaling drives total parenteral nutrition-associated injury", *Nutrients*, MDPI, Vol. 12 No. 5, p. 1493.

Ha, E., Wang, W. and Wang, Y.J. (2002), "Peroxide formation in polysorbate 80 and protein stability", *Journal of Pharmaceutical Sciences*, Elsevier, Vol. 91 No. 10, pp. 2252–2264.

Hair, A.B. and Good, M. (2023), "Dilemmas in feeding infants with intestinal failure: a neonatologist's perspective", *Journal of Perinatology*, Nature Publishing Group US New York, Vol. 43 No. 1, pp. 114–119.

Halliwell, B. (1996), "Commentary: vitamin C: antioxidant or pro-oxidant in vivo?", *Free Radical Research*, Taylor & Francis, Vol. 25 No. 5, pp. 439–454.

Halliwell, B. (2000), "Lipid peroxidation, antioxidants and cardiovascular disease: how should we move forward?", *Cardiovascular Research*, Elsevier Science, Vol. 47 No. 3, pp. 410–418.

HaMai, D. and Bondy, S.C. (2004), "Oxidative basis of manganese neurotoxicity", *Annals of the New York Academy of Sciences*, Wiley Online Library, Vol. 1012 No. 1, pp. 129–141.

Hamdan, M. and Puckett, Y. (2023), "Total Parenteral Nutrition", *Treasure Island (FL)*, StatPearls Publishing. Available at: https://www.ncbi.nlm.nih.gov/books/NBK559036/ (Accessed: 29 October 2024).

Han, X. and Gross, R.W. (2005), "Shotgun lipidomics: electrospray ionization mass spectrometric analysis and quantitation of cellular lipidomes directly from crude extracts of biological samples", *Mass Spectrometry Reviews*, Wiley Online Library, Vol. 24 No. 3, pp. 367–412.

Hardy, G. and Puzovic, M. (2009), "Formulation, stability, and administration of parenteral nutrition with new lipid emulsions", *Nutrition in Clinical Practice*, Wiley Online Library, Vol. 24 No. 5, pp. 616–625.

Harvey, K.A., Xu, Z., Pavlina, T.M., Zaloga, G.P. and Siddiqui, R.A. (2015), "Modulation of endothelial cell integrity and inflammatory activation by commercial lipid emulsions", *Lipids in Health and Disease*, Springer, Vol. 14, pp. 1–16.

Heinecke, J.W. (2006), "Lipoprotein oxidation in cardiovascular disease: chief culprit or innocent bystander?", *The Journal of Experimental Medicine*, Rockefeller University Press, Vol. 203 No. 4, pp. 813–816.

Helbock, H.J., Motchnik, P.A. and Ames, B.N. (1993), "Toxic hydroperoxides in intravenous lipid emulsions used in preterm infants", *Pediatrics*, American Academy of Pediatrics, Vol. 91 No. 1, pp. 83–87.

Hendershot, L.M., Valentine, V.A., Lee, A.S., Morris, S.W. and Shapiro, D.N. (1994), "Localization of the gene encoding human BiP/GRP78, the endoplasmic reticulum cognate of the HSP70 family, to chromosome 9q34", *Genomics*, Elsevier, Vol. 20 No. 2, pp. 281–284.

Hetz, C. (2012), "The unfolded protein response: controlling cell fate decisions under ER stress and beyond", *Nature Reviews Molecular Cell Biology*, Nature Publishing Group UK London, Vol. 13 No. 2, pp. 89–102.

Hill, C.H. (1975), "Interrelationships of selenium with other trace elements.", *Federation Proceedings*, Vol. 34, pp. 2096–2100.

Hoff, D.S. and Michaelson, A.S. (2009), "Effects of light exposure on total parenteral nutrition

and its implications in the neonatal population", *The Journal of Pediatric Pharmacology and Therapeutics*, Pediatric Pharmacy Advocacy Group, Vol. 14 No. 3, pp. 132–143.

Hong, L., Wu, J. and Cai, W. (2007), "Glutathione Decreased Parenteral Nutrition–Induced Hepatocyte Injury in Infant Rabbits", *Journal of Parenteral and Enteral Nutrition*, Wiley Online Library, Vol. 31 No. 3, pp. 199–204.

Hu, M. and Palić, D. (2020), "Micro-and nano-plastics activation of oxidative and inflammatory adverse outcome pathways", *Redox Biology*, Elsevier, Vol. 37, p. 101620.

Huang, H.-Y., Appel, L.J., Croft, K.D., Miller III, E.R., Mori, T.A. and Puddey, I.B. (2002), "Effects of vitamin C and vitamin E on in vivo lipid peroxidation: results of a randomized controlled trial", *The American Journal of Clinical Nutrition*, Elsevier, Vol. 76 No. 3, pp. 549–555.

Huber, L.-A., Lee, T., LeDrew, R.L., Dodge, M.E., Brunton, J.A. and Bertolo, R.F. (2019), "Photoprotection but not N-acetylcysteine improves intestinal blood flow and oxidation status in parenterally fed piglets", *Journal of Pediatric Gastroenterology and Nutrition*, LWW, Vol. 69 No. 6, pp. 719–725.

Ibrahim, I.M., Abdelmalek, D.H. and Elfiky, A.A. (2019), "GRP78: A cell's response to stress", *Life Sciences*, Elsevier, Vol. 226, pp. 156–163.

Imran, M., Titilayo, B., Adil, M., Mehmood, Q., Mustafa, S.H. and Shen, Q. (2024), "Ascorbyl palmitate: A comprehensive review on its characteristics, synthesis, encapsulation and applications", *Process Biochemistry*, Elsevier, Vol. 142, p. 68-80

Inagaki, T., Choi, M., Moschetta, A., Peng, L.I., Cummins, C.L., McDonald, J.G., Luo, G., *et al.* (2005), "Fibroblast growth factor 15 functions as an enterohepatic signal to regulate bile acid homeostasis", *Cell Metabolism*, Elsevier, Vol. 2 No. 4, pp. 217–225.

Isaksson, B., Hambraeus, L., Vinnars, E., Samuelson, G., Larsson, J. and Asp, N.-G. (2002), "In memory of Arvid Wretlind 1919-2002", *Food & Nutrition Research*, Swedish Nutrition Foundation, p. 117.

Isesele, P., Vaidya, H., Gill, R. and Cheema, S.K. (2022), "Treatment of preadipocytes with fish oil, mixed oil, or soybean oil-based lipid emulsions have differential effects on the regulation of lipogenic and lipolytic genes in mature 3T3-L1 adipocytes", *Prostaglandins, Leukotrienes and Essential Fatty Acids*, Elsevier, Vol. 177, p. 102396.

Iurlaro, R. and Muñoz-Pinedo, C. (2016), "Cell death induced by endoplasmic reticulum stress", *The FEBS Journal*, Wiley Online Library, Vol. 283 No. 14, pp. 2640–2652.

Iyer, K.R., Spitz, L. and Clayton, P. (1998), "New insight into mechanisms of parenteral nutrition—Associated cholestasis: Role of plant sterols", *Journal of Pediatric Surgery*, Elsevier, Vol. 33 No. 1, pp. 1–6.

Jain, A.K., Stoll, B., Burrin, D.G., Holst, J.J. and Moore, D.D. (2012), "Enteral bile acid treatment improves parenteral nutrition-related liver disease and intestinal mucosal atrophy in neonatal pigs", *American Journal of Physiology-Gastrointestinal and Liver Physiology*, American Physiological Society Bethesda, MD, Vol. 302 No. 2, pp. G218–G224.

Jain, A.K., Wen, J.X., Blomenkamp, K.S., Arora, S., Blaufuss, T.A., Rodrigues, J., Long, J.P., *et al.* (2016), "Oleanolic acid improves gut atrophy induced by parenteral nutrition", *Journal of Parenteral and Enteral Nutrition*, Wiley Online Library, Vol. 40 No. 1, pp. 67–72.

Jalabert, A., Grand, A., Steghens, J., Barbotte, E., Pigue, C. and Picaud, J. (2011), "Lipid peroxidation in all-in-one admixtures for preterm neonates: impact of amount of lipid, type of lipid emulsion and delivery condition", *Acta Paediatrica*, Wiley Online Library, Vol. 100 No. 9, pp. 1200–1205.

Janda, K., Kasprzak, M. and Wolska, J. (2015), "Vitamin C–structure, properties, occurrence and functions", *Pomeranian Journal of Life Sciences*, Vol. 61 No. 4.

Jankov, R.P., Negus, A. and Tanswell, A.K. (2001), "Antioxidants as therapy in the newborn: some words of caution", *Pediatric Research*, Nature Publishing Group, Vol. 50 No. 6, pp. 681–687.

Jansson, P.J., Lindqvist, C. and Nordström, T. (2005), "Iron prevents ascorbic acid (vitamin C) induced hydrogen peroxide accumulation in copper contaminated drinking water", *Free Radical Research*, Taylor & Francis, Vol. 39 No. 11, pp. 1233–1239.

Javid, P.J., Oron, A.P., Duggan, C.P., Squires, R.H., Horslen, S.P. and Consortium, P.I.F. (2018), "The extent of intestinal failure-associated liver disease in patients referred for intestinal rehabilitation is associated with increased mortality: an analysis of the Pediatric Intestinal Failure Consortium database", *Journal of Pediatric Surgery*, Elsevier, Vol. 53 No. 7, pp. 1399–1402.

Jeejeebhoy, K. (2009), "Zinc: an essential trace element for parenteral nutrition", *Gastroenterology*, Elsevier, Vol. 137 No. 5, pp. S7–S12.

Jensen, S.K., Engberg, R.M. and Hedemann, M.S. (1999), "All-rac-α-tocopherol acetate is a better vitamin E source than all-rac-α-tocopherol succinate for broilers", *The Journal of Nutrition*, Elsevier, Vol. 129 No. 7, pp. 1355–1360.

Jin, J., Mulesa, L. and Carrilero Rouillet, M. (2017), "Trace elements in parenteral nutrition: considerations for the prescribing clinician", *Nutrients*, MDPI, Vol. 9 No. 5, p. 440.

Johnson, P.J. (2013), "Review of macronutrients in parenteral nutrition for neonatal intensive care population", *Neonatal Network*, Springer, Vol. 33 No. 1, pp. 29–34.

Johnston, K.M., Gooch, K., Korol, E., Vo, P., Eyawo, O., Bradt, P. and Levy, A. (2014), "The economic burden of prematurity in Canada", *BMC Pediatrics*, Springer, Vol. 14, pp. 1–10.

Juan, C.A., Pérez de la Lastra, J.M., Plou, F.J. and Pérez-Lebeña, E. (2021), "The chemistry of reactive oxygen species (ROS) revisited: outlining their role in biological macromolecules (DNA, lipids and proteins) and induced pathologies", *International Journal of Molecular Sciences*, MDPI, Vol. 22 No. 9, p. 4642.

Kadac-Czapska, K., Ośko, J., Knez, E. and Grembecka, M. (2024), "Microplastics and Oxidative Stress—Current Problems and Prospects", *Antioxidants*, MDPI, Vol. 13 No. 5, p. 579.

Kalyanaraman, B. (2013), "Teaching the basics of redox biology to medical and graduate students: Oxidants, antioxidants and disease mechanisms", *Redox Biology*, Elsevier, Vol. 1

No. 1, pp. 244–257.

Kanike, N., Groh-Wargo, S., Thomas, M., Chien, E.K., Mhanna, M., Kumar, D., Worley, S., *et al.* (2020), "Risk of iodine deficiency in extremely low gestational age newborns on parenteral nutrition", *Nutrients*, MDPI, Vol. 12 No. 6, p. 1636.

Karthigesu, K., Bertolo, R.F. and Brown, R.J. (2021), "Parenteral nutrition and oxidant load in neonates", *Nutrients*, MDPI, Vol. 13 No. 8, p. 2631.

Karthigesu, K., Brown, R.J. and Bertolo, R.F. (2023), "In vitro effect of vitamins C and E on the lipid peroxidation of total parenteral nutrition for newborns", *Clinical Nutrition ESPEN*, Elsevier, Vol. 58, pp. 565–566.

Kathleen, M.G. (2019), "New Lipid Strategies to Prevent/Treat Neonatal Cholestasis", in Josef, N. and Brenda, P. (Eds.), *Gastroenterology and Nutrition*, Third Edit., Elsevier, pp. 133–155, doi: 10.1016/B978-0-323-54502-0.00009-8.

Kaźmierczak-Barańska, J., Boguszewska, K., Adamus-Grabicka, A. and Karwowski, B.T. (2020), "Two faces of vitamin C—antioxidative and pro-oxidative agent", *Nutrients*, MDPI, Vol. 12 No. 5, p. 1501.

Keitel, V., Stindt, J. and Häussinger, D. (2019), "Bile acid-activated receptors: GPBAR1 (TGR5) and other G protein-coupled receptors", *Bile Acids and Their Receptors*, Springer, pp. 19–49.

Kennedy, K.A., Tyson, J.E. and Chamnanvanikij, S. (2000), "Early versus delayed initiation of progressive enteral feedings for parenterally fed low birth weight or preterm infants.", *The Cochrane Database of Systematic Reviews*, Wiley, No. 2, pp. CD001970–CD001970.

Kerwin, J.L., Wiens, A.M. and Ericsson, L.H. (1996), "Identification of fatty acids by electrospray mass spectrometry and tandem mass spectrometry", *Journal of Mass Spectrometry*, Wiley Online Library, Vol. 31 No. 2, pp. 184–192.

Khan, S., Rayis, M., Rizvi, A., Alam, M.M., Rizvi, M. and Naseem, I. (2019), "ROS mediated antibacterial activity of photoilluminated riboflavin: A photodynamic mechanism against nosocomial infections", *Toxicology Reports*, Elsevier, Vol. 6, pp. 136–142.

Khashu, M., Harrison, A., Lalari, V., Gow, A., Lavoie, J.-C. and Chessex, P. (2006), "Photoprotection of parenteral nutrition enhances advancement of minimal enteral nutrition in preterm infants", *Seminars in Perinatology*, Vol. 30, Elsevier, pp. 139–145.

Kim, H., Kirschenbaum, L.J., Rosenthal, I. and Riesz, P. (1993), "Photosensitized formation of ascorbate radicals by riboflavin: an ESR study", *Photochemistry and Photobiology*, Wiley Online Library, Vol. 57 No. 5, pp. 777–784.

Kim, H.Y., Picciano, M.F. and Wallig, M.A. (1992), "Postnatal selenium repletion protects lungs of neonatal rats from hyperoxia", *The Journal of Nutrition*, Elsevier, Vol. 122 No. 9, pp. 1760–1767.

King, H.M., Cosslett, A.G., Thomas, C.P. and Price-Davies, R. (2018), "A HPLC method to monitor the occurrence of lipid peroxidation in intravenous lipid emulsions used in parenteral nutrition using in-line UV and charged aerosol detection", *Clinical Nutrition*

ESPEN, Elsevier, Vol. 28, pp. 96–102.

Kir, S., Beddow, S.A., Samuel, V.T., Miller, P., Previs, S.F., Suino-Powell, K., Xu, H.E., *et al.* (2011), "FGF19 as a postprandial, insulin-independent activator of hepatic protein and glycogen synthesis", *Science*, American Association for the Advancement of Science, Vol. 331 No. 6024, pp. 1621–1624.

Kleiber, N., Chessex, P., Rouleau, T., Nuyt, A.-M., Perreault, M. and Lavoie, J.-C. (2010), "Neonatal exposure to oxidants induces later in life a metabolic response associated to a phenotype of energy deficiency in an animal model of total parenteral nutrition", *Pediatric Research*, Nature Publishing Group, Vol. 68 No. 3, pp. 188–192.

Klinger, G., Shamir, R., Singer, P., Diamond, E.M., Josefsberg, Z. and Sirota, L. (1999), "Parenteral selenium supplementation in extremely low birth weight infants: inadequate dosage but no correlation with hypothyroidism", *Journal of Perinatology*, Nature Publishing Group, Vol. 19 No. 8, pp. 568–572.

Knafo, L., Chessex, P., Rouleau, T. and Lavoie, J.-C. (2005), "Association between hydrogen peroxide-dependent byproducts of ascorbic acid and increased hepatic acetyl-CoA carboxylase activity", *Clinical Chemistry*, Oxford University Press, Vol. 51 No. 8, pp. 1462–1471.

Koseesirikul, P., Chotinaruemol, S. and Ukarapol, N. (2012), "Incidence and risk factors of parenteral nutrition-associated liver disease in newborn infants", *Pediatrics International*, Wiley Online Library, Vol. 54 No. 3, pp. 434–436.

Kumar, J.A. and Teckman, J.H. (2015), "Controversies in the mechanism of total parenteral nutrition induced pathology", *Children*, MDPI, Vol. 2 No. 3, pp. 358–370.

Kurvinen, A., Nissinen, M.J., Andersson, S., Korhonen, P., Ruuska, T., Taimisto, M., Kalliomäki, M., *et al.* (2012), "Parenteral plant sterols and intestinal failure–associated liver disease in neonates", *Journal of Pediatric Gastroenterology and Nutrition*, LWW, Vol. 54 No. 6, pp. 803–811.

Laborie, S., Lavoie, J.-C. and Chessex, P. (1998), "Paradoxical role of ascorbic acid and riboflavin in solutions of total parenteral nutrition: implication in photoinduced peroxide generation", *Pediatric Research*, Nature Publishing Group, Vol. 43 No. 5, pp. 601–606.

Laborie, S., Lavoie, J.-C. and Chessex, P. (2000), "Increased urinary peroxides in newborn infants receiving parenteral nutrition exposed to light", *The Journal of Pediatrics*, Elsevier, Vol. 136 No. 5, pp. 628–632.

Laborie, S., Lavoie, J.-C., Pineault, M. and Chessex, P. (2000), "Contribution of multivitamins, air, and light in the generation of peroxides in adult and neonatal parenteral nutrition solutions", *Annals of Pharmacotherapy*, SAGE Publications, Vol. 34 No. 4, pp. 440–445.

Lacaille, F., Gupte, G., Colomb, V., D'Antiga, L., Hartman, C., Hojsak, I., Kolacek, S., *et al.* (2015), "Intestinal failure–associated liver disease: a position paper of the ESPGHAN Working Group of Intestinal Failure and Intestinal Transplantation", *Journal of Pediatric Gastroenterology and Nutrition*, LWW, Vol. 60 No. 2, pp. 272–283.

Lan, D., Yao, C., Li, X., Liu, H., Wang, D., Wang, Y. and Qi, S. (2022), "Tocopherol attenuates

the oxidative stress of BMSCs by inhibiting ferroptosis through the PI3k/AKT/mTOR pathway", *Frontiers in Bioengineering and Biotechnology*, Frontiers Media SA, Vol. 10, p. 938520.

Lauridsen, C., Engel, H., Jensen, S.K., Craig, A.M. and Traber, M.G. (2002), "Lactating sows and suckling piglets preferentially incorporate RRR-over all-rac-α-tocopherol into milk, plasma and tissues", *The Journal of Nutrition*, Elsevier, Vol. 132 No. 6, pp. 1258–1264.

Lavoie, J.-C., Bélanger, S., Spalinger, M. and Chessex, P. (1997), "Admixture of a multivitamin preparation to parenteral nutrition: the major contributor to in vitro generation of peroxides", *Pediatrics*, American Academy of Pediatrics Elk Grove Village, IL, USA, Vol. 99 No. 3, pp. e6–e6.

Lavoie, J.-C. and Chessex, P. (2019), "Parenteral nutrition and oxidant stress in the newborn: A narrative review", *Free Radical Biology and Medicine*, Elsevier, Vol. 142, pp. 155–167.

Lavoie, J.-C., Mohamed, I. and Teixeira, V. (2022), "Dose–response effects of glutathione supplement in parenteral nutrition on pulmonary oxidative stress and alveolarization in newborn guinea pig", *Antioxidants*, MDPI, Vol. 11 No. 10, p. 1956.

Lavoie, J.-C., Rouleau, T. and Chessex, P. (2004), "Interaction between ascorbate and light-exposed riboflavin induces lung remodeling", *Journal of Pharmacology and Experimental Therapeutics*, ASPET, Vol. 311 No. 2, pp. 634–639.

Lavoie, J.-C., Rouleau, T., Gagnon, C. and Chessex, P. (2002), "Photoprotection prevents TPN-induced lung procollagen mRNA in newborn guinea pigs", *Free Radical Biology and Medicine*, Elsevier, Vol. 33 No. 4, pp. 512–520.

Lavoie, J.-C., Spalinger, M. and Chessex, P. (1999), "Glutathione synthetic activity in the lungs in newborn guinea pigs", *Lung*, Springer, Vol. 177, pp. 1–7.

Le, H.D., De Meijer, V.E., Robinson, E.M., Zurakowski, D., Potemkin, A.K., Arsenault, D.A., Fallon, E.M., *et al.* (2011), "Parenteral fish-oil–based lipid emulsion improves fatty acid profiles and lipids in parenteral nutrition–dependent children", *The American Journal of Clinical Nutrition*, Elsevier, Vol. 94 No. 3, pp. 749–758.

Lee, A.S. (2005), "The ER chaperone and signaling regulator GRP78/BiP as a monitor of endoplasmic reticulum stress", *Methods*, Elsevier, Vol. 35 No. 4, pp. 373–381.

Lee, J.Y., Kim, H.J. and Cho, Y.S. (2023), "Selenium deficiency and supplementation in infants: a narrative review", *Annals of Clinical Nutrition and Metabolism*, The Korean Society of Surgical Metabolism and Nutrition, Vol. 15 No. 1, pp. 8–14.

Lee, J.Y., Shin, H.J., Bae, H.J., Jo, Y.H., Cho, Y.S., Shin, S.H., Kim, E., *et al.* (2022), "Effect of selenium-free parenteral nutrition on serum selenium of neonates and infants maintained on long-term parenteral nutrition", *Journal of Parenteral and Enteral Nutrition*, Wiley Online Library, Vol. 46 No. 5, pp. 1045–1053.

Lee, S.H., Oe, T. and Blair, I.A. (2001), "Vitamin C-induced decomposition of lipid hydroperoxides to endogenous genotoxins", *Science*, American Association for the Advancement of Science, Vol. 292 No. 5524, pp. 2083–2086.

Lee, S.R. (2018), “Critical role of zinc as either an antioxidant or a prooxidant in cellular systems”, *Oxidative Medicine and Cellular Longevity*, Wiley Online Library, Vol. 2018 No. 1, p. 9156285.

Leguina-Ruzzi, A.A. and Ortiz, R. (2018), “Current evidence for the use of smoflipid® emulsion in critical care patients for parenteral nutrition”, *Critical Care Research and Practice*, Wiley Online Library, Vol. 2018 No. 1, p. 6301293.

Lembo, C., Buonocore, G. and Perrone, S. (2021), “Oxidative stress in preterm newborns”, *Antioxidants*, MDPI, Vol. 10 No. 11, p. 1672.

Leung, F.Y. (1998), “Trace elements that act as antioxidants in parenteral micronutrition”, *The Journal of Nutritional Biochemistry*, Elsevier, Vol. 9 No. 6, pp. 304–307.

Lev-Ran, A., Johnson, M., Hwang, D.L., Askanazi, J., Weissman, C. and Gersovitz, M. (1987), “Double-Blind Study of Glycerol vs Glucose in Parenteral Nutrition of Postsurgical Insulin-Treated Diabetic Patients”, *Journal of Parenteral and Enteral Nutrition*, Wiley Online Library, Vol. 11 No. 3, pp. 271–274.

Lewandowski, A.J., Levy, P.T., Bates, M.L., McNamara, P.J., Nuyt, A.M. and Goss, K.N. (2020), “Impact of the vulnerable preterm heart and circulation on adult cardiovascular disease risk”, *Hypertension*, Am Heart Assoc, Vol. 76 No. 4, pp. 1028–1037.

Li, X., Xin, Y., Mo, Y., Marozik, P., He, T. and Guo, H. (2022), “The bioavailability and biological activities of phytosterols as modulators of cholesterol metabolism”, *Molecules*, MDPI, Vol. 27 No. 2, p. 523.

Li, Z., Shen, Y., Song, Y., Zhang, Y., Zhang, C., Ma, Y., Zhang, F., *et al.* (2021), “ER stress-related molecules induced by Hantaan virus infection in differentiated THP-1 cells”, *Cell Stress and Chaperones*, Elsevier, Vol. 26 No. 1, pp. 41–50.

Lin, J.H., Walter, P. and Yen, T.S.B. (2008), “Endoplasmic reticulum stress in disease pathogenesis”, *Annual Review of Pathology: Mechanisms of Disease*, Annual Reviews, Vol. 3 No. 1, pp. 399–425.

Lindeman, J.H.N., D Zoeren-Grobben, V., Schrijver, J., Speek, A.J., Poorthuis, B. and Berger, H.M. (1989), “The total free radical trapping ability of cord blood plasma in preterm and term babies”, *Pediatric Research*, Nature Publishing Group, Vol. 26 No. 1, pp. 20–24.

Linder, M.C. and Hazegh-Azam, M. (1996), “Copper biochemistry and molecular biology”, *The American Journal of Clinical Nutrition*, Elsevier, Vol. 63 No. 5, pp. 797S-811S.

Ling, W.H. and Jones, P.J.H. (1995), “Dietary phytosterols: a review of metabolism, benefits and side effects”, *Life Sciences*, Elsevier, Vol. 57 No. 3, pp. 195–206.

Liu, Y., Zhang, D., Hu, J., Liu, G., Chen, J., Sun, L., Jiang, Z., *et al.* (2015), “Visible light-induced lipid peroxidation of unsaturated fatty acids in the retina and the inhibitory effects of blueberry polyphenols”, *Journal of Agricultural and Food Chemistry*, ACS Publications, Vol. 63 No. 42, pp. 9295–9305.

Liu, Z., Liu, G., Ha, D.P., Wang, J., Xiong, M. and Lee, A.S. (2023), “ER chaperone GRP78/BiP translocates to the nucleus under stress and acts as a transcriptional regulator”, *Proceedings*

of the National Academy of Sciences, National Acad Sciences, Vol. 120 No. 31, p. e2303448120.

Liu, Z., Ren, Z., Zhang, J., Chuang, C.-C., Kandaswamy, E., Zhou, T. and Zuo, L. (2018), "Role of ROS and nutritional antioxidants in human diseases", *Frontiers in Physiology*, Frontiers, Vol. 9, p. 360203.

Loeffler, H., Jonitz-Heincke, A., Peters, K., Mueller-Hilke, B., Fiedler, T., Bader, R. and Klinder, A. (2020), "Comparison of inflammatory effects in THP-1 monocytes and macrophages after exposure to metal ions", *Materials*, MDPI, Vol. 13 No. 5, p. 1150.

Longmire, A.W., Swift, L.L., Roberts II, L.J., Awad, J.A., Burk, R.F. and Morrow, J.D. (1994), "Effect of oxygen tension on the generation of F2-isoprostanes and malondialdehyde in peroxidizing rat liver microsomes", *Biochemical Pharmacology*, Elsevier, Vol. 47 No. 7, pp. 1173–1177.

Loris, P., Enrico, R., Carola, Z., Lucia, S., Elena, I., Andrea, B., Alessandra, M., *et al.* (1998), "Lipid peroxidation and antioxidant status in adults receiving lipid-based home parenteral nutrition", *The American Journal of Clinical Nutrition*, Elsevier, Vol. 68 No. 4, pp. 888–893.

Lu, S.C. (2009), "Regulation of glutathione synthesis", *Molecular Aspects of Medicine*, Elsevier, Vol. 30 No. 1–2, pp. 42–59.

Lu, S.C. (2013), "Glutathione synthesis", *Biochimica et Biophysica Acta (BBA)-General Subjects*, Elsevier, Vol. 1830 No. 5, pp. 3143–3153.

Lubos, E., Loscalzo, J. and Handy, D.E. (2011), "Glutathione peroxidase-1 in health and disease: from molecular mechanisms to therapeutic opportunities", *Antioxidants & Redox Signaling,* Mary Ann Liebert, Vol. 15 No.7, pp 1957–1997.

Lucas, A., Morley, R., Cole, T.J., Lister, G. and Leeson-Payne, C. (1992), "Breast milk and subsequent intelligence quotient in children born preterm", *The Lancet*, Elsevier, Vol. 339 No. 8788, pp. 261–264.

Lunec, J. (1990), "Free radicals: their involvement in disease processes", *Annals of Clinical Biochemistry*, SAGE Publications Sage UK: London, England, Vol. 27 No. 3, pp. 173–182.

Luo, Y., Wan, Q., Xu, M., Zhou, Q., Chen, X., Yin, D., He, H., *et al.* (2019), "Nutritional preconditioning induced by astragaloside IV on isolated hearts and cardiomyocytes against myocardial ischemia injury via improving Bcl-2-mediated mitochondrial function", *Chemico-Biological Interactions*, Elsevier, Vol. 309, p. 108723.

Lushchak, V.I. (2012), "Glutathione homeostasis and functions: potential targets for medical interventions", *Journal of Amino Acids*, Wiley Online Library, Vol. 2012 No. 1, p. 736837.

Luu, T.M., Katz, S.L., Leeson, P., Thébaud, B. and Nuyt, A.-M. (2016), "Preterm birth: risk factor for early-onset chronic diseases", *Cmaj*, The Canadian Medical Association (CMA), Vol. 188 No. 10, pp. 736–746.

Ma, E., Ingram, K.H., Milne, G.L. and Garvey, W.T. (2017), "F2-isoprostanes reflect oxidative stress correlated with lean mass and bone density but not insulin resistance", *Journal of the*

Endocrine Society, Endocrine Society Washington, DC, Vol. 1 No. 5, pp. 436–448.

Madhyastha, H., Madhyastha, R., Chakraborty, E., Banerjee, K., Shah, K., Nakajima, Y., Chauhan, N.S., *et al.* (2023), "Fluro-protein C-phycocyanin docked silver nanocomposite accelerates cell migration through NFκB signaling pathway", *International Journal of Molecular Sciences*, MDPI, Vol. 24 No. 4, p. 3184.

Madnawat, H., Welu, A.L., Gilbert, E.J., Taylor, D.B., Jain, S., Manithody, C., Blomenkamp, K., *et al.* (2020), "Mechanisms of parenteral nutrition–associated liver and gut injury", *Nutrition in Clinical Practice*, Wiley Online Library, Vol. 35 No. 1, pp. 63–71.

Maltepe, E. and Saugstad, O.D. (2009), "Oxygen in health and disease: regulation of oxygen homeostasis-clinical implications", *Pediatric Research*, Nature Publishing Group, Vol. 65 No. 3, pp. 261–268.

Mandy, M. and Nyirenda, M. (2018), "Developmental origins of health and disease: the relevance to developing nations", *International Health*, Oxford University Press, Vol. 10 No. 2, pp. 66–70.

Martinelli, E., Granato, D., Azevedo, L., Gonçalves, J.E., Lorenzo, J.M., Munekata, P.E.S., Simal-Gandara, J., *et al.* (2021), "Current perspectives in cell-based approaches towards the definition of the antioxidant activity in food", *Trends in Food Science & Technology*, Elsevier, Vol. 116, pp. 232–243.

Martini, S., Aceti, A., Della Gatta, A.N., Beghetti, I., Marsico, C., Pilu, G. and Corvaglia, L. (2023), "Antenatal and postnatal sequelae of oxidative stress in preterm infants: a narrative review targeting pathophysiological mechanisms", *Antioxidants*, MDPI, Vol. 12 No. 2, p. 422.

Masenga, S.K., Kabwe, L.S., Chakulya, M. and Kirabo, A. (2023), "Mechanisms of oxidative stress in metabolic syndrome", *International Journal of Molecular Sciences*, MDPI, Vol. 24 No. 9, p. 7898.

Matyas, M. and Zaharie, G. (2019), "Antioxidants at newborns", *Antioxidants*, IntechOpen London, UK, Vol. 10, pp. 97–109.

Mawson, A.R. (2016), "A role for the liver in parturition and preterm birth", *Journal of Translational Science*, NIH Public Access, Vol. 2 No. 3, p. 154.

Mehta, N.M., Skillman, H.E., Irving, S.Y., Coss-Bu, J.A., Vermilyea, S., Farrington, E.A., McKeever, L., *et al.* (2017), "Guidelines for the provision and assessment of nutrition support therapy in the pediatric critically ill patient: Society of Critical Care Medicine and American Society for Parenteral and Enteral Nutrition", *Journal of Parenteral and Enteral Nutrition*, Wiley Online Library, Vol. 41 No. 5, pp. 706–742.

Merritt, R.J., Cohran, V., Raphael, B.P., Sentongo, T., Volpert, D., Warner, B.W. and Goday, P.S. (2017), "Intestinal rehabilitation programs in the management of pediatric intestinal failure and short bowel syndrome", *Journal of Pediatric Gastroenterology and Nutrition*, LWW, Vol. 65 No. 5, pp. 588–596.

Meyer, M.P., Haworth, C., Meyer, J.H. and Commerford, A. (1996), "A comparison of oral and intravenous iron supplementation in preterm infants receiving recombinant erythropoietin",

The Journal of Pediatrics, Elsevier, Vol. 129 No. 2, pp. 258–263.

Milne, G.L., Yin, H., Hardy, K.D., Davies, S.S. and Roberts, L.J. (2011), "Isoprostane generation and function", *Chemical Reviews*, ACS Publications, Vol. 111 No. 10, pp. 5973–5996.

Miloudi, K., Comte, B., Rouleau, T., Montoudis, A., Levy, E. and Lavoie, J.-C. (2012), "The mode of administration of total parenteral nutrition and nature of lipid content influence the generation of peroxides and aldehydes", *Clinical Nutrition*, Elsevier, Vol. 31 No. 4, pp. 526–534.

Miranda, C.L., Reed, R.L., Kuiper, H.C., Alber, S. and Stevens, J.F. (2009), "Ascorbic acid promotes detoxification and elimination of 4-hydroxy-2 (E)-nonenal in human monocytic THP-1 cells", *Chemical Research in Toxicology*, ACS Publications, Vol. 22 No. 5, pp. 863–874.

Mirtallo, J.M. (2001), "Parenteral formulas", *Clinical Nutrition: Parenteral Nutrition, 3rd Ed. WB Saunders*, pp. 118–137.

Moclair, A. and Bates, I. (1995), "The efficacy of heparin in maintaining peripheral infusions in neonates", *European Journal of Pediatrics*, Springer, Vol. 154, pp. 567–570.

Mohamed, I., Elremaly, W., Rouleau, T. and Lavoie, J. (2017), "Ascorbylperoxide contaminating parenteral nutrition is associated with bronchopulmonary dysplasia or death in extremely preterm infants", *Journal of Parenteral and Enteral Nutrition*, Wiley Online Library, Vol. 41 No. 6, pp. 1023–1029.

Mohamed, I., El Raichani, N., Otis, A.-S. and Lavoie, J.-C. (2023), "Parenteral Cysteine Supplementation in Preterm Infants: One Size Does Not Fit All", *Biomedicines*, MDPI, Vol. 12 No. 1, p. 63.

Morin, G., Guiraut, C., Perez Marcogliese, M., Mohamed, I. and Lavoie, J.-C. (2019), "Glutathione supplementation of parenteral nutrition prevents oxidative stress and sustains protein synthesis in guinea pig model", *Nutrients*, MDPI, Vol. 11 No. 9, p. 2063.

Morrow, J.D., Awad, J.A., Kato, T., Takahashi, K., Badr, K.F., Roberts, L.J. and Burk, R.F. (1992), "Formation of novel non-cyclooxygenase-derived prostanoids (F2-isoprostanes) in carbon tetrachloride hepatotoxicity. An animal model of lipid peroxidation.", *The Journal of Clinical Investigation*, Am Soc Clin Investig, Vol. 90 No. 6, pp. 2502–2507.

Mosa, S. and Nasef, N. (2015), "Light-exposed parenteral nutrition solutions and implications for preterm infants", *Diet and Nutrition in Critical Care, Springer, New York, NY*, pp. 2019–2035.

Mozuraityte, R., Kristinova, V., Standal, I.B., Carvajal, A.K. and Aursand, M. (2016), "Oxidative stability and shelf life of fish oil", *Oxidative Stability and Shelf Life of Foods Containing Oils and Fats*, Elsevier, pp. 209–231.

Mungala Lengo, A., Mohamed, I. and Lavoie, J.-C. (2024), "Glutathione Supplementation Prevents Neonatal Parenteral Nutrition-Induced Short-and Long-Term Epigenetic and Transcriptional Disruptions of Hepatic H2O2 Metabolism in Guinea Pigs", *Nutrients*, MDPI, Vol. 16 No. 6, p. 849.

Muto, M., Lim, D., Soukvilay, A., Field, C., Wizzard, P.R., Goruk, S., Ball, R.O., *et al.* (2017), "Supplemental Parenteral Vitamin E Into Conventional Soybean Lipid Emulsion Does Not Prevent Parenteral Nutrition–Associated Liver Disease in Full-Term Neonatal Piglets", *Journal of Parenteral and Enteral Nutrition*, Wiley Online Library, Vol. 41 No. 4, pp. 575–582.

Nandivada, P., Carlson, S.J., Chang, M.I., Cowan, E., Gura, K.M. and Puder, M. (2013), "Treatment of parenteral nutrition-associated liver disease: the role of lipid emulsions", *Advances in Nutrition*, Elsevier, Vol. 4 No. 6, pp. 711–717.

National Guideline Alliance, U. (2020), "Monitoring neonatal parenteral nutrition", *NICE Guideline, No. 154*, National Institute for Health and Care Excellence (NICE). Available at: https://www.nice.org.uk/guidance/ng154 (Accessed on: 29 October 2024).

Navarro-Yepes, J., Zavala-Flores, L., Anandhan, A., Wang, F., Skotak, M., Chandra, N., Li, M., *et al.* (2014), "Antioxidant gene therapy against neuronal cell death", *Pharmacology & Therapeutics*, Elsevier, Vol. 142 No. 2, pp. 206–230.

Neuzil, J., Darlow, B.A., Inder, T.E., Sluis, K.B., Winterbourn, C.C. and Stocker, R. (1995), "Oxidation of parenteral lipid emulsion by ambient and phototherapy lights: potential toxicity of routine parenteral feeding", *The Journal of Pediatrics*, Elsevier, Vol. 126 No. 5, pp. 785–790.

Ng, C.F., Schafer, F.Q., Buettner, G.R. and Rodgers, V.G.J. (2007), "The rate of cellular hydrogen peroxide removal shows dependency on GSH: mathematical insight into in vivo H2O2 and GPx concentrations", *Free Radical Research*, Taylor & Francis, Vol. 41 No. 11, pp. 1201–1211.

Ng, K., Stoll, B., Chacko, S., Saenz de Pipaon, M., Lauridsen, C., Gray, M., Squires, E.J., *et al.* (2016), "Vitamin E in new-generation lipid emulsions protects against parenteral nutrition–associated liver disease in parenteral nutrition–fed preterm pigs", *Journal of Parenteral and Enteral Nutrition*, Wiley Online Library, Vol. 40 No. 5, pp. 656–671.

Ni, Y. and Eng, C. (2012), "Vitamin E protects against lipid peroxidation and rescues tumorigenic phenotypes in cowden/cowden-like patient-derived lymphoblast cells with germline SDHx variants", *Clinical Cancer Research*, AACR, Vol. 18 No. 18, pp. 4954–4961.

Niinikoski, H., Stoll, B., Guan, X., Kansagra, K., Lambert, B.D., Stephens, J., Burrin, D.G., *et al.* (2004), "Onset of small intestinal atrophy is associated with reduced intestinal blood flow in TPN-fed neonatal piglets", *The Journal of Nutrition*, Elsevier, Vol. 134 No. 6, pp. 1467–1474.

Niki, E. (2007), "Tocopherylquinone and tocopherylhydroquinone", *Redox Report*, Taylor & Francis, Vol. 12 No. 5, pp. 204–210.

Niki, E. (2014), "Role of vitamin E as a lipid-soluble peroxyl radical scavenger: in vitro and in vivo evidence", *Free Radical Biology and Medicine*, Elsevier, Vol. 66, pp. 3–12.

Nimal, R., Selcuk, O., Kurbanoglu, S., Shah, A., Siddiq, M. and Uslu, B. (2022), "Trends in electrochemical nanosensors for the analysis of antioxidants", *TrAC Trends in Analytical*

Chemistry, Elsevier, Vol. 153, p. 116626.

Noh, K.-M. and Koh, J.-Y. (2000), "Induction and activation by zinc of NADPH oxidase in cultured cortical neurons and astrocytes", *The Journal of Neuroscience*, Soc Neuroscience, Vol. 20 No. 23, p. RC111.

Nowak, K. (2020), "Parenteral nutrition–associated liver disease", *Clinical Liver Disease*, LWW, Vol. 15 No. 2, pp. 59–62.

Nwachukwu, I.D., Sarteshnizi, R.A., Udenigwe, C.C. and Aluko, R.E. (2021), "A concise review of current in vitro chemical and cell-based antioxidant assay methods", *Molecules*, MDPI, Vol. 26 No. 16, p. 4865.

Nyyssönen, K., Poulsen, H.E., Hayn, M., Agerbo, P., Porkkala-Sarataho, E., Kaikkonen, J., Salonen, R., *et al.* (1997), "Effect of supplementation of smoking men with plain or slow release ascorbic acid on lipoprotein oxidation", *European Journal of Clinical Nutrition*, Nature Publishing Group, Vol. 51 No. 3, pp. 154–163.

Ohuma, E.O., Moller, A.-B., Bradley, E., Chakwera, S., Hussain-Alkhateeb, L., Lewin, A., Okwaraji, Y.B., *et al.* (2023), "National, regional, and global estimates of preterm birth in 2020, with trends from 2010: a systematic analysis", *The Lancet*, Elsevier, Vol. 402 No. 10409, pp. 1261–1271.

Oliveira, S.J., de Sousa, M. and Pinto, J.P. (2011), "ER stress and iron homeostasis: a new frontier for the UPR", *Biochemistry Research International*, Wiley Online Library, Vol. 2011 No. 1, p. 896474.

Oliver, C., Watson, C., Crowley, E., Gilroy, M., Page, D., Weber, K., Messina, D., *et al.* (2019), "Vitamin and mineral supplementation practices in preterm infants: a survey of Australian and New Zealand neonatal intensive and special care units", *Nutrients*, MDPI, Vol. 12 No. 1, p. 51.

Osland, E.J., Ali, A., Isenring, E., Ball, P., Davis, M. and Gillanders, L. (2014), "Australasian Society for Parenteral and Enteral Nutrition guidelines for supplementation of trace elements during parenteral nutrition", *Asia Pacific Journal of Clinical Nutrition*, Wiley Online Library, Vol. 23 No. 4, pp. 545–554.

Oteiza, P.I., Mackenzie, G.G. and Verstraeten, S. V. (2004), "Metals in neurodegeneration: involvement of oxidants and oxidant-sensitive transcription factors", *Molecular Aspects of Medicine*, Elsevier, Vol. 25 No. 1–2, pp. 103–115.

Ozcan, L. and Tabas, I. (2012), "Role of endoplasmic reticulum stress in metabolic disease and other disorders", *Annual Review of Medicine*, Annual Reviews, Vol. 63 No. 1, pp. 317–328.

Ozkan, H., Koksal, N., Dorum, B.A., Kocael, F., Ozarda, Y., Bozyigit, C., Dogan, P., *et al.* (2019), "New-generation fish oil and olive oil lipid for prevention of oxidative damage in preterm infants: single center clinical trial at university hospital in Turkey", *Pediatrics International*, Wiley Online Library, Vol. 61 No. 4, pp. 388–392.

Ozsurekci, Y. and Aykac, K. (2016), "Oxidative stress related diseases in newborns", *Oxidative Medicine and Cellular Longevity*, Wiley Online Library, Vol. 2016 No. 1, p. 2768365.

Pacheco-Ordaz, R., Antunes-Ricardo, M., Gutiérrez-Uribe, J.A. and González-Aguilar, G.A. (2018), "Intestinal permeability and cellular antioxidant activity of phenolic compounds from mango (Mangifera indica cv. Ataulfo) peels", *International Journal of Molecular Sciences*, MDPI, Vol. 19 No. 2, p. 514.

Padayatty, S.J. and Levine, M. (2016), "Vitamin C: the known and the unknown and Goldilocks", *Oral Diseases*, Wiley Online Library, Vol. 22 No. 6, pp. 463–493.

Park, J.H., Nordström, U., Tsiakas, K., Keskin, I., Elpers, C., Mannil, M., Heller, R., *et al.* (2023), "The motor system is exceptionally vulnerable to absence of the ubiquitously expressed superoxide dismutase-1", *Brain Communications*, Oxford University Press US, Vol. 5 No. 1, p. fcad017.

Park, M.N., Um, E.-S., Rahman, M.A., Kim, J.W., Park, S.S., Cho, Y., Song, H., *et al.* (2022), "Leonurus japonicus Houttuyn induces reactive oxygen species-mediated apoptosis via regulation of miR-19a-3p/PTEN/PI3K/AKT in U937 and THP-1 cells", *Journal of Ethnopharmacology*, Elsevier, Vol. 291, p. 115129.

Pathak, P., Xie, C., Nichols, R.G., Ferrell, J.M., Boehme, S., Krausz, K.W., Patterson, A.D., *et al.* (2018), "Intestine farnesoid X receptor agonist and the gut microbiota activate G-protein bile acid receptor-1 signaling to improve metabolism", *Hepatology*, Wiley Online Library, Vol. 68 No. 4, pp. 1574–1588.

Paulsen, C.E. and Carroll, K.S. (2013), "Cysteine-mediated redox signaling: chemistry, biology, and tools for discovery", *Chemical Reviews*, ACS Publications, Vol. 113 No. 7, pp. 4633–4679.

Pederson, A. (2011), *Book Review: The ASPEN Pediatric Nutrition Support Core Curriculum*, *Nutrition in Clinical Practice*, Vol. 26, Wiley Online Library, pp. 358–358, doi: 10.1177/0884533611405539.

Perin, J., Mulick, A., Yeung, D., Villavicencio, F., Lopez, G., Strong, K.L., Prieto-Merino, D., *et al.* (2022), "Global, regional, and national causes of under-5 mortality in 2000–19: an updated systematic analysis with implications for the Sustainable Development Goals", *The Lancet Child & Adolescent Health*, Elsevier, Vol. 6 No. 2, pp. 106–115.

Perks, P., Huynh, E., Kaluza, K. and Boullata, J.I. (2022), "Advances in Trace Element Supplementation for Parenteral Nutrition", *Nutrients*, MDPI, Vol. 14 No. 9, p. 1770, doi: 10.3390/NU14091770.

Perrone, S., Laschi, E. and Buonocore, G. (2019), "Biomarkers of oxidative stress in the fetus and in the newborn", *Free Radical Biology and Medicine*, Elsevier, Vol. 142, pp. 23–31.

Perrone, S., Negro, S., Tataranno, M.L. and Buonocore, G. (2010), "Oxidative stress and antioxidant strategies in newborns", *The Journal of Maternal-Fetal & Neonatal Medicine*, Taylor & Francis, Vol. 23 No. sup3, pp. 63–65.

Perrone, S., Santacroce, A., Picardi, A. and Buonocore, G. (2016), "Fetal programming and early identification of newborns at high risk of free radical-mediated diseases", *World Journal of Clinical Pediatrics*, Baishideng Publishing Group Inc, Vol. 5 No. 2, p. 172.

Pertkiewicz, M., Cosslett, A., Mühlebach, S. and Dudrick, S.J. (2009), "Basics in clinical

nutrition: Stability of parenteral nutrition admixtures", *E-SPEN, the European e-Journal of Clinical Nutrition and Metabolism*, Elsevier, Vol. 3 No. 4, pp. e117–e119.

Pezzati, M., Dani, C., Tronchin, M., Filippi, L., Rossi, S. and Rubaltelli, F.F. (2004), "Prediction of early tolerance to enteral feeding by measurement of superior mesenteric artery blood flow velocity: appropriate-versus small-for-gestational-age preterm infants", *Acta Paediatrica*, Wiley Online Library, Vol. 93 No. 6, pp. 797–802.

Pfaffenbach, K.T. and Lee, A.S. (2011), "The critical role of GRP78 in physiologic and pathologic stress", *Current Opinion in Cell Biology*, Elsevier, Vol. 23 No. 2, pp. 150–156.

Phelps, D.L. (1988), "The role of vitamin E therapy in high-risk neonates.", *Clinics in Perinatology*, Vol. 15 No. 4, pp. 955–963.

Pianese, P., Salvia, G., Campanozzi, A., D'Apolito, O., Russo, A. Dello, Pettoello-Mantovani, M. and Corso, G. (2008), "Sterol profiling in red blood cell membranes and plasma of newborns receiving total parenteral nutrition", *Journal of Pediatric Gastroenterology and Nutrition*, LWW, Vol. 47 No. 5, pp. 645–651.

Picaud, J.C., Steghens, J.P., Auxenfans, C., Barbieux, A., Laborie, S. and Claris, O. (2004), "Lipid peroxidation assessment by malondialdehyde measurement in parenteral nutrition solutions for newborn infants: a pilot study", *Acta Paediatrica*, Wiley Online Library, Vol. 93 No. 2, pp. 241–245.

Pincemail, J., Deby, C., Lismonde, M., Bertrand, Y., Camus, G. and Lamy, M. (1990), "Pentane measurement, an index of in vivo lipoperoxidation: Applications and limits of the method", *Free Radicals, Lipoproteins, and Membrane Lipids*, Springer, pp. 163–169.

Pinto, J.T. and Zempleni, J. (2016), "Riboflavin", *Advances in Nutrition*, American Society for Nutrition, Vol. 7 No. 5, p. 973.

Pioli, P.D. (2019), "Protocol: Hemocytometer cell counting". Available at: https://med.wmich.edu/sites/default/files/Hemacytometer_Cell_Counting.pdf (Accessed: 29 October 2024)

Pironi, L. and Sasdelli, A.S. (2019), "Intestinal failure-associated liver disease", *Clinics in Liver Disease*, Elsevier, Vol. 23 No. 2, pp. 279–291.

Pisoschi, A.M. and Pop, A. (2015), "The role of antioxidants in the chemistry of oxidative stress: A review", *European Journal of Medicinal Chemistry*, Elsevier, Vol. 97, pp. 55–74.

Pitkäken, O.M. (1992), "Peroxidation of lipid emulsions: a hazard for the premature infant receiving parenteral nutrition?", *Free Radical Biology and Medicine*, Elsevier, Vol. 13 No. 3, pp. 239–245.

Pizzino, G., Irrera, N., Cucinotta, M., Pallio, G., Mannino, F., Arcoraci, V., Squadrito, F., *et al.* (2017), "Oxidative stress: harms and benefits for human health", *Oxidative Medicine and Cellular Longevity*, Wiley Online Library, Vol. 2017 No. 1, p. 8416763.

Plaisance, V., Brajkovic, S., Tenenbaum, M., Favre, D., Ezanno, H., Bonnefond, A., Bonner, C., *et al.* (2016), "Endoplasmic reticulum stress links oxidative stress to impaired pancreatic beta-cell function caused by human oxidized LDL", *PLoS One*, Public Library of Science

San Francisco, CA USA, Vol. 11 No. 9, p. e0163046.

Pluth, M.D., Tomat, E. and Lippard, S.J. (2011), "Biochemistry of mobile zinc and nitric oxide revealed by fluorescent sensors", *Annual Review of Biochemistry*, Annual Reviews, Vol. 80 No. 1, pp. 333–355.

Podmore, I.D., Griffiths, H.R., Herbert, K.E., Mistry, N., Mistry, P. and Lunec, J. (1998), "Vitamin C exhibits pro-oxidant properties", *Nature*, Nature Publishing Group UK London, Vol. 392 No. 6676, p. 559.

Pohanka, M., Pejchal, J., Snopková, S., Havlickova, K., Z Karasova, J., Bostik, P. and Pikula, J. (2012), "Ascorbic acid: an old player with a broad impact on body physiology including oxidative stress suppression and immunomodulation: a review", *Mini Reviews in Medicinal Chemistry*, Bentham Science Publishers, Vol. 12 No. 1, pp. 35–43.

Poljsak, B., Šuput, D. and Milisav, I. (2013), "Achieving the balance between ROS and antioxidants: when to use the synthetic antioxidants", *Oxidative Medicine and Cellular Longevity*, Wiley Online Library, Vol. 2013 No. 1, p. 956792.

Pollak, A., Hayde, M., Hayn, M., Herkner, K., Lombard, K.A., Lubec, G., Weninger, M., *et al.* (2001), "Effect of intravenous iron supplementation on erythropoiesis in erythropoietin-treated premature infants", *Pediatrics*, American Academy of Pediatrics Elk Grove Village, IL, USA, Vol. 107 No. 1, pp. 78–85.

Porkkala-Sarataho, E., Salonen, J.T., Nyyssönen, K., Kaikkonen, J., Salonen, R., Ristonmaa, U., Diczfalusy, U., *et al.* (2000), "Long-term effects of vitamin E, vitamin C, and combined supplementation on urinary 7-hydro-8-oxo-2′-deoxyguanosine, serum cholesterol oxidation products, and oxidation resistance of lipids in nondepleted men", *Arteriosclerosis, Thrombosis, and Vascular Biology*, Am Heart Assoc, Vol. 20 No. 9, pp. 2087–2093.

Prasad, A.S., Brewer, G.J., Schoomaker, E.B. and Rabbani, P. (1978), "Hypocupremia induced by zinc therapy in adults", *Jama*, American Medical Association, Vol. 240 No. 20, pp. 2166–2168.

Premkumar, M.H., Carter, B.A., Hawthorne, K.M., King, K. and Abrams, S.A. (2014), "Fish oil–based lipid emulsions in the treatment of parenteral nutrition-associated liver disease: an ongoing positive experience", *Advances in Nutrition*, Elsevier, Vol. 5 No. 1, pp. 65–70.

Prolla, I., Tomlinson, C., Pencharz, P.B., Porto, B., Elango, R. and Courtney-Martin, G. (2022), "Amino acid requirements of total parenteral nutrition (TPN) fed neonates: a narrative review of current knowledge and the basis for a new amino acid solution in neonatal nutrition", *Pediatric Medicine*, AME Publishing Company, Vol. 5.

Pulido, R., Bravo, L. and Saura-Calixto, F. (2000), "Antioxidant activity of dietary polyphenols as determined by a modified ferric reducing/antioxidant power assay", *Journal of Agricultural and Food Chemistry*, ACS Publications, Vol. 48 No. 8, pp. 3396–3402.

Raftery, M.J. (2014), "Determination of oxidative protein modifications using mass spectrometry", *Redox Report*, Taylor & Francis, Vol. 19 No. 4, pp. 140–147.

Raichani, N. El, Guiraut, C., Morin, G., Mohamed, I. and Lavoie, J. (2022), "Stability of glutathione added as a supplement to parenteral nutrition", *Journal of Parenteral and*

Enteral Nutrition, Wiley Online Library, Vol. 46 No. 5, pp. 1080–1087.

Raman, M., Almutairdi, A., Mulesa, L., Alberda, C., Beattie, C. and Gramlich, L. (2017), "Parenteral nutrition and lipids", *Nutrients*, MDPI, Vol. 9 No. 4, p. 388.

Rayman, M.P. (2000), "The importance of selenium to human health", *The Lancet*, Elsevier, Vol. 356 No. 9225, pp. 233–241.

Rea, M.S. and Figueiro, M.G. (2016), "The NICU lighted environment", *Newborn and Infant Nursing Reviews*, Elsevier, Vol. 16 No. 4, pp. 195–202.

Read, A. and Schröder, M. (2021), "The unfolded protein response: an overview", *Biology*, MDPI, Vol. 10 No. 5, p. 384.

Reis, A. and Spickett, C.M. (2012), "Chemistry of phospholipid oxidation", *Biochimica et Biophysica Acta (BBA)-Biomembranes*, Elsevier, Vol. 1818 No. 10, pp. 2374–2387.

Restaino, R.M., Deo, S.H., Parrish, A.R., Fadel, P.J. and Padilla, J. (2017), "Increased monocyte-derived reactive oxygen species in type 2 diabetes: role of endoplasmic reticulum stress", *Experimental Physiology*, Wiley Online Library, Vol. 102 No. 2, pp. 139–153.

Riss, T.L., Moravec, R.A., Niles, A.L., Duellman, S., Benink, H.A., Worzella, T.J. and Minor, L. (2016), "Cell viability assays", *Assay Guidance Manual [Internet]*, Eli Lilly & Company and the National Center for Advancing Translational Sciences. Available at: https://www.ncbi.nlm.nih.gov/books/NBK144065/ (Accessed: 29 October 2024)

Ristoff, E. and Larsson, A. (2007), "Inborn errors in the metabolism of glutathione", *Orphanet Journal of Rare Diseases*, Springer, Vol. 2, pp. 1–9.

Rizvi, S., Raza, S.T., Ahmed, F., Ahmad, A., Abbas, S. and Mahdi, F. (2014), "The role of vitamin E in human health and some diseases", *Sultan Qaboos University Medical Journal*, Sultan Qaboos University, Vol. 14 No. 2, p. e157.

Rizzo, V., Capozza, M., Panza, R., Laforgia, N. and Baldassarre, M.E. (2022), "Macronutrients and micronutrients in parenteral nutrition for preterm newborns: a narrative review", *Nutrients*, MDPI, Vol. 14 No. 7, p. 1530.

Roberts, L.J., Fessel, J.P. and Davies, S.S. (2005), "The biochemistry of the isoprostane, neuroprostane, and isofuran pathways of lipid peroxidation", *Brain Pathology*, Wiley Online Library, Vol. 15 No. 2, pp. 143–148.

Robinson, D.T., Ayers, P., Fleming, B., Gura, K.M., Gutsul, L., Michalski, A., Holcombe, B., *et al.* (2021), "Recommendations for photoprotection of parenteral nutrition for premature infants: An ASPEN position paper", *Nutrition in Clinical Practice*, Wiley Online Library, Vol. 36 No. 5, pp. 927–941.

Robinson, J., Moseley, M.J. and Fielder, A.R. (1990), "Illuminance of neonatal units.", *Archives of Disease in Childhood*, BMJ Publishing Group Ltd, Vol. 65 No. 7 Spec No, pp. 679–682.

Rochling, F.A. and Catron, H.A. (2019), "Intestinal failure-associated liver disease: Causes, manifestations and therapies", *Current Opinion in Gastroenterology*, LWW, Vol. 35 No. 2, pp. 126–133.

Rodríguez, R.G., Pattini, A.E., Roberto, G.R. and Andrea, E.P. (2016), "Neonatal intensive care unit lighting: update and recommendations", *Archivos Argentinos De Pediatria*, Vol. 114 No. 4, pp. 361–367.

Roggero, P., Mosca, F., Giannì, M.L., Orsi, A., Amato, O., Migliorisi, E., Longini, M., *et al.* (2010), "F2-isoprostanes and total radical-trapping antioxidant potential in preterm infants receiving parenteral lipid emulsions", *Nutrition*, Elsevier, Vol. 26 No. 5, pp. 551–555.

Ron, D. and Walter, P. (2007), "Signal integration in the endoplasmic reticulum unfolded protein response", *Nature Reviews Molecular Cell Biology*, Nature Publishing Group UK London, Vol. 8 No. 7, pp. 519–529.

Rubio, C.P. and Cerón, J.J. (2021), "Spectrophotometric assays for evaluation of Reactive Oxygen Species (ROS) in serum: General concepts and applications in dogs and humans", *BMC Veterinary Research*, Springer, Vol. 17 No. 1, p. 226.

Ryan, M.J., Dudash, H.J., Docherty, M., Geronilla, K.B., Baker, B.A., Haff, G.G., Cutlip, R.G., *et al.* (2010), "Vitamin E and C supplementation reduces oxidative stress, improves antioxidant enzymes and positive muscle work in chronically loaded muscles of aged rats", *Experimental Gerontology*, Elsevier, Vol. 45 No. 11, pp. 882–895.

Rydlewski, A.A., Manin, L.P., Pizzo, J.S., Silva, P.D., da Silveira, R., Tavares, C.B.G., de Paula, M., *et al.* (2021), "Lipid profile by direct infusion ESI-MS and fatty acid composition by GC-FID in human milk: Association with nutritional status of donors", *Journal of Food Composition and Analysis*, Elsevier, Vol. 100, p. 103797.

Sadu Singh, B.K., Narayanan, S.S., Khor, B.H., Sahathevan, S., Abdul Gafor, A.H., Fiaccadori, E., Sundram, K., *et al.* (2020), "Composition and functionality of lipid emulsions in parenteral nutrition: examining evidence in clinical applications", *Frontiers in Pharmacology*, Frontiers, Vol. 11, p. 476933.

Sae-Khow, K., Charoensappakit, A., Chiewchengchol, D. and Leelahavanichkul, A. (2022), "High-dose intravenous ascorbate in sepsis, a pro-oxidant enhanced microbicidal activity and the effect on neutrophil functions", *Biomedicines*, MDPI, Vol. 11 No. 1, p. 51.

Salazar, G., Huang, J., Feresin, R.G., Zhao, Y. and Griendling, K.K. (2017), "Zinc regulates Nox1 expression through a NF-κB and mitochondrial ROS dependent mechanism to induce senescence of vascular smooth muscle cells", *Free Radical Biology and Medicine*, Elsevier, Vol. 108, pp. 225–235.

Sato, K., Niki, E. and Shimasaki, H. (1990), "Free radical-mediated chain oxidation of low density lipoprotein and its synergistic inhibition by vitamin E and vitamin C", *Archives of Biochemistry and Biophysics*, Elsevier, Vol. 279 No. 2, pp. 402–405.

Saugstad, O.D. (2003), "Bronchopulmonary dysplasia—oxidative stress and antioxidants", *Seminars in Neonatology*, Elsevier, Vol.8, pp. 39–49.

Saugstad, O.D. (2005), "Room air resuscitation—two decades of neonatal research", *Early Human Development*, Elsevier, Vol. 81 No. 1, pp. 111–116.

Savini, S., D'Ascenzo, R., Biagetti, C., Serpentini, G., Pompilio, A., Bartoli, A., Cogo, P.E., *et al.* (2013), "The effect of 5 intravenous lipid emulsions on plasma phytosterols in preterm

infants receiving parenteral nutrition: a randomized clinical trial", *The American Journal of Clinical Nutrition*, Elsevier, Vol. 98 No. 2, pp. 312–318.

Sen, S. and Chakraborty, R. (2011), "The role of antioxidants in human health", *Oxidative Stress: Diagnostics, Prevention, and Therapy*, ACS Publications, pp. 1–37.

Seo, M.Y. and Lee, S.M. (2002), "Protective effect of low dose of ascorbic acid on hepatobiliary function in hepatic ischemia/reperfusion in rats", *Journal of Hepatology*, Elsevier, Vol. 36 No. 1, pp. 72–77, doi: 10.1016/S0168-8278(01)00236-7.

Serrano-Negrón, J.E., Zhang, Z., Rivera-Ruiz, A.P., Banerjee, A., Romero-Nutz, E.C., Sánchez-Torres, N., Baksi, K., *et al.* (2018), "Tunicamycin-induced ER stress in breast cancer cells neither expresses GRP78 on the surface nor secretes it into the media", *Glycobiology*, Oxford University Press, Vol. 28 No. 2, pp. 61–68.

Shah, P.S., McDonald, S.D., Barrett, J., Synnes, A., Robson, K., Foster, J., Pasquier, J.-C., *et al.* (2018), "The Canadian Preterm Birth Network: a study protocol for improving outcomes for preterm infants and their families", *Canadian Medical Association Open Access Journal*, Canadian Medical Association Open Access Journal, Vol. 6 No. 1, pp. E44–E49.

Shahidi, F. and Zhong, Y. (2015), "Measurement of antioxidant activity", *Journal of Functional Foods*, Elsevier, Vol. 18, pp. 757–781.

Shattuck, K.E., Bhatia, J., Grinnell, C. and Rassin, D.K. (1995), "The Effects of Light Exposure on the In Vitro Hepatic Response to an Amino Acid—Vitamin Solution", *Journal of Parenteral and Enteral Nutrition*, Wiley Online Library, Vol. 19 No. 5, pp. 398–402.

Shichiri, M. (2014), "The role of lipid peroxidation in neurological disorders", *Journal of Clinical Biochemistry and Nutrition*, SOCIETY FOR FREE RADICAL RESEARCH JAPAN, Vol. 54 No. 3, pp. 151–160.

Shike, M. (2009), "Copper in parenteral nutrition", *Gastroenterology*, Elsevier, Vol. 137 No. 5, pp. S13–S17.

Sies, H. (1999), "Glutathione and its role in cellular functions", *Free Radical Biology and Medicine*, Elsevier, Vol. 27 No. 9–10, pp. 916–921.

Silva, E., González, T., Edwards, A.M. and Zuloaga, F. (1998), "Visible light induced lipoperoxidation of a parenteral nutrition fat emulsion sensitized by flavins", *The Journal of Nutritional Biochemistry*, Elsevier, Vol. 9 No. 3, pp. 149–154.

Silvers, K.M., Darlow, B.A. and Winterbourn, C.C. (2001), "Lipid peroxide and hydrogen peroxide formation in parenteral nutrition solutions containing multivitamins", *Journal of Parenteral and Enteral Nutrition*, Wiley Online Library, Vol. 25 No. 1, pp. 14–17.

Silvers, K.M., Sluis, K.B., Darlow, B.A., McGill, F., Stocker, R. and Winterbourn, C.C. (2001), "Limiting light-induced lipid peroxidation and vitamin loss in infant parenteral nutrition by adding multivitamin preparations to Intralipid", *Acta Paediatrica*, Wiley Online Library, Vol. 90 No. 3, pp. 242–249.

Singer, P., Bursztein, S., Kirvelä, O., Mansour, B., Yoshimura, N., Blaustein, J. and Askanazi, J. (1992), "Hypercaloric glycerol in injured patients", *Surgery*, Elsevier, Vol. 112 No. 3, pp.

509–514.

Smith, E.M., Bartosova, Z., Wagner, M., Jaspers, V.L.B. and Monclús, L. (2024), "Exposure to microplastics affects fatty acid composition in the Japanese quail depending on sex and particle size", *Science of the Total Environment*, Elsevier, Vol. 912, p. 169019.

Soghier, L.M. and Brion, L.P. (2006), "Cysteine, cystine or N-acetylcysteine supplementation in parenterally fed neonates", *Cochrane Database of Systematic Reviews*, John Wiley & Sons, Ltd, No. 4.

Spencer, A.U., Yu, S., Tracy, T.F., Aouthmany, M.M., Llanos, A., Brown, M.B., Brown, M., *et al.* (2005), "Parenteral nutrition–associated cholestasis in neonates: multivariate analysis of the potential protective effect of taurine", *Journal of Parenteral and Enteral Nutrition*, Wiley Online Library, Vol. 29 No. 5, pp. 337–344.

Statistics Canada. (2021), "Table 13-10-0404-01 Low birth weight babies, by province and territory", available: https://www150.statcan.gc.ca/t1/tbl1/en/tv.action?pid=1310040401 (Accessed: 5 June 2021).

Steger, P.J.K. and Mühlebach, S.F. (2000), "Lipid peroxidation of intravenous lipid emulsions and all-in-one admixtures in total parenteral nutrition bags: the influence of trace elements", *Journal of Parenteral and Enteral Nutrition*, Wiley Online Library, Vol. 24 No. 1, pp. 37–41.

Stehle, P., Stoffel-Wagner, B. and Kuhn, K.S. (2016), "Parenteral trace element provision: recent clinical research and practical conclusions", *European Journal of Clinical Nutrition*, Nature Publishing Group, Vol. 70 No. 8, pp. 886–893.

Stetler, R.A., Gan, Y., Zhang, W., Liou, A.K., Gao, Y., Cao, G. and Chen, J. (2010), "Heat shock proteins: cellular and molecular mechanisms in the central nervous system", *Progress in Neurobiology*, Elsevier, Vol. 92 No. 2, pp. 184–211.

Suita, S., Ikeda, K., Hayashida, Y., Naito, K., Handa, N. and Doki, T. (1984), "Zinc and copper requirements during parenteral nutrition in the newborn", *Journal of Pediatric Surgery*, J Pediatr Surg, Vol. 19 No. 2, pp. 126–130.

Sullivan, J.L. (1988), "Iron, plasma antioxidants, and the'oxygen radical disease of prematurity'", *American Journal of Diseases of Children*, American Medical Association, Vol. 142 No. 12, pp. 1341–1344.

Sullivan, J.L. and Newton, R.B. (1988), "Serum antioxidant activity in neonates.", *Archives of Disease in Childhood*, BMJ Publishing Group Ltd, Vol. 63 No. 7 Spec No, pp. 748–750.

Talbot, G. (2011), "The Stability and Shelf Life of Fats and Oils", *The Stability and Shelf Life of Food*, Elsevier, pp. 461–503.

Tao, J., Ruan, X., Li, H., Li, X.X., John, E.M., Zac, V. and Li, X.X. (2009), "Endoplasmic reticulum stress is involved in acetylated low-density lipoprotein induced apoptosis in THP-1 differentiated macrophages", *Chinese Medical Journal*, Chinese Medical Journals Publishing House Co., Ltd., Vol. 122 No. 15, pp. 1794–1799.

Tebbe, B., Wu, S., Geilen, C.C., Eberle, J., Kodelja, V. and Orfanos, C.E. (1997), "L-ascorbic

acid inhibits UVA-induced lipid peroxidation and secretion of IL-1α and IL-6 in cultured human keratinocytes in vitro", *Journal of Investigative Dermatology*, Elsevier, Vol. 108 No. 3, pp. 302–306.

Tessier, D.M., Khalil, A., Trottier, L. and Fülöp, T. (2009), "Effects of vitamin C supplementation on antioxidants and lipid peroxidation markers in elderly subjects with type 2 diabetes", *Archives of Gerontology and Geriatrics*, Elsevier, Vol. 48 No. 1, pp. 67–72.

Thomas, S.R. and Stocker, R. (2000), "Molecular action of vitamin E in lipoprotein oxidation:: Implications for atherosclerosis", *Free Radical Biology and Medicine*, Elsevier, Vol. 28 No. 12, pp. 1795–1805.

Ticho, A.L., Malhotra, P., Dudeja, P.K., Gill, R.K. and Alrefai, W.A. (2019), "Bile acid receptors and gastrointestinal functions", *Liver Research*, Elsevier, Vol. 3 No. 1, pp. 31–39.

Tindell, R. and Tipple, T. (2018), "Selenium: implications for outcomes in extremely preterm infants", *Journal of Perinatology*, Nature Publishing Group US New York, Vol. 38 No. 3, pp. 197–202.

Tobin, A.J., Noel, N.P., Christian, S.L. and Brown, R.J. (2021), "Lipoprotein lipase hydrolysis products induce pro-inflammatory cytokine expression in triple-negative breast cancer cells", *BMC Research Notes*, Springer, Vol. 14, pp. 1–6.

Traber, M.G. and Atkinson, J. (2007), "Vitamin E, antioxidant and nothing more", *Free Radical Biology and Medicine*, Elsevier, Vol. 43 No. 1, pp. 4–15.

Traber, M.G. and Stevens, J.F. (2011), "Vitamins C and E: beneficial effects from a mechanistic perspective", *Free Radical Biology and Medicine*, Elsevier, Vol. 51 No. 5, pp. 1000–1013.

Treml, J., Večeřová, P., Herczogová, P. and Šmejkal, K. (2021), "Direct and indirect antioxidant effects of selected plant phenolics in cell-based assays", *Molecules*, MDPI, Vol. 26 No. 9, p. 2534.

Tsotsolis, N., Tsirgogianni, K., Kioumis, I., Pitsiou, G., Baka, S., Papaiwannou, A., Karavergou, A., *et al.* (2015), "Pneumothorax as a complication of central venous catheter insertion", *Annals of Translational Medicine*, AME Publications, Vol. 3 No. 3.

Unal, S., Demirel, N., Erol, S., Isik, D.U., Kulali, F., Iyigun, F. and Bas, A.Y. (2018), "Effects of two different lipid emulsions on morbidities and oxidant stress statuses in preterm infants: an observational study", *The Journal of Maternal-Fetal & Neonatal Medicine*, Taylor & Francis, Vol. 31 No. 7, pp. 850–856.

Vanek, V.W., Borum, P., Buchman, A., Fessler, T.A., Howard, L., Jeejeebhoy, K., Kochevar, M., *et al.* (2012), "ASPEN position paper: recommendations for changes in commercially available parenteral multivitamin and multi–trace element products", *Nutrition in Clinical Practice*, Wiley Online Library, Vol. 27 No. 4, pp. 440–491.

Vento, M., Moro, M., Escrig, R., Arruza, L., Villar, G., Izquierdo, I., Roberts, L.J., *et al.* (2009), "Preterm resuscitation with low oxygen causes less oxidative stress, inflammation, and chronic lung disease", *Pediatrics*, American Academy of Pediatrics, Vol. 124 No. 3, pp. e439–e449.

Vercauteren, M., Panneel, L., Jorens, P.G., Covaci, A., Cleys, P., Mulder, A., Janssen, C.R., *et al.* (2024), "An Ex Vivo Study Examining Migration of Microplastics from an Infused Neonatal Parenteral Nutrition Circuit", *Environmental Health Perspectives*, Vol. 132 No. 3, p. 37703.

Verrastro, I., Pasha, S., Tveen Jensen, K., Pitt, A.R. and Spickett, C.M. (2015), "Mass spectrometry-based methods for identifying oxidized proteins in disease: advances and challenges", *Biomolecules*, MDPI, Vol. 5 No. 2, pp. 378–411.

Vitetta, L. and Linnane, A.W. (2014), "Endocellular regulation by free radicals and hydrogen peroxide: key determinants of the inflammatory response", *Inflammopharmacology*, Springer, Vol. 22 No. 2, pp. 69–72.

Vivekanandan-Giri, A., Byun, J. and Pennathur, S. (2011), "Quantitative analysis of amino acid oxidation markers by tandem mass spectrometry", *Methods in Enzymology*, Vol. 491, Elsevier, pp. 73–89.

Waitzberg, D.L. and Torrinhas, R.S. (2009), "Fish oil lipid emulsions and immune response: what clinicians need to know", *Nutrition in Clinical Practice*, Wiley Online Library, Vol. 24 No. 4, pp. 487–499.

Waitzberg, D.L., Torrinhas, R.S. and Jacintho, T.M. (2006), "New parenteral lipid emulsions for clinical use", *Journal of Parenteral and Enteral Nutrition*, Wiley Online Library, Vol. 30 No. 4, pp. 351–367.

Wang, N., Wang, J., Zhang, T., Huang, L., Yan, W., Lu, L., Jia, J., *et al.* (2021), "Alterations of gut microbiota and serum bile acids are associated with parenteral nutrition-associated liver disease", *Journal of Pediatric Surgery*, Elsevier, Vol. 56 No. 4, pp. 738–744.

Wanten, G.J.A. and Calder, P.C. (2007), "Immune modulation by parenteral lipid emulsions", *The American Journal of Clinical Nutrition*, Elsevier, Vol. 85 No. 5, pp. 1171–1184.

Watrobska-Swietlikowska, D. and Szlagatys-Sidorkiewicz, A. (2015), "Evaluation of physical stability of all in one parenteral admixtures for pediatric home care with high electrolytes concentrations", *Nutricion Hospitalaria*, Sociedad Española de Nutrición Parenteral y Enteral, Vol. 31 No. 1, pp. 236–243.

Wolfe, K.L., Kang, X., He, X., Dong, M., Zhang, Q. and Liu, R.H. (2008), "Cellular antioxidant activity of common fruits", *Journal of Agricultural and Food Chemistry*, ACS Publications, Vol. 56 No. 18, pp. 8418–8426.

Wolfe, K.L. and Liu, R.H. (2007), "Cellular antioxidant activity (CAA) assay for assessing antioxidants, foods, and dietary supplements", *Journal of Agricultural and Food Chemistry*, ACS Publications, Vol. 55 No. 22, pp. 8896–8907.

Wolff, S.P. and Woff, S.P. (1994), "[18] Ferrous ion oxidation in presence of ferric ion indicator xylenol orange for measurement of hydroperoxides", *Methods in Enzymology*, Vol. 233, Elsevier, pp. 182–189.

Wongkittichote, P., Lasio, M.L.D., Magistrati, M., Pathak, S., Sample, B., Carvalho, D.R., Ortega, A.B., *et al.* (2023), "Phenotypic, molecular, and functional characterization of COQ7-related primary CoQ10 deficiency: Hypomorphic variants and two distinct disease

entities", *Molecular Genetics and Metabolism*, Elsevier, Vol. 139 No. 4, p. 107630.

Wu, F., Tian, F.-J. and Lin, Y. (2015), "Oxidative stress in placenta: health and diseases", *BioMed Research International*, Wiley Online Library, Vol. 2015 No. 1, p. 293271.

Wu, J., Chen, S., Liu, H., Zhang, Z., Ni, Z., Chen, J., Yang, Z., *et al.* (2018), "Tunicamycin specifically aggravates ER stress and overcomes chemoresistance in multidrug-resistant gastric cancer cells by inhibiting N-glycosylation", *Journal of Experimental & Clinical Cancer Research*, Springer, Vol. 37, pp. 1–12.

Xia, Z., Miao, J., Chen, B., Guo, J., Ou, Y., Liang, X., Yin, Y., *et al.* (2022), "Purification, identification, and antioxidative mechanism of three novel selenium-enriched oyster antioxidant peptides", *Food Research International*, Elsevier, Vol. 157, p. 111359.

Xu, X.-T., Huang, H., Tian, M.-X., Hu, R.-C., Dai, Z. and Jin, X. (2021), "A four-oil intravenous lipid emulsion improves markers of liver function, triglyceride levels and shortens length of hospital stay in adults: a systematic review and meta-analysis", *Nutrition Research*, Elsevier, Vol. 92, pp. 1–11.

Yailian, A.-L., Serre, C., Fayard, J., Faucon, M., Thomaré, P., Filali, S., Pivot, C., *et al.* (2019), "Production and stability study of a hospital parenteral nutrition solution for neonates", *Journal of Pharmaceutical Analysis*, Elsevier, Vol. 9 No. 2, pp. 83–90.

Yamazaki, E., Horikawa, M. and Fukushima, R. (2011), "Vitamin C supplementation in patients receiving peripheral parenteral nutrition after gastrointestinal surgery", *Nutrition*, Elsevier, Vol. 27 No. 4, pp. 435–439.

Yanase, F., Raman, S., Naorungroj, T., McCarthy, A., Cree, M., Schlapbach, L.J. and Bellomo, R. (2021), "Efficacy and safety of parenteral high-dose vitamin C therapy in pediatric patients: A scoping review", *Pediatric Critical Care Medicine*, LWW, Vol. 22 No. 6, pp. 561–571.

Yimcharoen, M., Kittikunnathum, S., Suknikorn, C., Nak-On, W., Yeethong, P., Anthony, T.G. and Bunpo, P. (2019), "Effects of ascorbic acid supplementation on oxidative stress markers in healthy women following a single bout of exercise", *Journal of the International Society of Sports Nutrition*, Springer, Vol. 16, pp. 1–9.

Yoshida, Y. and Niki, E. (2004), "Detection of lipid peroxidation in vivo: total hydroxyoctadecadienoic acid and 7-hydroxycholesterol as oxidative stress marker.", *Free Radical Research*, Vol. 38 No. 8, pp. 787–794.

Żalikowska-Gardocka, M. and Przybyłkowski, A. (2020), "Review of parenteral nutrition-associated liver disease", *Clinical and Experimental Hepatology*, Termedia, Vol. 6 No. 2, pp. 65–73.

Zarban, A., Toroghi, M.M., Asli, M., Jafari, M., Vejdan, M. and Sharifzadeh, G. (2015), "Effect of vitamin C and E supplementation on total antioxidant content of human breastmilk and infant urine", *Breastfeeding Medicine*, Mary Ann Liebert, Inc. 140 Huguenot Street, 3rd Floor New Rochelle, NY 10801 USA, Vol. 10 No. 4, pp. 214–217.

Zhang, K. and Kaufman, R.J. (2008), "Chapter Twenty Identification and Characterization of Endoplasmic Reticulum Stress-Induced Apoptosis In Vivo", *Methods in Enzymology*,

Elsevier, Vol. 442, pp. 395–419.

Zhang, W., Xiao, S. and Ahn, D.U. (2013), "Protein oxidation: basic principles and implications for meat quality", *Critical Reviews in Food Science and Nutrition*, Taylor & Francis, Vol. 53 No. 11, pp. 1191–1201.

Zhang, X., Noisa, P., Hamzeh, A. and Yongsawatdigul, J. (2024), "Glycation of tilapia protein hydrolysate decreases cellular antioxidant activity upon in vitro gastrointestinal digestion", *Food Chemistry: X*, Elsevier, Vol. 21, p. 101228.

Zitka, O., Skalickova, S., Gumulec, J., Masarik, M., Adam, V., Hubalek, J., Trnkova, L., *et al.* (2012), "Redox status expressed as GSH: GSSG ratio as a marker for oxidative stress in paediatric tumour patients", *Oncology Letters*, Spandidos Publications, Vol. 4 No. 6, pp. 1247–1253.

APPENDICES

Appendix I: Composition of TPN given to neonates

1.1 Proteins

Both essential and non-essential amino acids are provided in a balanced mixture for TPN to support the growth and development of neonates **(Table 1.1)**.

Table 1.1: Amino acid composition for TPN. Primene 10% (*w/v*) is an injectable amino acid solution designed specifically for infants under one year old. It is a product of Baxter Corporation, Canada. The dosage is determined based on per kilogram per day requirement for newborns.

Amino acids	**Primene® (Baxter) 10% *w/v* (500 mL)**	**Dose rate at kg/day (40 mL) (g)**
Indispensable amino acids (g/100 mL)		
Isoleucine	0.67	0.0536
Leucine	1.0	0.08
Valine	0.76	0.0608
Phenylalanine	0.42	0.0336
Tryptophan	0.2	0.016
Methionine	0.24	0.0192
Lysine	1.1	0.088
Threonine	0.37	0.0296
Histidine	0.38	0.0304
Dispensable amino acids		
Alanine	0.8	0.064
Aspartate	0.6	0.048
Glutamate	1.0	0.08
Serine	0.4	0.032
Conditionally indispensable amino acids		
Cysteine	0.189	0.01512
Tyrosine	0.045	0.0036
Glycine	0.4	0.032
Arginine	0.84	0.0672

Proline	0.3	0.024
Asparagine	-	-
Glutamine	-	-
Non-protein amino acids		
Ornithine hydrochloride	0.318	0.02544
Taurine	0.060	0.0048

Source*: Baxter Corporation, Mississauga, Ontario, Canada. Product monograph for Primene 10%* (Prolla *et al.*, 2022).

1.2 Lipids

Table 1.2 shows the commercially available lipid emulsions with their compositions. The fatty acid composition of Intralipid™, SMOFlipid®, and Omegaven® emulsions is given in Table 1.3.

Table 1.2: Commercially available intravenous lipid emulsions. Available forms, the ratio of plant and animal oils, fatty acid composition, omega-6 to -3 ratio, vitamin E composition, and phytosterol levels are given. Coconut oil is the source of medium-chain triacylglycerols (MCT). Soybean oil is a 100% plant-based oil. ω-6:ω-3 ratio: ratio of omega-6 to omega-3 fatty acids; N/A: not available; MUFA: monounsaturated fatty acids (Oleic acid).

	Intralipid® 10%*, 20%, 30%	**Structolipid® 20%**	**Lipofundin® 10%, 20%**	**ClinOleic® 20%**	**SMOFlipid® 20%**	**Omegaven® 10%**
Composition of oils	100% soybean oil	64% soybean oil and 36% coconut oil	50% soybean oil and 50% coconut oil	20% soybean oil and 80% olive oil	30% soybean oil, 30% MCT, 25% olive oil, and 15% fish oil	100% fish oil
Generation	First generation 1960s-1970s	Second generation Since 1985	Second generation Since 1985	Third generation Since 1990	Fourth generation Since 2000	Fourth generation Since 2000
SFA	15	46.3	59.4	14.5	36.9	21.2
MUFA						
Oleic Acid (%)	24	14	11	62.3	55.3	15.1
Omega-6 FAs						
Linoleic acid (%)	53	35	29.1	18.7	37.2	4.4
Arachidonic Acid (%)	0.1	0.15	0.2	0.5	1.0	2.1
Omega-3 FAs						
α-linolenic acid (%)	8	5	4.5	2.3	4.7	1.8
Eicosapentaenoic acid (%)	NA	NA	NA	NA	4.7	19.2
Docosahexaenoic acid (%)	NA	NA	NA	0.5	4.4	12.1
ω6:ω3 ratio	7:1	7:1	7:1	9:1	2.5:1	1:8
Vitamins						
All-rac-α-tocopherol (mg/L)	38	6.9	85	32	200	250
Plant sterol						
Phytosterol (mg/L)	343	NA	278	274	297	-
Glycerol (g/L)	22	25	25	25	25	25
Purified egg phospholipid (g/L)	12	12	12	12	12	12
Sodium Oleate (mg/L)	N/A				300	
Total Energy (L)	4600 kJ (1100 kcal)	8200 kJ (1960 kcal)	8095 (1935 kcal)		8400 kJ (2000 kcal)	470 kJ (1120 kcal)
Osmolality (mOsm/kg water)	300	350	380	270	380	308-376

*Compositions of fatty acids and other components, including energy and osmolarity are derived from a given percentage containing lipid emulsion. Adopted from: Fresenius Kabi. Product Information of lipid emulsions. 'Fresenius Kabi Official Website. Available at: https://www.fresenius-kabi.com/; Product monograph of SMOFlipid 20 of 20%, Fresenius Kabu Canada Ltd (Deshpande and Cai, 2020; King *et al.*, 2018; Raman *et al.*, 2017; Sadu Singh *et al.*, 2020).

Table: 1.3 The fatty acid composition of commonly used lipid emulsions, namely Intralipid™, SMOFlipid®, and Omegaven®.

	Carbon number: double bond	**Intralipid™ (%)**	**SMOFlipid® (%)**	**Omegaven® (%)**
Oleic acid	C18:1	19-30	23-35	4-11
Linoleic acid	C18:2	44-62	14-25	1.5
Caprylic acid	C8:0	-	13-24	-
Palmitic acid	C16:0	7-14	7-12	-
Capric acid	C10:0	-	5-15	-
Stearic acid	C18:0	1.4-5.5	1.5-4	-
α-linolenic acid	C18:3	4-11	1.5-3.5	1.1
Arachidonic acid	C20:4	-	-	0.2-2
EPA	C20:5	-	1-3.5	13-26
DHA	22:6	-	1-3.5	14-27

Adopted from (King *et al.*, 2018); EPA: eicosapentaenoic acid; DHA: Docosahexaenoic acid.

1.3 Micronutrients

The composition of vitamins, including fat-soluble and water-soluble vitamins, with dose rates are given in **Table 1.4**. Trace elements (TEs) added to the TPN are given in **Table 1.5**. Electrolytes such as calcium, magnesium, potassium, chloride, and phosphorus are also added to the TPN to support the vital functions of neonates (**Table 1.6**).

Table 1.4: Composition of vitamins for parenteral nutrition. Janeway Children Hospital, St. John's, NL uses multi-12/K_1® pediatric multiple vitamins for infusion.

Vitamins	Multi-12/K1 Pediatric	Per/kg/day at 150mL TPN/kg
Vial 1 (4 mL)		
Ascorbic acid	80 mg	24 mg
Vitamin A	2300 IU	690 IU
Vitamin D	400 IU	120 IU
Thiamine (as hydrocholoride)	1.2 mg	0.36 mg
Riboflavin (as phosphate)	1.4 mg	0.42 mg
Pyridoxine hydrochloride	1 mg	0.3 mg
Niacinamide	17 mg	5.1 mg
d-Panthenol	5 mg	1.5 mg
Vitamin E (DL-alpha tocopherol acetate)	7 IU	2.1 IU
Vitamin K_1	0.2 mg	0.06 mg
Vial 2 (1 mL)		
Biotin	20 µg	6 µg
Folic acid	140 µg	42 µg
Vitamin B12 (cyanocobalamin)	1 µg	0.3 µg
Other ingredients		
Polysorbate 80	1.25%	-
Sodium hydroxide or HCl or Sodium citrate or citric acid	To adjust pH	-
Mannitol	7.5%	-

Source: SANDOZ Canada Inc., Boucherville, Quebec, Canada J4B 1E6

Table 5: Trace elements (TEs) added to the TPN. Micro+6 contains six TEs for pediatric TPN.

	Micro+6 SANDOZ Canada Incorporated	**Per kg/day**
Zinc sulfate	3 mg/mL	0.3 mg
Copper sulfate	0.4 mg/mL	0.04 mg
Manganese sulfate	0.1 mg/ml	0.01 mg
Chromic chloride	4 µg/mL	0.4 µg
Selenious acid	20 µg/mL	2 µg
Sodium iodine	60 µg/mL	6 µg

Table 6: Electrolytes for TPN with manufacturer name and dose rate for newborns.

Electrolytes	Manufacturer	Dose rate
Magnesium sulfate injection USP 20% (200 mg/mL or 0.8 mmol/mL)	SANDOZ, Canada	0.2 mmol/kg/day
Calcium gluconate injection, USP 10% (100 mg/mL or 0.465 mEq/mL/0.232 mmol/mL	Fresenius Kabi, Canada	0.46 mmol/day
Potassium phosphates injection, USP (15 mL) • Phosphorus (3 mmol/mL) • Potassium (4.4 mEq/mL	Fresenius Kabi, Canada	Phosphorus (4.08 mmol/kg/day) Potassium (5.98 mEq/kg/day
Sodium Acetate injection, USP 32.8% (4 mmol/mL or 4 mEq) anhydrous	Omega, Canada	3 mmol/kg/day

Appendix II: Standard curves of the experiments

2.1. Standard curves for ferrous oxidation-xylenol orange (FOX) version II assay

A standard curve was plotted using 0, 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, and 0.7 mM concentrations of 30% hydrogen peroxide solution (#H1009, Sigma Aldrich, Oakville, ON, Canada) (**Figure 2.1**).

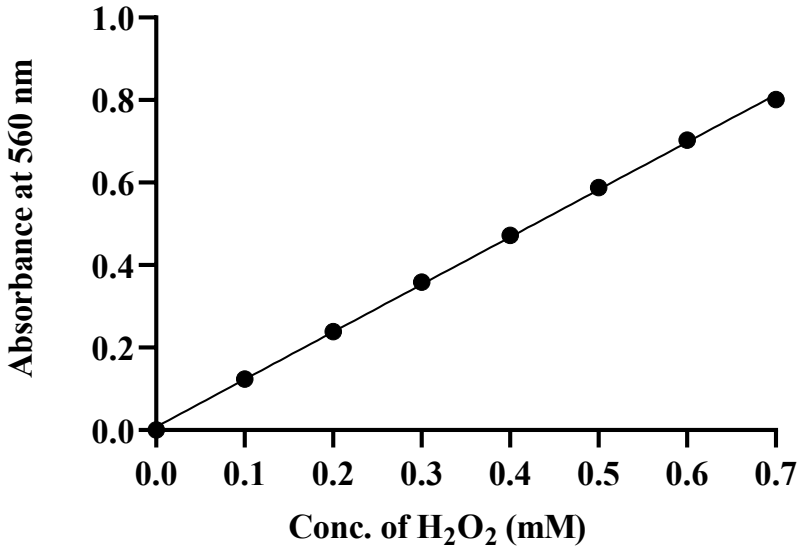


Figure 2.1: The standard curve for FOX version II assay to determine the peroxide levels generated in TPN, equivalent to H_2O_2. The liner regression curve was plotted using GraphPad Prism v6.0; r^2=0.99 and $p < 0.0001$. The unknown peroxide levels were determined by interpolation of the absorbance values of known concentration of H_2O_2 from the linear regression curve.

2.2. Standard curve for ferric reducing antioxidant power (FRAP) assay

A standard curve was plotted using the concentrations of 0 μg/mL (0 μM), 5 μg/mL (20 μM), 10 μg/mL (40 μM), 15 μg/mL (60 μM), 30 μg/mL (120 μM), 45 μg/mL (180 μM), 60 μg/mL (240 μM), 75 μg/mL (300 μM), and 90 μg/mL (360 μM) of Trolox (**Figure 2.2**).

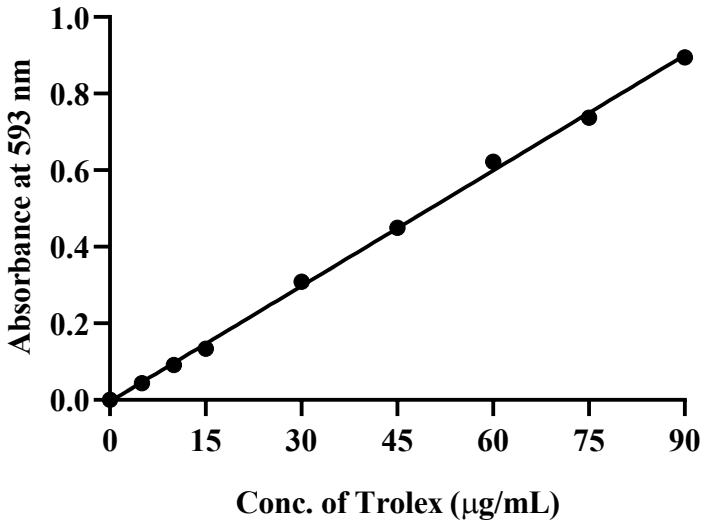


Figure 2.2: Standard curve for FRAP assay to determine the antioxidant power of optimized TPN solution, equivalent to Trolox (TE) in μg/mL. The liner regression curve was plotted using GraphPad Prism v6.0; r^2=0.99 and $p < 0.0001$. The unknown antioxidant power in TE (μg/mL) were determined by interpolation of the absorbance values of known concentration of Trolox from the linear regression curve.

2.3 Standard curve for bicinchoninic acid (BCA) assay

A standard curve was plotted for bovine serum albumin (BSA) concentrations from 0, 6.25, 12.5, 25, 50, 100, 200, and 400 μg/mL (**Figure 2.3)**

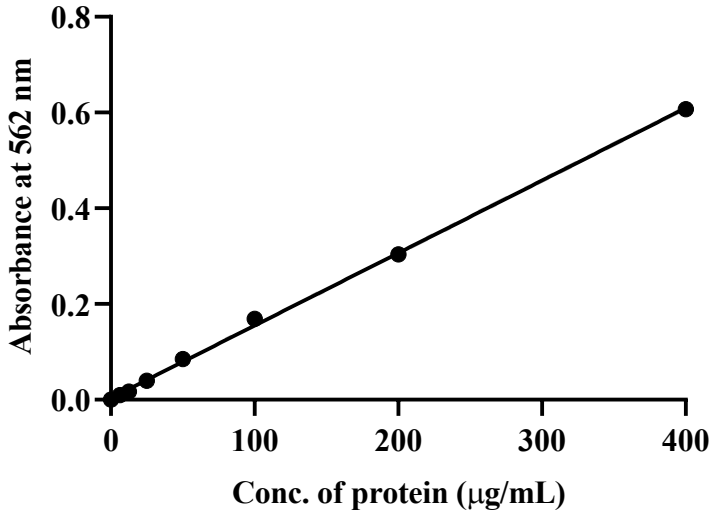


Figure 2.3: Standard curve for BCA protein assay to determine concentration of total protein in a cell lysate. The blank was used for baseline correction. The unknown amount of total protein (μg/mL) was calculated by interpolation of the absorbance values measured at 562 nm from the linear regression curve determined by GraphPad Prism v6.0. Slope parameters: r^2=0.99; $p <$ 0.0001.

2.4 Standard curve for thiobarbituric acid reactive substances (TBAR) assay

A standard was plotted using the concentrations of 0, 0.26, 0.52, 1.04, 2.09, 4.18, and 8.35 μM of MDA (**Figure 2.4**).

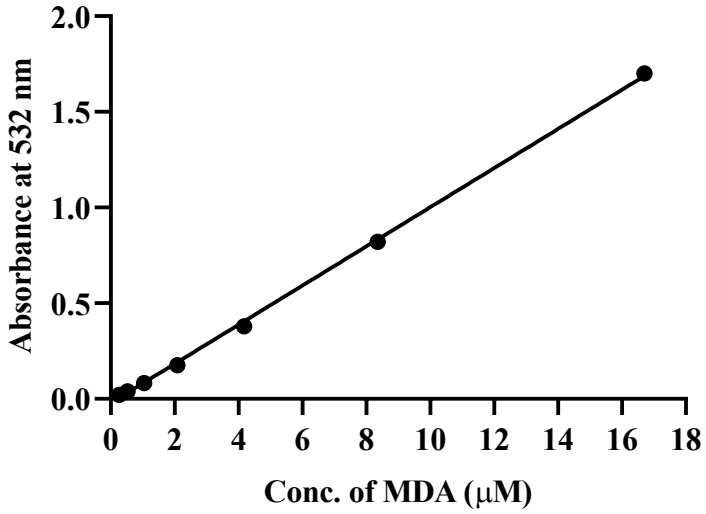
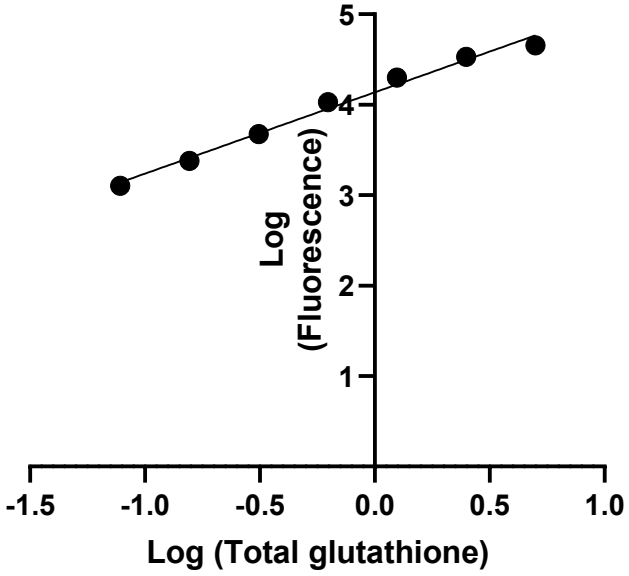


Figure 2.4: Standard curve for MDA to determine concentration of TBARS in the media. The blank was used for baseline correction. The unknown amount of MDA (μM) was determined by interpolation of the absorbance values measured at 532 nm from the linear regression curve determined by GraphPad Prism v6.0. Slope parameters: r^2=0.99; $p < 0.0001$.

2.5 Standard curves for reduced form of glutathione (GSH) and oxidized form of glutathione (GSSG)

A standard was plotted using the concentrations of 0, 0.1563, 0.3125, 0.625, 1.25, 2.5, 5, and 10 μM for GSH and 0, 0.0781, 0.1563, 0.3125, 0.625, 1.25, 2.5, and 5 μM for total glutathione (GSH + GSSG). The best smooth curve was plotted through the corrected fluorescence reading. A logarithmic linear regression (log (y)=a+ $b$log (x)) was used to obtain the sample concentrations (**Figure 2.5 A and B**).

(A)


5
4
3
2
1
Log
(Fluorescence)
-1.5
-1.0
-0.5
0.0
0.5
1.0
Log (Total glutathione)

(B)

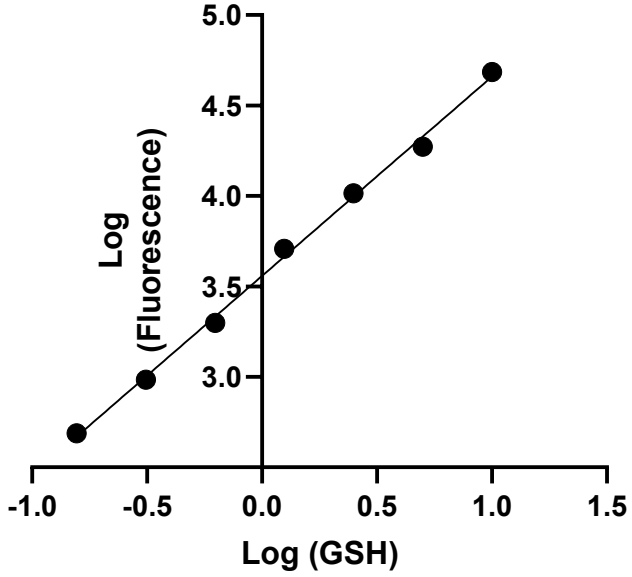

5.0
4.5
4.0
3.5
3.0
Log
(Fluorescence)
-1.0
-0.5
0.0
0.5
1.0
1.5
Log (GSH)

Figure 2.5: Standard curve for total glutathione (GSH + GSSG) to determine concentrations of reduced form of glutathione (GSH) and oxidized form of glutathione (GSSG) in the cell lysates. The blank was used for baseline correction. Log transformed linear regression of **A:** Total glutathione **B:** GSH. The unknown amount of total glutathione (μM) was determined by interpolation of the absorbance values measured at Ex/Em = 490/520 nm and the linear regression model determined by GraphPad Prism v6.0. Slope parameter of the log linear model: R^2=0.99; $p < 0.0001$.

Appendix III: Oxidized fatty acids and spectra of LC-MS/MS

Table 3.1: Oxidized fatty acids of standard AIO-TPN (*n*=3). Oxidized fatty acids were assumed to have a shift of *m/z* by 16 amu, representing the breakdown of a single double bond.

Oxidized fatty acids	Percentage
C12:1	0.11 (0.01)
C16:1	0.11 (0.01)
C18:3	0.83 (0.04)
C18:2	1.26 (0.08)
C18:1	1.15 (0.07)
C20:5	0.09 (0.01)
C20:4	0.11 (0.0)
C20:6	0.21 (0.02)

Figure 3.1: Mass spectrum of light protected (LP) optimized TPN for 24 hours

Lipid of the LP AIO-TPN mixture was extracted and injected into a TSQ Quantis^{TM} mass spectrometer under electrospray ionization (ESI) to obtain the spectrum.

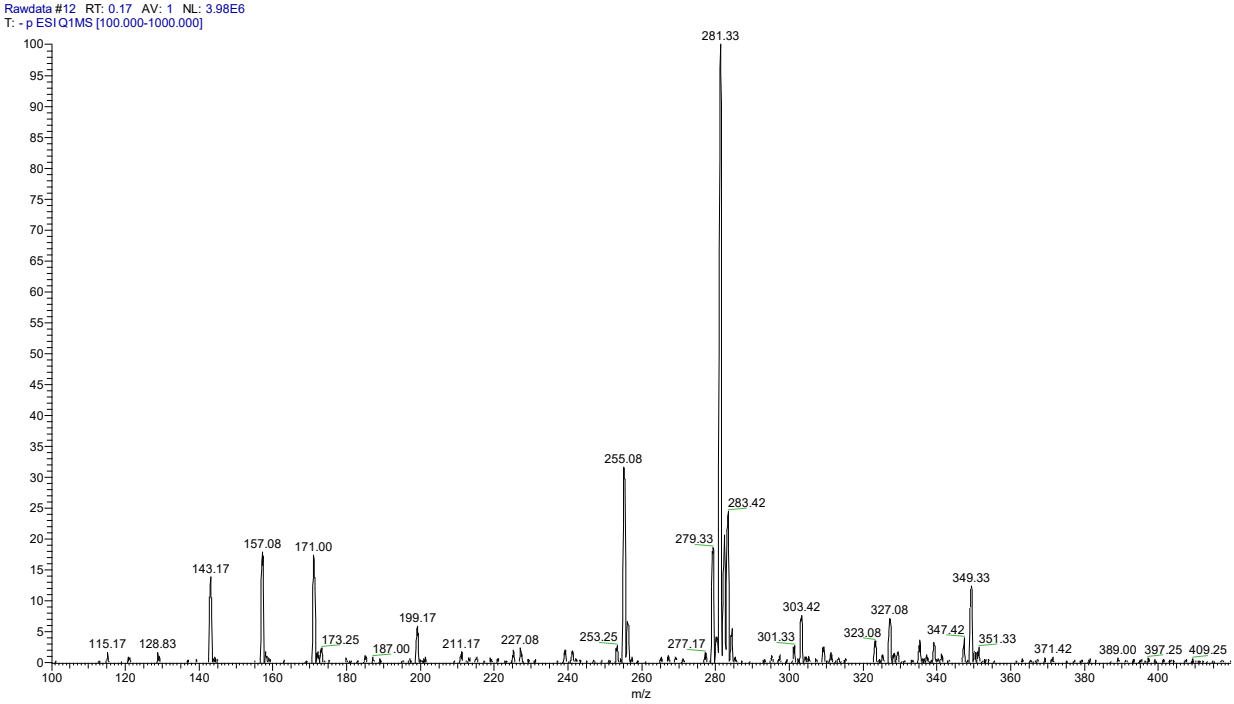


Figure 3.2: Mass spectrum of light exposed (LE) optimized TPN for 24 hours.

Lipid of the LP AIO-TPN mixture was extracted and injected into a TSQ Quantis^{TM} mass spectrometer under electrospray ionization (ESI) to obtain the spectrum.

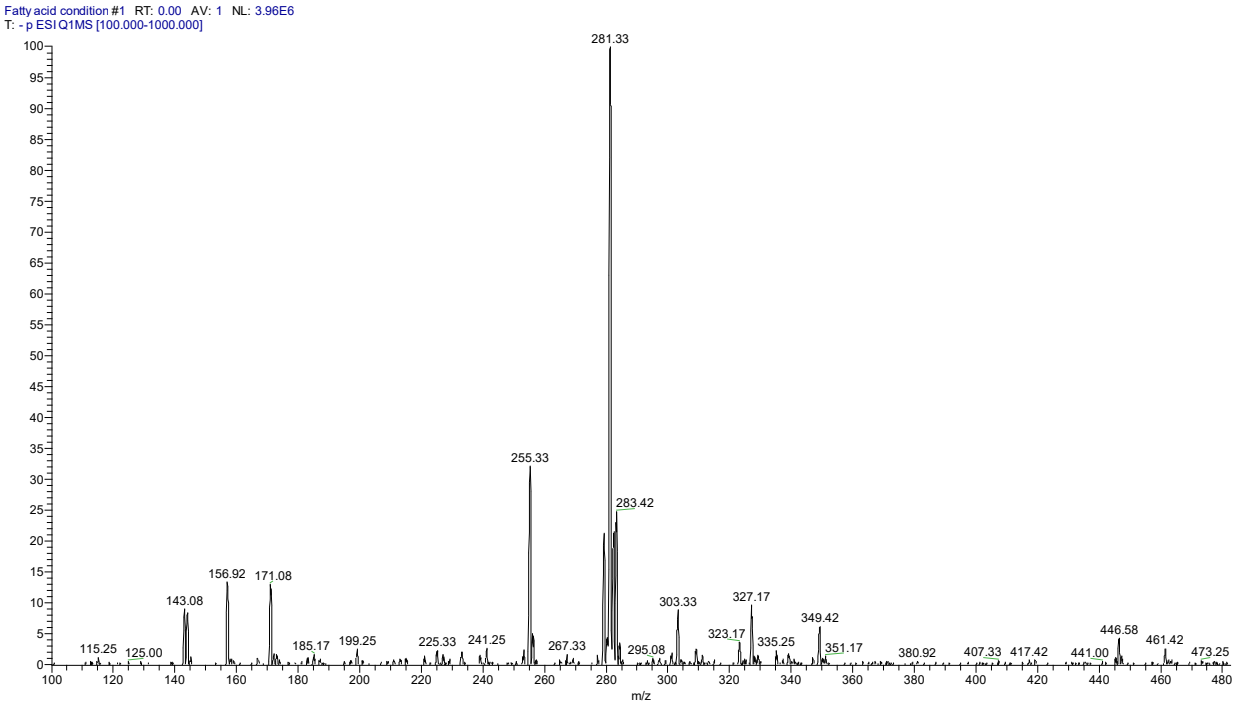


Figure 3.3: Mass spectrum of F_2-isoprostane fragmentation.

F_2-isoprostane (8-isoprostane $F_{2\alpha}$) is an oxidized product of arachidonic acid. The precursor ion is detected at *m/z* 353 and product ion is detected at *m/z* 193.

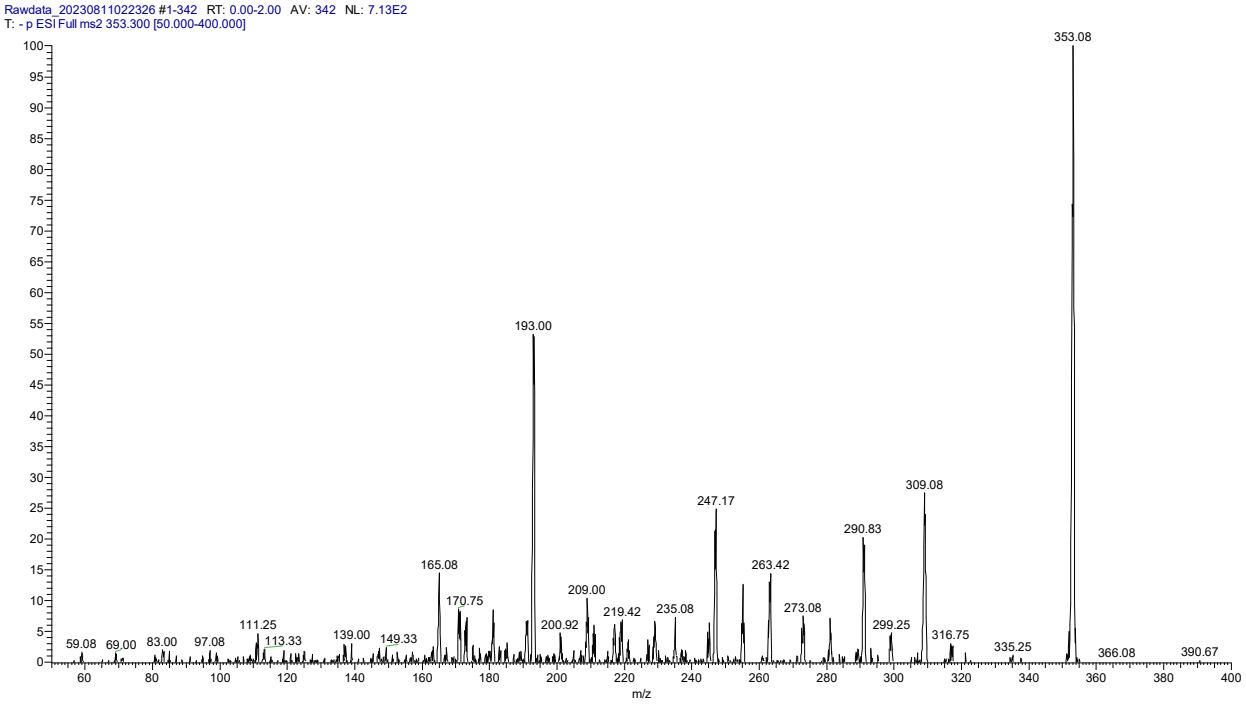


Figure 3.4: Mass spectrum of TPN sample for F_2-isoprostane (8-isoprostane $F_{2\alpha}$) identification