

CHAPTER V

DIFFERENTIAL MOLECULAR RESPONSES AND FATTY ACID METABOLISM INDUCED BY UNENCAPSULATED TUNA OIL AND GLUCOSE TRANSPORTER-TARGETED NANOPARTICLES

5.1 Abstract

Strategy to enhance the nutritional value of meat, particularly in slow-growing chicken breeds, challenges remain in maximizing the bioavailability and stability of essential fatty acids, such as omega-3 and omega-6 polyunsaturated fatty acids (PUFAs), including the need to mitigate lipid oxidation during feed processing and improve their utilization in animal systems. Encapsulation technologies, such as lipid nanoparticles, influence biological processes related to protein mechanisms, but their specific effects remain unclear. This study investigated the molecular impacts of tuna oil (positive control, PC) and tuna oil encapsulated in lipid nanoparticles (targeted nanoparticles, TNP) on slow-growing Korat chickens using proteomics analysis. Protein samples from the iliotibialis muscle (6 from PC, 6 from TNP, and 5 from the negative control (NC)) were analyzed using nanoLC-MS/MS. Differential abundant proteins (DAPs) were identified through pairwise comparisons between conditions (NC vs PC, and NC vs TNP) to highlight specific biological responses unique to each dietary treatment. In the TNP condition, energy metabolism shifted toward aerobic pathways, with increased β -oxidation and reduced reliance on alternative pathways, such as the creatine, to support the TCA cycle. By contrast, the PC condition exhibited high lipid peroxidation and by-products, triggering robust antioxidant (PRDX1, PRDX6, ESD, TXN, GSTM2, ADPRHL2) and detoxification responses (AKR7A2, ALDH9A1, ALDH5A1, GSTM2), as well as membrane repair mechanisms (ANXA2P2, ANXA4, PDCD6). However, TNP group showed ROS defense mechanism but obviously lower, interestingly, enhanced expression of proteins related to vesicle trafficking (AHNAK2 and TCP1). Lipid peroxidation in the PC group also led to calcium influx to regulated calcium homeostasis and stabilize muscle contraction altering muscle responses to oxidative

stress. Lipid peroxidation in the PC group also led to calcium influx oxidative stress. Lipid peroxidation in the PC group also led to calcium influx to regulated calcium regulated calcium homeostasis and stabilize muscle contraction altering muscle responses to oxidative stress. This included upregulation of proteins involved in calcium pumps in the sarcoplasmic reticulum (ATP2A2 and SRL) and proteins that stabilize muscle contraction (MYOM1, MYOM2, MYLK2, MYLPF), while the TNP group adapt to the demands of enhanced contractile activity (PDLIM3, MYO7L2, MYH1A, MYH1D, MYH1F, MYH1E). In terms of immune responses, the PC group demonstrated stronger MHC-based immunosurveillance (CALR3 and PDIA3), showing robust antioxidant defenses under stress from lipid oxidation. Interestingly, while TNP conditions exhibited lower immune responses to oxidative stress, they maintained immune surveillance through selective protein expression patterns. These findings highlight the distinct cellular strategies employed by PC and TNP conditions in responding to oxidative stress and immune challenges, with potential implications for optimizing lipid delivery systems in therapeutic applications.

Keywords: Korat chicken, lipid-based nanoparticle, n-3 PUFAs, proteomics

5.2 Introduction

n-3 PUFAs are widely recognized for their health benefits, including cardiovascular protection and anti-inflammatory properties (Simopoulos, 2008; Calder, 2015). These benefits have prompted the incorporation of n-3 PUFAs into poultry diets as a strategy to enhance the nutritional value of meat, particularly in slow-growing chicken breeds (Hang et al., 2018; Khosinklang et al., 2023). However, challenges remain in maximizing the bioavailability and stability of these essential fatty acids, including the need to mitigate lipid oxidation during feed processing and improve their utilization in animal systems.

Lipid-based nanotechnology offers a promising solution for targeted delivery of n-3 PUFAs, addressing these challenges. Nanoparticles protect n-3 PUFAs from oxidative degradation (Chanburee et al., 2017; Chang et al., 2018) and enhance their absorption (Tian et al., 2017), facilitating their incorporation into animal tissues (Tunki et al., 2019). These advancements could significantly improve the nutritional profile of

meat products. Recent research has demonstrated that glucose transporter-targeted nanoparticles can be absorbed by intestinal cells in vitro (Wangngae et al., 2023) and subsequently reach target tissues such as skeletal muscle in vivo (Yeh et al., 2014). These observations point to the potential of nanoencapsulation technologies to optimize n-3 PUFA delivery and functionality in meat production.

Despite these promising developments, comprehensive evaluations of nanoencapsulation in agricultural contexts remain limited. There is a critical need to investigate how these technologies influence biological processes, protein expression, and overall meat quality in livestock. Such insights are essential to bridge the gap between laboratory advancements and their practical applications in sustainable animal production systems (Jones & Ricke, 2021).

This study addresses this gap by comparing the effects of unencapsulated and nanoencapsulated tuna oil on fatty acid profiles and protein expression in Korat chickens. By elucidating the distinct impacts of these n-3 PUFA delivery methods, the research aims to uncover the advantages of nanoencapsulation in improving the functional quality of meat while supporting sustainable and economically viable poultry production.

5.3 Materials and methods

5.3.1 Ethics statement

The ethics statement was conducted at farm of Suranaree university of technology as described in chapter IV, section 4.3.2.

5.3.2 Birds and housing management

The birds and housing management was described in chapter IV, section 4.3.3.

5.3.3 Experiment chicken and tissue collection

The experimental model was a completely randomized design, which included three dietary treatments, 5 replications and twenty-five 49-d-old female chickens per replication. A basal diet was formulated based on corn-soybean containing 6% of rice bran oil as the negative control (NC), 3% of the RBO oil content were replaced with 3% tuna oil as positive control group (PC) or 3% tuna oil in targeted

nanoparticles (TNP). All experimental diets were formulated to provide 3,100 Kcal/Kg ME and 19% CP for the finisher (d 49 to 70) as presented in Table 4.1 of chapter IV.

At 70 days of age, 30 chickens (2 females per replicate) were randomly selected and euthanized for molecular analysis. Thigh meat samples (Iliotibialis muscles) were collected from 10 females in each group and stored at -20°C until fatty acid profile analysis. Lipid extraction and methylation procedures were conducted according to the methods described in chapter IV, section 4.3.5.4. The n-6/n-3 ratio was calculated based on the detected quantities of n-6 and n-3 polyunsaturated fatty acids (PUFAs) derived from their respective chromatogram peaks. Based on the fatty acid profiling results, six samples were manually selected representing two experimental conditions and five negative controls chosen according to the n-6/n-3 ratio (see in Supplementary Table 5.1) for preparation for proteomic analysis.

PCA was conducted to evaluate the variation in fatty acid profiles among the experimental groups. The dataset, comprising fatty acid variables and samples, was imported into OriginPro 9.85 (OriginLab, Northampton, Massachusetts, USA) for multivariate analysis.

5.3.4 Samples preparation for proteomic analysis

The frozen muscle samples approximately 100 mg of each sample were ground and homogenized in liquid nitrogen. Muscle samples were resuspended in 40 mM Tris-HCl (pH 8) (Laville et al., 2009), 2 mM EDTA, and a protease inhibitor cocktail (P2714, Sigma) at the ratio of 1:4 (wt/vol) under agitation (1000 rpm on ThermoMixer™ C at 4°C), during 30 min. And they were homogenized with a sonicator (5 x 20 s). After homogenization, samples were centrifuged at 10,000 g for 10 min at 4°C, and supernatants were stored at -20°C.

Protein concentration was determined using the Uptima BC Assay Protein Quantitation kit (Interchim) according to the manufacturer's instructions and using bovine serum albumin as the standard.

5.3.5 SDS-PAGE separation of proteins

Protein extraction efficiency was assessed by separating 25 µg protein samples on 4-20% SDS-PAGE gels alongside molecular weight markers. Samples, prepared in 2X buffer and heated at 95°C, were electrophoresed at 200 V for 48 min,

followed by overnight Coomassie Blue staining. Gels were destained in 30% ethanol/10% acetic acid and stored in 2% acetic acid.

5.3.6 SDS-PAGE without fractionation

Each sample (25 µg) was mixed with 2X sample buffer (125 mM Tris-HCl pH 6.8, 4% SDS, 20% glycerol, 10% TCEP, and 0.004% bromophenol blue), heated at 95°C for 5 min, and separated using 10% polyacrylamide gels under SDS-PAGE at 50 V for 25 min. The gels were stained overnight with 5% Coomassie Blue R-350 in 30% ethanol/10% acetic acid, followed by destaining. A single protein band per lane was excised for in-gel digestion.

5.3.7 in-gel digestion

Gel pieces were washed in water:acetonitrile solution (1:1, 5 min) followed by 100% acetonitrile (10 min). Reduction and cysteine alkylation was performed by successive incubation with 10mM dithiothreitol in 50 mM NH_4HCO_3 (30 min, 56°C), then 55mM iodoacetamide in 50 mM NH_4HCO_3 (20 min, RT, in dark). Pieces were then incubated with 50mM NH_4HCO_3 and acetonitrile (1:1, 10 min) followed by acetonitrile (15 min). Proteolytic digestion was carried out overnight using 25mM NH_4HCO_3 with 12.5 ng/µl Trypsin (Sequencing grade, Roche diagnostics, Paris, France). Resultant peptides were extracted by incubation in 5% formic acid (sonicated) with the supernatant removed and saved, followed by incubation in water:acetonitrile and 1% formic acid (1:1, 10 min) and a final incubation with acetonitrile (5 min), again supernatant was removed and saved. These two peptide extractions were pooled and dried using a SPD1010 speedvac system (Thermosavant, Thermofisher Scientific, Bremen, Germany). The resultant peptide mixture was enriched with SPIN Columns C18 (Millipore) and dried again before being analyzed by nanoflow liquid chromatography tandem mass spectrometry (Nano LC-MS/MS).

5.3.8 Mass spectrometry LC1D-nanoESI-LTQ-Orbitrap analysis

Peptide mixtures were analyzed by on-line nanoflow liquid chromatography tandem mass spectrometry (nano LC-MS/MS). All experiments were performed on a dual linear ion trap Fourier Transform Mass Spectrometer (FT-MS) LTQ Orbitrap Velos Pro (Thermo Fisher Scientific, Bremen, Germany) coupled to an Ultimate® 3000 RSLC Ultra High-Pressure Liquid Chromatographer (Thermo Fisher Scientific, Bremen, Germany) controlled by Chromeleon Software (version 6.80 SR13).

Samples were desalted and concentrated for 10 min at 5 μ L/min on an LCPackings trap column (Acclaim PepMap 100 C18, 75 μ m inner diameter x 2 cm long, 3 μ m particles, 100 Å pores). The peptide separation was conducted using a LCPackings nano-column (Acclaim PepMap C18, 75 μ m inner diameter x 50 cm long, 2 μ m particles, 100 Å pores) at 300nL/min by applying gradient consisted of 2-45% B during 120 min. Mobile phases consisted of (A) 0.1% formic acid, 97.9% water, 2% acetonitrile (v/v/v) and (B) 0.1% formic acid, 19.9% water, 80% acetonitrile (v/v/v).

Data were acquired using Xcalibur version 3.0.63 software (Thermo Fisher Scientific, San Jose, CA), in positive data-dependent mode in the 300-1800 m/z mass range. Resolution in the Orbitrap was set at R=60,000. The 20 most intense peptide ions with charge states ≥ 2 were sequentially isolated (isolation width 2 m/z, 1 microscan) and fragmented in the high-pressure linear ion trap using CID (collision induced dissociation) mode (collision energy 35%, activation time 10 ms, Qz 0.25). Dynamic exclusion was activated during 30 seconds with a repeat count of 1. The lock mass was enabled for accurate mass measurements. Polydimethylcyclsiloxane (m/z, 445.1200025, $(\text{Si}(\text{CH}_3)_2\text{O})_6$) ion was used for internal recalibration of the mass spectra MS/MS ion searches were performed using Mascot search engine version 2.7.0.1 (Matrix Science, London, UK) via Proteome Discoverer 2.5 software (ThermoFisher Scientific, Bremen, Germany) against NCBIprot_Gallus database (2023/01). The search parameters included trypsin as a protease with two allowed missed cleavages and carbamidomethylcysteine, methionine oxidation and acetylation of N-term protein as variable modifications. The tolerance of the ions was set to 5ppm for parent and 0.8Da for-fragment ion matches. Mascot results obtained from the target and decoy databases searches were subjected to Scaffold Q+S v5.2.2 and Scaffold Quant v5.0.3 softwares (Proteome Software, Portland, USA) using the protein cluster analysis option (assemblage of proteins into clusters based on shared peptide evidence). Peptide and protein identifications were accepted if they could be established at greater than 95.0% probability as specified by the Peptide Prophet algorithm and by the Protein Prophet algorithm, respectively. Protein identifications were accepted if they contained at least two identified peptides.

After applying a protein identification filter to at least 2 unique peptides and 95% probability of identification for peptides and proteins, the data was

normalized and quantified according to the "XIC" (eXtracted Ion Chromatogram) method in Scaffold Quant. The "XIC" or "Precursor Intensity (normalized)" is based on the normalized values of the intensities of the chromatographic peaks corresponding to the peptides of a protein. XIC data were Log transformed (Log10 Precursor Intensity), and statistical tests such as Anova and t-test were conducted. A Log2 Fold Change Precursor Intensity was also calculated between 2 conditions to identify the level of abundance of a protein. The data was then exported to Excel. The protein list was filtered according to the criteria: p-value ≤ 0.05 ; ratio of 2 ($\text{Log}_2(\text{FC}) \leq -0.234$ or ≥ 0.263) (or $\text{FC} < 0.85$ or > 1.2) to highlight differential proteins.

5.3.9 Bioinformatics analysis of differential abundant proteins (DAPs)

The thresholds of $\text{Log}_2(\text{FC}) \leq -0.234$ or ≥ 0.263 and P-value < 0.05 were set to identify DAPs. Gene ontology (GO) enrichment including biological process (BP), cellular component (CC), molecular function (MF) and Kyoto Encyclopedia of Gene and Genomes (KEGG) pathways enrichment analyses were performed using ClueGo plug-in via Cytoscape software (version 3.10.3). The GO enriched proteins and KEGG pathways were considered enriched with Right-sided hypergeometric test, a P-value < 0.05 , correcting by FDR with Benjamini-Hochberg method ($\text{FDR} < 0.05$). Go terms and KEGG pathways grouping was based on the following criteria: Kappa score threshold was set at 0.45 for functional relatedness, and %Gene/Term was used for term overview. Then visualized by ggplot2 package in R version 4.4.2 (R Core Team, 2020). Protein – protein interaction and MCL clustering was performed via STRING (version 12.0, <https://string-db.org>) against the *Gallus gallus* database and considering a high confidence score of 0.4 for interaction.

5.4 Results and discussion

The dietary interventions, consisting of 3% tuna oil (PC) and 3% tuna oil encapsulated in lipid nanoparticles (TNP), induced distinct changes in both fatty acid composition (Figure 5.1) and protein expression profiles, as demonstrated by the PCA, volcano plots and heatmap (Figures 5.2 – 5.3).

5.4.1 Principal component analysis of fatty acid profiles in Korat chicken received tuna oil in lipid nanoparticles

The PCA biplot of fatty acid profiles from experimental groups displayed in Figure 5.1, delineating the impact of dietary supplementation with different forms of n-3 PUFAs on the fatty acid composition in Korat chickens. The groups analyzed include the negative control group fed a diet high in n-6 PUFAs (NC), a positive control group supplemented with 3% tuna oil (PC), and a treatment group receiving 3% tuna oil encapsulated in lipid nanoparticles (TNP).

The first principal component (PC1, 45.1% of total variance) predominantly captures variations in long-chain polyunsaturated fatty acids, especially those related to n-3 PUFAs such as EPA (C20:5n-3) and DHA (C22:6n-3), as evidenced by their strong loadings on this axis. This axis also shows a separation between the TNP group and the other groups, suggesting that nanoencapsulation significantly enhances the bioavailability and assimilation of these fatty acids in the chicken tissues.

The second principal component (PC2, 25.3% of total variance) highlights differences mostly in saturated fatty acids (SFAs) and monounsaturated fatty acids (MUFAs), with significant loadings from C14:0, C16:0, and C18:1n-9. This axis differentiates the NC group from the supplemented groups (PC and TNP), indicating changes in lipid metabolism resulting from n-3 PUFA supplementation.

The 95% confidence ellipses illustrate clear clustering distinctions among the three groups. The NC group, marked by black circles, clusters tightly around high levels of n-6 fatty acids and lower levels of n-3 PUFAs, reflecting their high n-6 PUFA diet. The PC group, indicated by red circles, shows a moderate intake of n-3 PUFAs, slightly overlapping with the TNP group but generally separate, indicating differences in fatty acid profiles due to the form of tuna oil administration. The TNP group, represented by green circles, is distinctly separated along the PC1 axis, underscoring the enhanced incorporation of n-3 PUFAs like EPA and DHA due to the nanoencapsulation process.

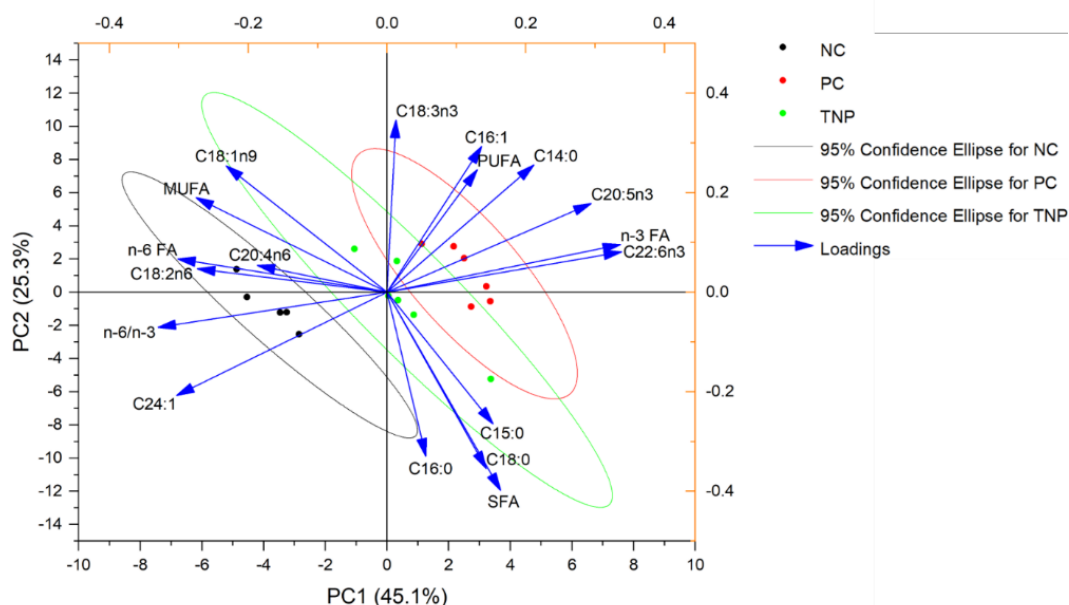


Figure 5.1 Principal Component Analysis (PCA) of fatty acid profiles from experimental groups. The biplot represents the distribution of fatty acids across three dietary treatments: Negative control (NC), Positive control (PC, 3% tuna oil), and Targeted-lipid nanoparticles (TNP, 3% tuna oil in lipid nanoparticles). Each point represents a sample, with NC indicated by black circles, PC by red circles, and TNP by green circles. The ellipses denote 95% confidence intervals for each group. The vectors (blue arrows) correspond to specific fatty acids and illustrate their contribution to the first two principal components, PC1 (45.1% of total variance) and PC2 (25.3% of total variance).

The PCA results of fatty acid profiles reveal significant shifts in lipid metabolism associated with dietary supplementation, particularly in the TNP group, where nanoencapsulation of n-3 PUFAs enhanced the assimilation of long-chain polyunsaturated fatty acids such as EPA and DHA. These changes in fatty acid composition are likely to influence downstream biological processes, as lipids play a central role in cellular signaling and metabolic regulation. The distinct separation of the TNP group along PC1 suggests a potential link between the enhanced bioavailability of n-3 PUFAs and alterations in protein expression profiles. To further

explore this relationship, differential protein expression analysis was conducted, revealing molecular mechanisms and pathways modulated by n-3 PUFA supplementation in its unencapsulated and nanoencapsulated forms.

5.4.2 Differential expression proteins of thigh muscle

5.4.2.1 Principal component analysis (PCA)

Principal Component Analysis (PCA) provided in Figure 5.2, was employed to elucidate the effects of dietary supplementation with n-3 PUFAs on protein expression in Korat chickens, the differential protein expression was presented in the Supplementary Table S5.2. Comprehensive group comparison (NC vs PC vs TNP) was presented in the initial PCA plot (Figure 5.2 A) displayed substantial differentiation among all three groups, with PC1 accounting for 60% of the variance and effectively separating the PC group from both NC and TNP. This suggests that unencapsulated tuna oil significantly alters protein expression compared to other treatments. Meanwhile, PC2, explaining 29% of the variance, differentiated TNP from NC, indicating subtle yet distinct effects imparted by nanoencapsulation on protein profiles.

Focused on comparison, the NC vs PC dedicated PCA plot (Figure 5.2 B), there was a pronounced separation between NC and PC along PC1 (63% variance), underscoring the impactful change in protein expression induced by unencapsulated tuna oil. The comparison between NC and TNP (Figure 5.2 C) showed a significant shift (72% variance on PC1), illustrating the strong influence of nanoencapsulation on protein expression beyond that of the control diet. Another plot revealed that despite identical n-3 PUFA content (Figure 5.2 D), nanoencapsulation in TNP modified protein expression distinctly from unencapsulated oil in PC, with a 61% variance described by PC1.

Ellipses representing the 95% confidence intervals for each treatment group confirmed the consistency within groups and validated the differential impact of each dietary intervention on protein expression profiles. These ellipses highlight not only the distinct clustering of each group but also the reproducibility of the dietary effects across biological replicates.

These PCA results clearly demonstrate that the form of n-3 PUFA delivery either unencapsulated or nanoencapsulated significantly influences the protein expression profiles in Korat chickens. The distinct clustering of treatment groups confirms our hypothesis that nanoencapsulation enhances the bioavailability and

efficacy of n-3 PUFAs, potentially offering a targeted mechanism for modulating specific biological pathways. Furthermore, the clear separation between NC and supplemented groups (both PC and TNP) reinforces the potent bioactive properties of n-3 PUFAs, regardless of the delivery form.

5.4.2.2 Volcano plots, Heatmap and Venn diagram

The volcano plots (Figure 5.2 E, F, and G) and heatmap (Figure 5.3) (the Supplementary Table S5.2) illustrating the DAPs between pairwise dietary treatment groups. These plots compare protein expression levels in pairwise fashion including NC vs PC, NC vs TNP, and PC vs TNP. Volcano plots revealed a significant number of proteins upregulated totally 92 proteins (shown in green) in the PC group compared to NC. The x-axis represents the log 2-fold change in protein intensity, indicating that several proteins associated with the intake of unencapsulated tuna oil were significantly overexpressed with log 2-fold change greater than 1.2. The y-axis, representing the $-\log_{10}$ of the p-value, highlights that many of these changes were statistically significant, surpassing the threshold indicated by the red horizontal line.

The Venn diagram (Fig. 2 H and see protein list in the Supplementary Table S5.2) illustrated the DAP expression patterns among the three pairwise dietary treatments. A total of 85 proteins were differentially expressed between NC and PC comparison, with 33 proteins uniquely altered in this group, while 75 proteins were differentially expressed in the NC and TNP comparison, with 29 proteins specific to that comparison. The overlap of 43 proteins between NC vs PC and NC vs TNP suggested that both unencapsulated and encapsulated tuna oil induce similar biological responses compared to the n-6 PUFA-rich control. Notably, only 9 proteins were differentially expressed in the PC vs TNP comparison, with 6 of these being unique to this group, and no overlap observed between NC vs TNP and PC vs TNP.

To gain a comprehensive understanding of the molecular responses elicited by dietary interventions, all differentially abundant proteins (DAPs) from the pairwise comparisons (NC vs PC, NC vs TNP, and PC vs TNP) were included for analysis.

5.4.3 Molecular responses to dietary interventions

To comprehensively understand the molecular responses elicited by dietary interventions, all DAPs from the pairwise comparisons (NC vs PC, NC vs TNP, and PC vs TNP) were analyzed for enriched Gene Ontology (GO) terms and KEGG

pathways. The analysis identified a total of 152 biological process (BP), 16 cellular component (CC), 24 molecular function (MF), and 21 KEGG pathways for NC vs PC condition and 167 BP, 15 CC, 22 MF and 21 KEGG pathways for NC vs TNP condition (see in supplementary file S5.3). Notably, while the comparisons between NC vs PC and NC vs TNP yielded enriched biological processes, cellular components, and molecular functions, the comparison between PC vs TNP did not reveal significant enrichment of any terms or pathways, suggesting limited molecular differentiation between these two conditions.

To further understand the molecular responses from dietary interventions, we used ClueGO to group enriched GO terms and KEGG pathways from the NC vs PC and NC vs TNP comparisons. This analysis categorizes terms into functional groups, highlighting differences in metabolic, cellular, and molecular processes.

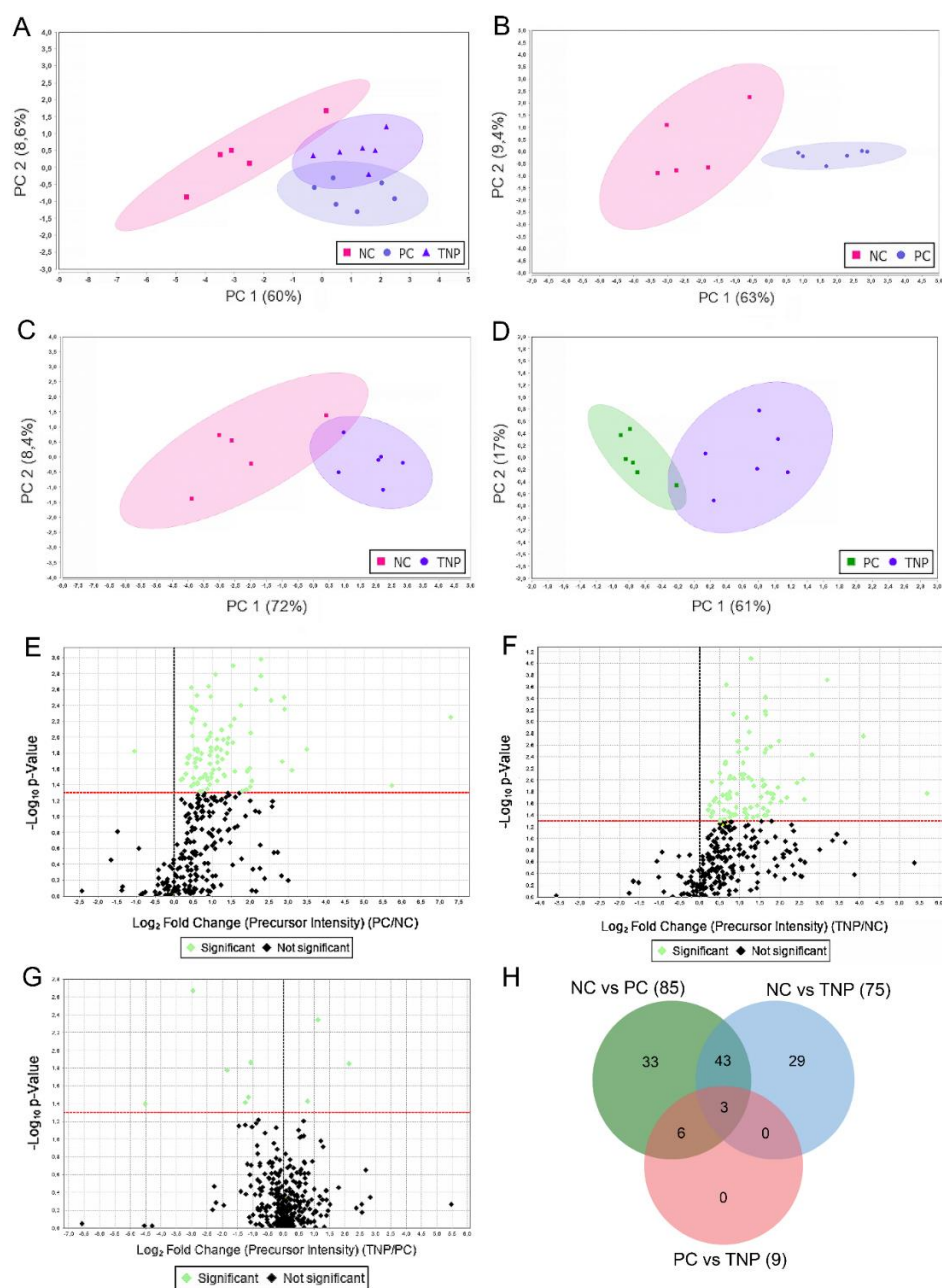


Figure 5.2 Principal Component Analysis (PCA) plots (A, B, C and D), illustrating the separation of dietary treatment groups based on differential protein expression profiles including NC (6% rice bran oil), PC (3% tuna oil), and TNP (3% tuna oil loaded in lipid nanoparticles). Volcano plots (E, F, and G) and Venn diagram (H) illustrating of the three pairwise groups. The ellipses in PCA plot represent the 95% confidence intervals for each group, indicating the variability and clustering within each treatment

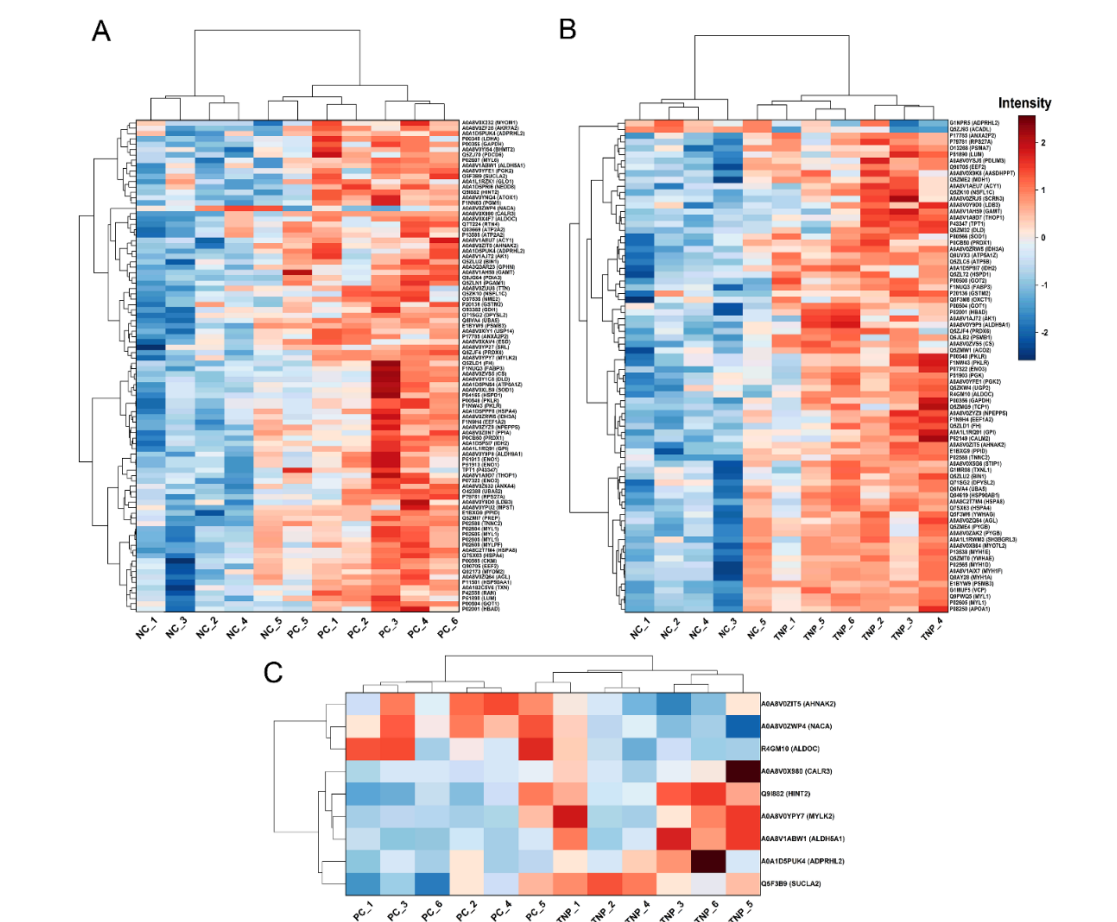


Figure 5.3 Heatmap illustrations of differential protein expression profiles across different samples including A, Negative control and Positive control (NC vs PC); B, Negative control and C, targeted-lipid nanoparticles (NC vs TNP), and Positive control and targeted-lipid nanoparticles (PC vs TNP). The samples are labeled along the x-axis, and the protein are listed along the y-axis. Hierarchical clustering is represented by dendrograms along both axes, categorizing both proteins and samples based on similarity in

expression patterns. The color scale on the right of each heatmap indicates the intensity levels of protein expression ranging from low (blue) to high (red).

5.4.4 Functional grouping of molecular responses to dietary interventions using GO terms and KEGG pathways clustering

This grouping highlights distinct biological functions influenced by each intervention, categorized into key processes such as energy metabolism, oxidative stress management, immune function, and protein synthesis. The analysis provides an organized view of the molecular shifts occurring under each condition, as illustrated in Figure 5.4 and detailed in Table 5.1. Below are the main grouped terms and pathways identified.

For the NC vs. PC condition classified into seven categories, The representative enrichment terms and pathways were arranged based on group-adjusted p-values using the ClueGO plugin. These categories including **Central metabolism and energy production** (Pyruvate metabolism, Citrate cycle (TCA cycle), Fumarate metabolic process, and Isocitrate dehydrogenase activity, Phosphopyruvate hydratase complex), **Amino acid metabolism and protein biosynthesis and inhibition** (Glycine, serine, and threonine metabolism, Aspartate family amino acid biosynthetic process, Arginine and proline metabolism, and Cellular modified amino acid biosynthetic process, and Aspartic-type endopeptidase inhibitor activity) **Cellular structure and dynamics** (Outer mitochondrial membrane organization, Protein insertion into mitochondrial outer membrane, T-tubule organization, Regulation of actin filament-based movement, and Intramolecular transferase activity, phosphotransferases), **Binding and transport mechanisms** (Tau protein binding, Magnesium ion binding, MHC class I peptide loading complex, ADP binding, and Progesterone receptor binding), **Stress response and immune function** (Peroxiredoxin activity, Response to interleukin-7, and Positive regulation by host of viral process), **Nucleotide metabolism and ribosomal activity** (Pentose phosphate pathway, purine ribonucleoside triphosphate metabolic process, Cytosolic small ribosomal subunit, and Translation elongation factor activity), and **Specialized cellular functions and interactions** (Interaction with symbiont, and Disordered domain specific binding).

For the NC vs. TNP condition classified into seven categories, the enrichment terms and pathways were arranged based on group-adjusted p-values using the ClueGO plugin. These categories including **Energy production and mitochondrial function** (Citrate cycle (TCA cycle), Isocitrate dehydrogenase activity, Hydro-lyase activity, and Oxidative phosphorylation), **Carbohydrate and energy metabolism** (Malate metabolic process, Glycolysis / Gluconeogenesis, Starch and sucrose metabolism, and polysaccharide catabolic process), **Protein and amino acid metabolism** (Proteasome core complex, and Translation elongation factor activity), **Cellular stress response and redox homeostasis** (cellular response to oxygen radical, Cell redox homeostasis, and Peroxiredoxin activity), **Muscle function and structural integrity** (Myosin complex, Proton-transporting ATP synthase complex, catalytic core F(1), and Muscle alpha-actinin binding), **Lipid transport and metabolic regulation** (High-density lipoprotein particle binding), and **Immune response and cellular communication** (Positive regulation by host of viral process, and Response to interleukin-7).

Differential pathway enrichment in n-3 PUFA delivery forms were identified key differences and similarities in the biological pathways enriched under two different conditions. The data revealed distinct patterns of pathway engagement that delineate the mechanisms through which each form of n-3 PUFA administration influences cellular and metabolic processes.

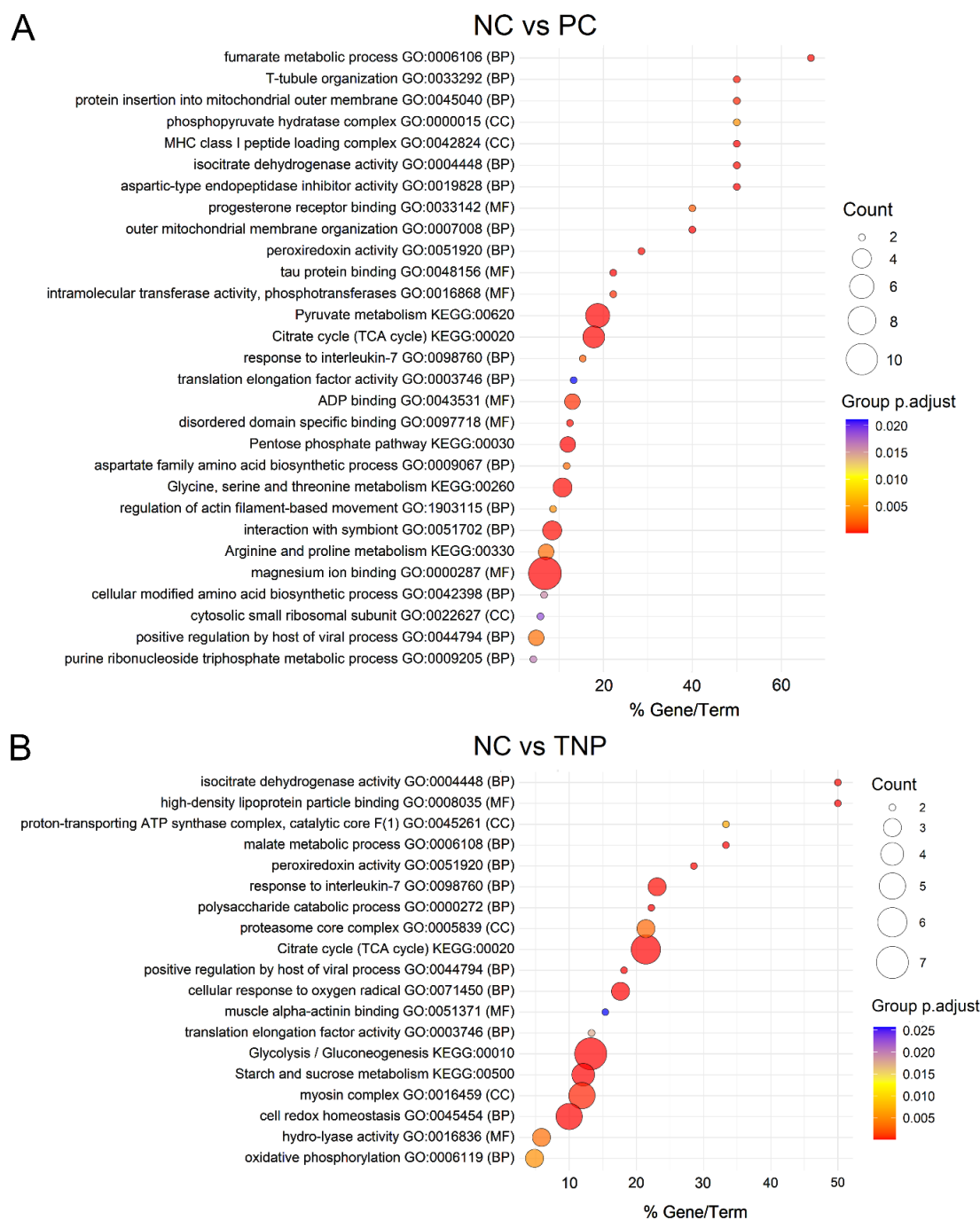


Figure 5.4 Comparative analysis of GO enrichment terms and KEGG pathways involvement in dietary treatments, NC vs PC (A) and NC vs TNP (B).

Table 5.1 Functional grouping of enriched representative (highest %gene/term) GO terms and KEGG pathways in dietary interventions (NC vs PC and NC vs TNP) by ClueGO plug-in.

Go/KEGG ID	Description	Group Adj. P-value ¹	% Gene/Term	Proteins (gene symbol)
NC vs PC				
KEGG:00330	Arginine and proline metabolism	2.61E-03	7.14	ALDH9A1, GAMT, GOT1
KEGG:00260	Glycine, serine and threonine metabolism	4.36E-05	10.81	BHMT2, DLD, GAMT, PGAM1
KEGG:00620	Pyruvate metabolism	8.50E-06	18.75	ALDH9A1, DLD, FH, GLO1, LDHA, PKLR
KEGG:00020	Citrate cycle (TCA cycle)	3.07E-05	17.86	DLD, FH, IDH2, IDH3A, SUCLA2
KEGG:00030	Pentose phosphate pathway	6.47E-06	12.00	ALDOC, GPI, PGM1
GO:0000287 (MF)	magnesium ion binding	2.34E-08	6.88	ADPRHL2, ENO1, ENO3, IDH2, IDH3A, MYL6, PDCD6, PGM1, PKLR, RAN, SUCLA2
GO:0043531 (MF)	ADP binding	5.53E-04	13.04	ATP5A1Z, MYL6, PGK2
GO:0016868 (MF)	intramolecular transferase activity, phosphotransferases	5.07E-04	22.22	PGAM1, PGM1
GO:0097718 (MF)	disordered domain specific binding	7.09E-05	12.50	GAPDH, HSP90AA1
GO:0033142 (MF)	progesterone receptor binding	1.87E-03	40.00	HSP90AA1, PPID
GO:0048156 (MF)	tau protein binding	7.72E-11	22.22	BIN1, HSP90AA1
GO:0022627 (CC)	cytosolic small ribosomal subunit	1.86E-02	5.88	RPS27A, UBA52

Table 5.1 (continued).

Go/KEGG ID	Description	Group Adj. P-value ¹	% Gene/Term	Proteins (gene symbol)
GO:0000015 (CC)	phosphopyruvate hydratase complex	4.37E-03	50.00	ENO1, ENO3
GO:0042824 (CC)	MHC class I peptide loading complex	1.43E-06	50.00	CALR3, PDIA3
GO:0042398 (BP)	cellular modified amino acid biosynthetic process	1.58E-02	6.67	CKM, GAMT
GO:0003746 (BP)	translation elongation factor activity	2.11E-02	13.33	EEF1A2, EEF2
GO:0009205 (BP)	purine ribonucleoside triphosphate metabolic process	1.65E-02	4.26	ATP5A1Z, RAN
GO:1903115 (BP)	regulation of actin filament-based movement	4.23E-03	8.70	BIN1, MYLK2
GO:0004448 (BP)	isocitrate dehydrogenase activity	6.67E-05	50.00	IDH2, IDH3A
GO:0098760 (BP)	response to interleukin-7	1.88E-03	15.38	HSPD1, PDIA3
GO:0051702 (BP)	interaction with symbiont	6.86E-05	8.51	ANXA2P2, GAPDH, HSPA8, HSPD1
GO:0045040 (BP)	protein insertion into mitochondrial outer membrane	3.74E-04	50.00	HSP90AA1, HSPA4
GO:0009067 (BP)	aspartate family amino acid biosynthetic process	2.48E-03	11.76	BHMT2, GOT1

Table 5.1 (continued).

Go/KEGG ID	Description	Group Adj. P-value ¹	% Gene/Term	Proteins (gene symbol)
GO:0006106 (BP)	fumarate metabolic process	4.80E-05	66.67	FH, GOT1
GO:0033292 (BP)	T-tubule organization	1.44E-04	50.00	ATP2A2, BIN1
GO:0051920 (BP)	peroxiredoxin activity	4.73E-05	28.57	PRDX1, PRDX6
GO:0044794 (BP)	positive regulation by host of viral process	2.68E-03	4.92	ANXA2P2, HSPA8
GO:0019828 (BP)	aspartic-type endopeptidase inhibitor activity	1.35E-07	50.00	BIN1, GAPDH
GO:0007008 (BP)	outer mitochondrial membrane organization	1.04E-08	40.00	HSP90AA1, HSPA4
NC vs TNP				
KEGG:00010	Glycolysis / Gluconeogenesis	5.24E-08	13.21	ALDH9A1, ALDOC, DLD, GAPDH, GPI, PGK2, PKLR
KEGG:00500	Starch and sucrose metabolism	8.54E-08	12.12	AGL, GPI, PYGB, UGP2
KEGG:00020	Citrate cycle (TCA cycle)	5.31E-13	21.43	ACO2, DLD, FH, IDH2, IDH3A, MDH1
GO:0051371 (MF)	muscle alpha-actinin binding	2.57E-02	15.38	LDB3, PDLIM3
GO:0016836 (MF)	hydro-lyase activity	3.16E-03	5.88	ACO2, ENO3, FH

Table 5.1 (continued).

Go/KEGG ID	Description	Group Adj. P-value ¹	% Gene/Term	Proteins (gene symbol)
GO:0008035 (MF)	high-density lipoprotein particle binding	8.92E-09	50.00	APOA1, HSPD1
GO:0016459 (CC)	myosin complex	3.60E-04	11.90	MYH1A, MYH1D, MYH1E, MYH1F, MYO7L2
GO:0045261 (CC)	proton-transporting ATP synthase complex, catalytic core F(1)	6.84E-03	33.33	ATP5A1Z, ATP5B
GO:0005839 (CC)	proteasome core complex	2.84E-03	21.43	PSMA7, PSMB1, PSMB3
GO:0006119 (BP)	oxidative phosphorylation	5.19E-03	4.84	ATP5B, DLD, PGK2
GO:0006108 (BP)	malate metabolic process	3.26E-12	33.33	FH, MDH1
GO:0000272 (BP)	polysaccharide catabolic process	2.89E-06	22.22	AGL, PYGB
GO:0045454 (BP)	cell redox homeostasis	8.13E-06	10.00	DLD, PRDX1, PRDX6, SH3BGRL3, TXNL1
GO:0098760 (BP)	response to interleukin-7	6.70E-05	23.08	ATP5B, HSPD1, STIP1
GO:0071450 (BP)	cellular response to oxygen radical	7.95E-10	17.65	ADPRHL2, PRDX1, SOD1
GO:0004448 (BP)	isocitrate dehydrogenase activity	7.03E-05	50.00	IDH2, IDH3A
GO:0051920 (BP)	peroxiredoxin activity	3.83E-05	28.57	PRDX1, PRDX6
GO:0003746 (BP)	translation elongation factor activity	1.75E-02	13.33	EEF1A2, EEF2
GO:0044794 (BP)	positive regulation by host of viral process	3.26E-06	18.18	ANXA2P2, HSPA8

¹ Corrected with Benjamini-Hochberg false discovery rate (FDR).

5.4.5 Interaction network of DAPs relates to biological function in each comparison

Analysis of the PPI networks (Figure 5.5 and Table 5.2) revealed the majority of DAPs interacted with each other and formed clusters. For the NC vs PC comprising with glycolysis (ALDOC, DLD, ENO1, GAPDH, GPI, LDHA, PGAM1, PGK2, PGM1, PKLR), TCA cycle (DLD, FH, IDH2, IDH3A, SUCLA2), and starch/sucrose metabolism (AGL, GPI, PGM1). A similar metabolic cluster appears in NC vs TNP, with additional focus on glycolysis-related enzymes (e.g., GPI, PGK1, PKLR) while starch/sucrose metabolism, glycogen breakdown and carbohydrate phosphorylase (UGP2, PYGB, AGL) which UGP2 and PYGB are uniquely found in this condition. Additionally, NC vs TNP also identified proteins related to lipid metabolism for energy production, including those involved in high-density lipoprotein particle binding (APOA1, HSPD1) and PPAR signaling (FABP3, APOA1, ACADL). Both interventions enhance energy metabolism via TCA cycle and glycolysis, but TNP uniquely activates carbohydrate-specific pathways like starch and sucrose metabolism, reflecting enhanced glucose utilization.

The pathways related to oxidative stress and antioxidant activity shown in NC vs PC, proteins like ALDH9A1, PRDX1, PRDX6 cluster near oxidative stress-related terms, highlighting their role in neutralizing ROS. In comparison to NC vs TNP, the antioxidant enzymes (PRDX1, SOD1) are grouped with ATP metabolism, indicating a combined role in redox balance and energy production. Both treatments enhance antioxidant defenses, with TNP having stronger enrichment of glutathione-related pathways, supporting its ability to combat oxidative stress more effectively.

The pathways related to muscle function as observed in the comparison between NC vs PC, proteins like MYL6, MYLPF, and MYOM1 are grouped, indicating structural regulation. While the NC vs TNP, a prominent muscle cluster includes MYH1, MYO7L2, MYL1, and TNNC2, emphasizing the role of TNP in enhancing muscle contraction and structure. PC impacts muscle function through immune modulation and actin regulation, while TNP directly targets muscle contraction and structure via myosin-related pathways, highlighting its potential for improving muscle health.

Pathways related to immune function shown in NC vs PC, proteins such as HSP90AA1, CALR3, and PDIA3 are prominently associated with immune-related terms, indicating their central roles in antigen processing and cytokine response

pathways. These proteins are involved in enhancing antigen presentation through the MHC Class I peptide-loading complex (PLC), which is crucial for immune surveillance and cytotoxic T cell activation. In comparison to NC vs TNP, immune-related proteins like HSPA8 and STIP1, which support general cellular stress responses rather than being directly linked to antigen processing, are present but appear to play fewer central roles compared to the unencapsulated oil condition.

Protein folding and degradation pathways depicted in the NC vs PC analysis, Protein folding-related proteins like HSP90AA1, HSPA4 cluster with elongation factors. When exploring NC and TNP, proteasome proteins (PSMA7, PSMB1, PSMB3) and chaperones (HSPD1, TCP1) form distinct clusters. Both conditions enhance protein homeostasis, but TNP uniquely supports protein degradation via the proteasome, suggesting its role in maintaining muscle protein quality to facilitates protein turnover, ensuring removal of damaged proteins and maintaining muscle integrity.

The PPI networks confirm and expand on the enriched pathways from the GO/KEGG table, providing a functional map of interactions. TNP's molecular impact shows stronger alignment with muscle-specific pathways, antioxidant responses, and glucose handling, whereas PC enhances immune and protein folding mechanisms.

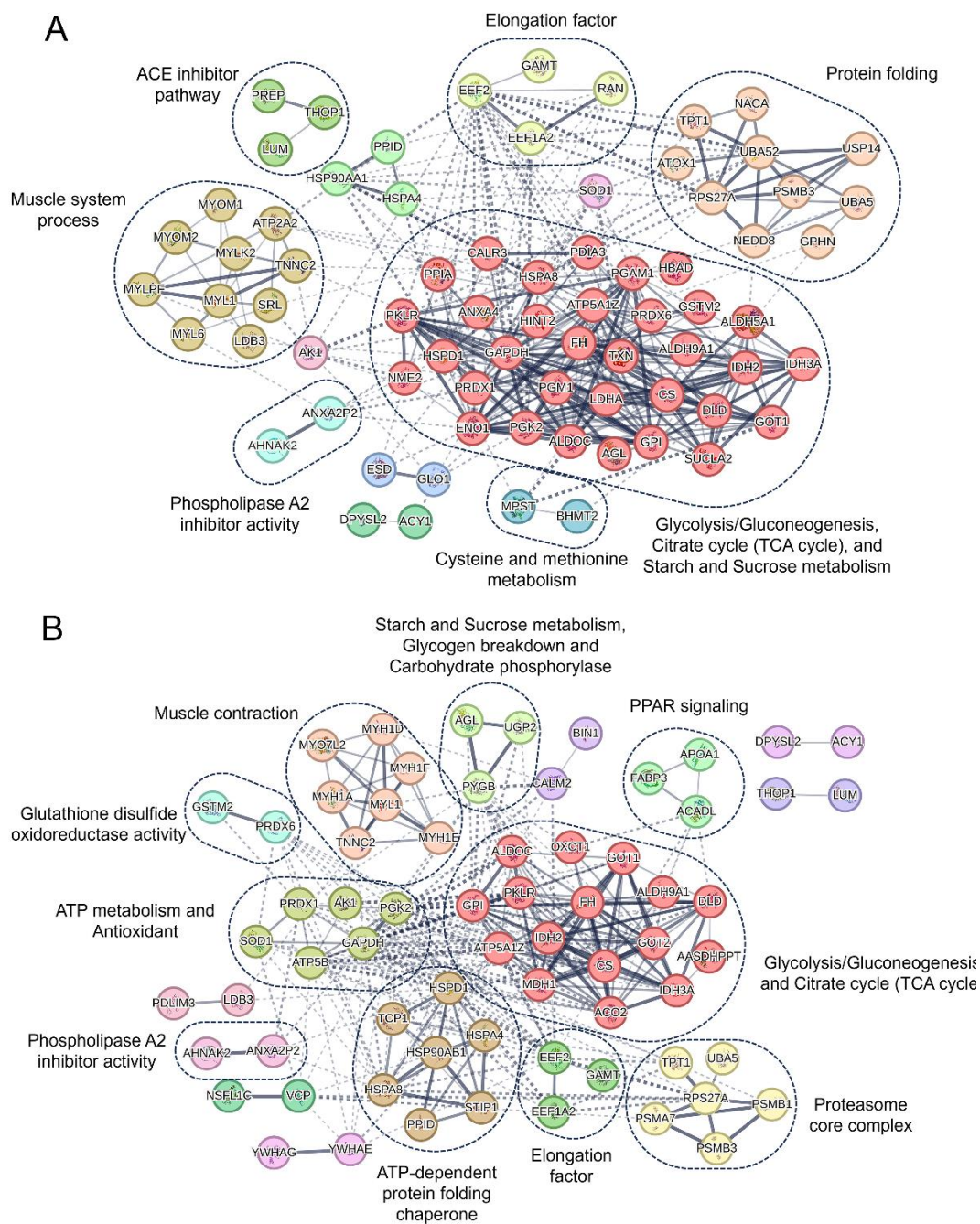


Figure 5.5 Protein-protein interaction networks in pairwise dietary comparisons, NC vs PC (A) and NC vs TNP (B).

Table 5.2 The DAPs from pairwise comparisons of NC vs PC and NC vs TNP conditions classified via PPI.

Accession Number	Gene symbol	Protein Name	NC vs PC		NC vs TNP	
			Log 2 FC	FDR	Log 2 FC	FDR
Starch/sucrose metabolism and glycolysis						
XP_015146231.2	AGL	glycogen debranching enzyme isoform X2 [Gallus gallus]	1.920	0.0270	1.600	0.0300
NP_001026205.2	PYGB	glycogen phosphorylase, brain form [Gallus gallus]	-	-	1.700	0.0160
NP_989442.2	UGP2	UTP--glucose-1-phosphate uridylyltransferase [Gallus gallus]	-	-	1.220	0.0090
AAQ10887.1	PGM1	phosphoglucomutase 1 [Gallus gallus]	0.454	0.0024	-	-
NP_001006128.2	GPI	glucose-6-phosphate isomerase [Gallus gallus]	0.483	0.0043	0.507	0.0077
XP_040512781.1	ALDOC	fructose-bisphosphate aldolase A [Gallus gallus]	0.344	0.0210	1.650	0.00038
NP_989636.1	GAPDH	glyceraldehyde-3-phosphate dehydrogenase [Gallus gallus]	0.577	0.0046	0.308	0.0110
NP_990316.3	PGK2	phosphoglycerate kinase [Gallus gallus]	0.572	0.0190	0.401	0.0210
NP_001026727.1	PGAM1	phosphoglycerate mutase 1 [Gallus gallus]	0.500	0.0059	-	-
XP_046787115.1	ENO1	alpha-enolase isoform X3 [Gallus gallus]	0.309	0.0290	-	-
NP_990450.1	ENO3	beta-enolase [Gallus gallus]	0.458	0.0042	0.530	0.0110
XP_015134281.1	PKLR	pyruvate kinase PKM isoform X3 [Gallus gallus]	0.366	0.0180	0.320	0.0370
NP_990615.2	LDHA	L-lactate dehydrogenase A chain [Gallus gallus]	0.458	0.0066	-	-

Table 5.2 (continued).

Accession Number	Gene symbol	Protein Name	NC vs PC		NC vs TNP	
			Log 2 FC	FDR	Log 2 FC	FDR
TCA cycle and amino acid metabolism						
NP_001006395.1	MDH1	malate dehydrogenase, cytoplasmic isoform MDH1 [Gallus gallus]	-	-	0.764	0.0087
NP_990652.1	GOT1	aspartate aminotransferase, cytoplasmic [Gallus gallus]	1.130	0.0140	-	-
XP_046781333.1	GOT2	aspartate aminotransferase, mitochondrial isoform X3 [Gallus gallus]	-	-	0.667	0.00023
AAN63562.1	ACO2	aconitase precursor [Gallus gallus]	-	-	0.671	0.0180
NP_001026770.2	IDH2	isocitrate dehydrogenase [NADP], mitochondrial [Gallus gallus]	0.0089	2.0200	1.590	0.0130
XP_015134372.1	IDH3A	isocitrate dehydrogenase [NAD] subunit alpha, mitochondrial isoform X1 [Gallus gallus]	0.941	0.0390	1.180	0.0029
NP_001025898.2	DLD	dihydrolipoyl dehydrogenase, mitochondrial [Gallus gallus]	1.310	0.0300	1.620	0.0120
NP_001006271.2	SUCLA2	succinate--CoA ligase [ADP-forming] subunit beta, mitochondrial [Gallus gallus]	2.850	0.0200	-	-
NP_001383574.1	FH	fumarate hydratase, mitochondrial isoform b [Gallus gallus]	1.030	0.0260	1.240	0.0015
NP_990838.1	CKM	creatine kinase M-type [Gallus gallus]	0.610	0.0390	-	-

Table 5.2 (continued).

Accession Number	Gene symbol	Protein Name	NC vs PC		NC vs TNP	
			Log 2 FC	FDR	Log 2 FC	FDR
XP_015155460.2	GAMT	guanidinoacetate N-methyltransferase isoform X1 [Gallus gallus]	1.100	0.0140	-	-
XP_046792434.1	BHMT2	betaine--homocysteine S-methyltransferase 1 [Gallus gallus]	0.680	0.0330	-	-
NP_001026562.2	ATP5B	ATP synthase subunit beta, mitochondrial precursor [Gallus gallus]	-	-	1.280	< 0.0001
PPAR signaling and fatty acid metabolism						
AAA48597.1	APOA1	apolipoprotein A-I [Gallus gallus]	-	-	1.570	0.0100
NP_001006511.1	ACADL	long-chain specific acyl-CoA dehydrogenase, mitochondrial [Gallus gallus]	-	-	2.440	0.0110
NP_001026060.2	FABP3	fatty acid-binding protein, heart [Gallus gallus]	1.230	0.0140	1.180	0.00085
Muscle contractility and calcium transporter						
AAA49097.1	TNNC2	troponin C [Gallus gallus]	5.730	0.0410	5.690	0.0170
XP_040559416.1	BIN1	myc box-dependent-interacting protein 1 isoform X34 [Gallus gallus]	1.130	0.0110	1.070	0.00940
NP_001038097.2	MYL1	myosin light chain 1, skeletal muscle isoform [Gallus gallus]	1.140	0.0170	1.630	0.0034
NP_990850.1	ATP2A2	sarcoplasmic/endoplasmic reticulum calcium ATPase 1 [Gallus gallus]	0.508	0.0330	-	-

Table 5.2 (continued).

Accession Number	Gene symbol	Protein Name	NC vs PC		NC vs TNP	
			Log 2 FC	FDR	Log 2 FC	FDR
XP_046783251.1	SRL	Sarcalumenin isoform X1 [Gallus gallus]	0.618	0.0370	-	-
XP_046774509.1	MYOM1	myomesin-1 isoform X1 [Gallus gallus]	1.330	0.0310	-	-
XP_046769147.1	MYOM2	M-protein, striated muscle isoform X1 [Gallus gallus]	2.050	0.0360	-	-
AAA73168.1	MYLK2	Myosin light chain kinase 2, skeletal/cardiac muscle	2.900	0.0045	-	-
NP_001185673.2	MYLPF	myosin regulatory light chain 11 [Gallus gallus]	1.910	0.0460	-	-
P02607.3	MYL6	Myosin light polypeptide 6 [Gallus gallus]	2.280	0.0010	-	-
XP_046775952.1	LDB3	LIM domain-binding protein 3 isoform X1 [Gallus gallus]	0.225	0.0340	0.285	0.0420
XP_015140913.2	PDLIM3	PDZ and LIM domain protein 3 isoform X1 [Gallus gallus]	-	-	0.758	0.0430
AAM88910.1	MYH1A	fast myosin heavy chain HCIII [Gallus gallus]	-	-	2.030	0.0240
XP_040505903.1	MYH1D	myosin-1B [Gallus gallus]	-	-	1.670	0.0400
NP_001305944.2	MYH1F	myosin, heavy chain 1F, skeletal muscle [Gallus gallus]	-	-	2.180	0.0200
NP_001384338.1	MYH1E	myosin, heavy chain 1E, skeletal muscle [Gallus gallus]	-	-	1.780	0.0170

Table 5.2 (continued).

Accession Number	Gene symbol	Protein Name	NC vs PC		NC vs TNP	
			Log 2 FC	FDR	Log 2 FC	FDR
Antioxidant defenses and downstream detoxification mechanisms						
NP_989542.2	SOD1	superoxide dismutase [Mn], mitochondrial precursor [Gallus gallus]	0.821	0.0290	0.861	0.0093
NP_001258861.1	PRDX1	peroxiredoxin-1 [Gallus gallus]	0.971	0.0150	0.720	0.0170
NP_001034418.2	PRDX6	peroxiredoxin-6 [Gallus gallus]	0.964	0.0031	0.695	0.0210
NP_001264720.1	ESD	S-formylglutathione hydrolase [Gallus gallus]	1.180	0.0250	-	-
XP_015742718.1	TXN	thioredoxin-like protein 1 isoform X4 [Coturnix japonica]	0.622	0.0150	-	-
XP_024999699.3	GSTM2	glutathione S-transferase 2-like isoform X1 [Gallus gallus]	1.600	0.0270	1.190	0.0400
XP_025001230.1	ADPRHL2	protein ADP-ribosylarginine hydrolase isoform X6 [Gallus gallus]	3.490	0.0140	0.720	0.0350
XP_010723427.1	TXNL1	thioredoxin-like protein 1 [Meleagris gallopavo]	-	-	0.511	0.0500
XP_040508134.1	SH3BGRL3	SH3 domain-binding glutamic acid-rich protein isoform X3 [Gallus gallus]	-	-	1.170	0.0450
XP_040545153.1	AKR7A2	aflatoxin B1 aldehyde reductase member 2 [Gallus gallus]	2.150	0.0025	-	-

Table 5.2 (continued).

Accession Number	Gene symbol	Protein Name	NC vs PC		NC vs TNP	
			Log 2 FC	FDR	Log 2 FC	FDR
XP_040533917.1	ALDH9A1	4-trimethylaminobutyraldehyde dehydrogenase [Gallus gallus]	0.680	0.0480	0.754	0.0160
NP_001376401.1	ALDH5A1	aldehyde dehydrogenase, mitochondrial [Gallus gallus]	1.40	0.0120	-	-
XP_419481.1	GLO1	lactoylglutathione lyase isoform X1 [Gallus gallus]	1.39	0.016	-	-
Phospholipid and vesicle trafficking management						
NP_990682.1	ANXA2P2	annexin A2 [Gallus gallus]	1.930	0.0260	1.840	0.0180
NP_001034418.2	PRDX6	peroxiredoxin-6 [Gallus gallus]	0.964	0.0031	0.695	0.0210
XP_040528062.1	AHNAK2	protein AHNAK2 isoform X1 [Gallus gallus]	1.990	0.0250	4.10	0.0018
XP_040545559.1	ANXA4	annexin A4 [Gallus gallus]	1.780	0.0480	-	-
NP_001026164.1	PDCD6	programmed cell death 6-interacting protein [Gallus gallus]	0.688	0.0190	-	-
NP_001006405.1	TCP1	T-complex protein 1 subunit alpha [Gallus gallus]	-	-	1.140	0.0310
AAA48597.1	APOA1	apolipoprotein A-I [Gallus gallus]	-	-	1.570	0.0100
NP_001026060.2	FABP3	fatty acid-binding protein, heart [Gallus gallus]	1.230	0.0140	1.180	0.00085
XP_040551122.1	MYO7L2	myosin-7 [Gallus gallus]	-	-	1.710	0.0340

Table 5.2 (continued).

Accession Number	Gene symbol	Protein Name	NC vs PC		NC vs TNP	
			Log 2 FC	FDR	Log 2 FC	FDR
Immune response						
XP_040549017.1	CALR3	calreticulin [Gallus gallus]	7.280	0.0056	-	-
NP_989441.1	PDIA3	protein disulfide-isomerase A3 precursor [Gallus gallus]	2.550	0.0035	-	-
NP_001026562.2	ATP5B	ATP synthase subunit beta, mitochondrial precursor [Gallus gallus]	-	-	1.280	< 0.0001
XP_040511080.1	STIP1	LOW QUALITY PROTEIN: stress-induced-phosphoprotein 1 isoform X1 [Gallus gallus]	-	-	1.13	0.035
XP_040539066.1	HSPA4	heat shock 70 kDa protein 4 [Gallus gallus]	0.856	0.032	1.03	0.008
NP_001310129.1	HSPA8	heat shock cognate 71 kDa protein	1.25	0.0081	1.3	0.0099
NP_001274134.1	RPS27A	ubiquitin-40S ribosomal protein S27a [Gallus gallus]	0.812	0.007	-	-
NP_990406.1	UBA52	ubiquitin-60S ribosomal protein L40 [Gallus gallus]	1.29	0.011	-	-
XP_040521491.1	USP14	ubiquitin carboxyl-terminal hydrolase 14 isoform X1 [Gallus gallus]	1.25	0.0082	-	-
Proteasome complex						
NP_001007906.1	PSMB1	proteasome subunit beta type-1 [Gallus gallus]	-	-	1.99	0.0021
XP_015155007.1	PSMB3	proteasome subunit beta type-3 [Gallus gallus]	2.9	0.0032	2.81	0.0037
NP_989944.2	PSMA7	proteasome subunit alpha type-7 isoform 1 [Gallus gallus]	-	-	0.778	0.038

5.4.6 n-3 PUFAs encapsulated in lipid nanoparticles induce a metabolic shift in skeletal muscle, favoring aerobic metabolism

n-3 PUFAs delivered via lipid nanoparticles may drive a unique metabolic shift in skeletal muscle, favoring aerobic metabolism and enhanced mitochondrial efficiency. This shift is evident from the distinct protein expression patterns observed in this condition. While both unencapsulated oil and nanoparticle forms of n-3 PUFAs upregulated proteins involved in energy-related processes such as starch and carbohydrate metabolism, glycolysis, the TCA cycle, and fatty acid metabolism, the lipid nanoparticle condition specifically suppresses anaerobic pathways, as indicated by the absence of L-lactate dehydrogenase A chain (**LDHA**) expression. This reduction in anaerobic reliance, coupled with lower succinate CoA ligase (**SUCLA2**) levels, suggests a decreased demand for GTP/ATP synthesis from substrate-level phosphorylation (Jannas-Vela et al., 2023). Instead, the TNP group increases NAD^+ demand through elevated expression of key oxidative enzymes such as isocitrate dehydrogenase subunit alpha (**IDH3A**), dihydrolipoyl dehydrogenase (**DLD**), and fumarate hydratase (**FH**), highlighting a shift toward enhanced TCA cycle activity (Lalia et al., 2017). Additionally, the upregulation of glycogen phosphorylase (**PYGB**), glycogen debranching enzyme (**AGL**), and UTP-glucose-1-phosphate uridylyltransferase (**UGP2**) along with fructose-bisphosphate aldolase C (**ALDOC**), points to active glycolysis and glycogen mobilization, ensuring a steady supply of glucose for oxidative metabolism (Ahmed et al., 2014). The upregulation of ATP synthase subunit beta (**ATP5B**) in the lipid nanoparticle group further supports enhanced mitochondrial efficiency by promoting ATP production through oxidative phosphorylation. Uniquely upregulated proteins like aconitase precursor (**ACO2**), malate dehydrogenase (**MDH1**), aspartate aminotransferase, cytoplasmic (**GOT1**) and aspartate aminotransferase, mitochondrial (**GOT2**) further reinforce this metabolic shift by strengthening mitochondrial-cytosolic coupling including malate-aspartate shuttle, optimizing energy flux through aerobic pathways (Ahmed et al., 2014; Jannas-Vela et al., 2023). The activation of PPAR signaling, evidenced by the upregulation of apolipoprotein A-I (**APOA1**) and long-chain specific acyl-CoA dehydrogenase, mitochondrial (**ACADL**), promotes fatty acid β -oxidation and mitochondrial biogenesis (Tachtsis et al., 2018; Tomczyk, 2024). Suggest that n-3 PUFAs encapsulated in lipid nanoparticles exert a

stronger and more targeted effect on skeletal muscle metabolism, optimizing energy production through aerobic pathways while efficiently utilizing glycogen reserves.

5.4.7 Lipid nanoparticle supplementation drives amino acid metabolism toward enhanced mitochondrial efficiency

The delivery methods of n-3 PUFAs produce distinct metabolic shifts in skeletal muscle. In the unencapsulated oil condition, metabolism is geared towards glycolysis and rapid energy buffering. Proteins such as creatine kinase M-type (**CKM**) support creatine synthesis and ATP regeneration via the creatine-phosphocreatine system, ensuring rapid regeneration of ATP in energy-intensive conditions (Gerling et al., 2019; Tomczyk, 2024), guanidinoacetate N-methyltransferase (**GAMT**) converse guanidinoacetate to form creatine by methylation (Schulze et al., 2016). Guanidinoacetate is synthesized from glycine and arginine (Nabuurs et al., 2013), crucial for meeting immediate energy demands during muscle activity (Bonilla et al., 2021).

Additionally, pathways associated with amino acid metabolism are activated. Betaine-homocysteine S-methyltransferase (**BHMT2**) catalyzes the conversion of homocysteine into methionine, an essential amino acid. Methionine is then converted into S-adenosylmethionine (**SAM**), a universal methyl donor involved in numerous methylation reactions, including the synthesis of creatine. This process supports both methylation-dependent biochemical pathways and energy metabolism (Garibotto et al., 2023). while GOT1 plays a key role in linking amino acid metabolism with the TCA cycle by catalyzing the conversion of aspartate to oxaloacetate (Li & Hoppe, 2023). Proteins such as DLD, involved in the TCA cycle and oxidative metabolism (Yang et al., 2019b), phosphoglycerate mutase 1 (**PGAM1**), As part of glycolysis (Katsnelson & Ceddia, 2020; Li & Liu, 2020), ensuring the energy required for amino acid biosynthesis and other anabolic processes (Hitosugi et al., 2012).

In contrast, lipid nanoparticles enhance oxidative metabolism and mitochondrial efficiency. Proteins such as DLD, which catalyzes redox reactions in the TCA cycle, and GAMT, which supports creatine synthesis for energy buffering, show higher expression compared to the unencapsulated oil condition. However, the activity of CKM is notably reduced, indicating a shift away from reliance on the creatine-phosphocreatine energy buffering system. Additionally, the upregulation of GOT2, a mitochondrial enzyme involved in the malate-aspartate shuttle, facilitates efficient

mitochondrial-cytosolic coupling. This enhances the transfer of reducing equivalents and supports sustained energy production through oxidative phosphorylation. These adaptations emphasize the lipid nanoparticle condition's ability to optimize mitochondrial function and promote aerobic metabolism more effectively than the unencapsulated oil condition. Overall, lipid nanoparticles prioritize oxidative metabolism and mitochondrial efficiency, while unencapsulated oil supports a broader range of metabolic processes, including glycolysis and amino acid metabolism. These insights highlight the potential of lipid nanoparticles to optimize energy output for applications requiring prolonged metabolic activity.

5.4.8 Impact of lipid nanoparticles on cellular antioxidant and detoxification mechanisms

The cellular response to lipid oxidation under n-3 PUFA conditions involves a coordinated network of upstream antioxidant defenses and downstream detoxification mechanisms, enabling the cell to maintain redox balance and mitigate oxidative damage caused by reactive oxygen species (ROS) and lipid peroxidation byproducts.

5.4.8.1 Cellular antioxidant

The cellular response to lipid oxidation under n-3 PUFA conditions involves a coordinated network of upstream antioxidant defenses and downstream detoxification mechanisms. These responses work collectively to maintain redox balance and mitigate oxidative damage. The distinct protein expression patterns observed in the unencapsulated oil and lipid nanoparticle conditions highlight how n-3 PUFAs delivery systems influence cellular adaptations to oxidative stress by modulating specific protein interactions and workflows.

In the unencapsulated oil condition, the higher oxidative burden, due to the susceptibility of free n-3 PUFAs (EPA and DHA) to lipid peroxidation, triggers a comprehensive antioxidant and detoxification response. Superoxide dismutase (**SOD1**), with moderate upregulation, acts as the first line of defense by neutralizing superoxide radicals ($O_2^{\cdot-}$), preventing further ROS propagation (Kusunoki et al., 2013). Its activity is supported downstream by peroxiredoxin-1 (**PRDX1**) and peroxiredoxin-6 (**PRDX6**), which reduce hydrogen peroxide (H_2O_2) (Jackson et al., 2022) and lipid hydroperoxides (ROOH) (Burillo et al., 2016), preventing damage to cellular macromolecules. These

peroxiredoxins (**PRDX1** and **PRDX6**) rely on glutathione, helps maintain PRDX6 activity, as PRDX6 also uses glutathione to reduce lipid hydroperoxide. The glutathione regenerated by S-formylglutathione hydrolase (**ESD**), detoxifies S-formylglutathione from formaldehyde (Gonzalez et al., 2006). thioredoxin-like protein 1 (**TXN**) complements these processes by reducing the disulfide bond of oxidized peroxiredoxins, ensuring their continuous function (AlOkda & Raamsdonk, 2023). glutathione S-transferase 2-like (**GSTM2**) and protein ADP-ribosylarginine hydrolase (**ADPRHL2**) manage the downstream detoxification of lipid peroxidation byproducts. GSTM2 conjugates reactive aldehydes like MDA and 4-Hydroxyhexenal (4-HHE) with glutathione for excretion (Soulage et al., 2020). The unencapsulated oil condition generates a higher level of oxidized proteins due to increased ROS production and lipid peroxidation. This oxidative stress often leads to the ADP-ribosylation of proteins, a post-translational modification that regulates damaged proteins and cellular stress responses. ADPRHL2 plays a critical role in this process by removing ADP-ribose modifications from oxidized proteins, thereby restoring their functionality and maintaining protein homeostasis (Beijer et al., 2021). This tightly integrated network ensures robust protection against oxidative stress in the unencapsulated oil condition.

In the lipid nanoparticle condition, encapsulation protects n-3 PUFAs from oxidation, leading to a reduced oxidative burden and a more streamlined antioxidant response. SOD1 remains critical for superoxide neutralization, but lower expression levels of PRDX1 and PRDX6 reflect the reduced need for extensive ROS detoxification. In this condition, the absence of TXN is compensated by the upregulation of thioredoxin-like protein 1 (**TXNL1**), which specializes in maintaining redox balance in specific cellular compartments (Zhao & Qi, 2021), particularly to support the mitochondria function. TXNL1 likely collaborates with the uniquely expressed SH3 domain-binding glutamic acid-rich protein (**SH3BGRL3**), an adapter protein interacts with SH3 domains of various proteins (Bin, 2016), to regulate redox-sensitive signaling pathways and support mitochondrial protein maintenance. Although GSTM2 continues to detoxify lipid peroxidation byproducts, its lower expression aligns with the reduced oxidative stress. ADPRHL2 also plays a role in repairing oxidized proteins, though at a diminished level, consistent with the lower demand for extensive repair processes. The absence of ESD and TXN further highlights the reduced need for

aldehyde detoxification and broad-spectrum redox regulation, with cellular resources redirected toward efficient mitochondrial function and signaling optimization.

These findings illustrate that the unencapsulated oil condition requires a highly interconnected network of antioxidant and detoxification pathways to combat high oxidative stress, while the lipid nanoparticle condition employs a more focused strategy, prioritizing mitochondrial function and specialized redox signaling. These distinct workflows underscore the influence of n-3 PUFAs delivery systems on cellular stress adaptation.

5.4.8.2 Detoxification mechanisms

Even though in both conditions, occurs protein plays a critical upstream role by neutralizing superoxide anions into hydrogen peroxide, reducing the formation of lipid peroxidation and preventing excessive oxidative damage. Under n-3 PUFA conditions, detoxification pathways are activated to manage reactive aldehydes and carbonyl compound generated from lipid peroxidation, such as 4-Hydroxynonenal (4-HNE), 4-HHE, malondialdehyde (MDA), and methylglyoxal (MGO), with distinct enzyme responses observed in unencapsulated oil and lipid nanoparticle conditions.

In the PC group, enzymes aflatoxin B1 aldehyde reductase member 2 (AKR7A2), 4-trimethylaminobutyraldehyde dehydrogenase (ALDH9A1), aldehyde dehydrogenase, mitochondrial (ALDH2), and GSTM2 are upregulated, reflecting a need for comprehensive aldehyde detoxification due to higher oxidative stress levels. AKR7A2 a cytosolic enzyme, acts as the first line of defense against toxic lipid-derived aldehydes, such as 4-HHE and MDA, by reducing them into less reactive alcohols using NADPH as a cofactor. AKR7A2, a cytosolic enzyme, initiates the detoxification of lipid-derived aldehydes by reducing reactive aldehydes, such as 4-HHE (Barski et al., 2008; Penning, 2015; Mol et al., 2017), into their corresponding alcohols, such as 4-hydroxyhexanol, using NADPH as a cofactor. This process transfers electrons from NADPH to the aldehyde (-CHO) group, neutralizing its reactivity and regenerating NADP^+ (Penning, 2015). ALDH2, a mitochondrial enzyme, plays a critical role in the second step of detoxification by directly oxidizing aldehydes, including 4-HNE, MDA, and certain amino acid-derived aldehydes, into their corresponding carboxylic acids (Vistoli et al., 2013; Wakabayashi et al., 2020; Karan et al., 2023). For example, ALDH2 catalyzes the oxidation of the aldehyde (-CHO) group in 4-HNE to produce 4-hydroxynonenoic acid (4-HNA), and similarly

converts MDA to malonic acid. This reaction uses NAD^+ as a cofactor, reducing it to NADH and ensuring the complete removal of reactive aldehydes (Ahmed Laskar & Younus, 2019; Wakabayashi et al., 2020). ALDH9A1, another aldehyde dehydrogenase, complements the detoxification process by oxidizing alcohol intermediates, such as 4-hydroxyhexanol (produced by AKR7A2), into their corresponding carboxylic acids. It also oxidizes some aldehydes directly, preparing them for safe excretion from the body (Makia et al., 2011). These enzymes form a coordinated detoxification pathway, protecting cells from the toxic effects of lipid-derived aldehydes and their intermediates by neutralizing and processing them into stable, excretable metabolites. GSTM2 provides an additional layer of protection by conjugating remaining aldehydes and alcohols with glutathione, making them water-soluble and facilitating their excretion via urine or bile (Singhal et al., 2015).

In contrast, the lipid nanoparticle condition upregulates ALDH9A1, and GSTM2, but not AKR7A2 and ALDH2, indicating a streamlined detoxification response with lower reliance on amino acid metabolism pathways. This condition focuses primarily on handling aldehydes from lipid peroxidation. AKR7A2 continues to play a critical role by reducing aldehydes to alcohols, albeit at a slight level, while ALDH9A1 ensures these alcohol intermediates are further oxidized into carboxylic acids, facilitating their safe excretion from the body. GSTM2 works alongside these enzymes by conjugating residual reactive species with glutathione, bolstering antioxidant defenses and facilitating detoxification. The absence of ALDH2 suggests reduce the generating of the lipid oxidative by-product.

Among the proteins involved in detoxification, lactoylglutathione lyase (GLO1) was upregulated in PC group, plays a critical role as a key component of the glyoxalase system. GLO1 is responsible for detoxifying harmful metabolic by-products, particularly reactive dicarbonyl compounds such as methylglyoxal (MGO), which is highly toxic due to its ability to form advanced glycation end-products (AGEs) that impair cellular function and promote oxidative stress. While MGO is a natural by-product of glycolysis (Allaman et al., 2015), it is also found in high amounts in fish oil (Suh et al., 2017). GLO1 initiates this process by converting methylglyoxal and reduced glutathione (GSH) into S-D-lactoylglutathione, which is further processed by GLO2 into D-lactate, simultaneously regenerating GSH in the process (Syed et al., 2023). This

glyoxalase pathway is essential for mitigating glycation stress and maintaining cellular homeostasis, particularly in conditions of elevated MGO levels.

These enzyme systems, starting with SOD1 in oxidative stress management, highlight the differential metabolic adaptations under distinct n-3 PUFA delivery, with the PC group providing a broad detoxification and energy-linked approach, while the TNP group optimizes the handling of lipid peroxidation products with streamlined efficiency.

5.4.9 The DAPs in lipid nanoparticles: preventing phospholipid damage and supporting trafficking

As we know, sources high in EPA and DHA provide precursors for essential metabolites, with enzymatic pathways (e.g., PLA2, LOX, COX) contributing to the production of biologically active lipid mediators that regulate inflammation and cellular homeostasis (Manson et al., 2023). However, under high n-3 PUFA levels, cells face the challenge of oxidative stress, which leads to radical-mediated lipid peroxidation. This process generates oxidized phospholipids, which, if left unchecked, can disrupt membrane integrity and cellular function. In this study, several key proteins, including annexin A2 (**ANXA2P2**), PRDX6, protein ANNAK2 (**AHNAK2**), annexin A4 (**ANXA4**), and programmed cell death 6-interacting protein (**PDCD6**), were identified as critical for preventing and managing phospholipid damage under these conditions, ensuring membrane stability and cellular resilience.

ANXA2P2 was identified as an oxidant-sensitive protein, functions as a membrane stabilizer during oxidative stress and role in pathogen-host cell interaction (Monastyrskaya et al., 2009; Defour et al., 2017; Dallacasagrande & Hajjar, 2020). Upon calcium activation, Annexin A2 binds peroxidized phospholipids, compartmentalizing them to prevent the spread of lipid peroxidation and providing a scaffold for other repair proteins (Jaiswal et al., 2014; Hajjar et al., 2015; Demonbreun et al., 2016; Defour et al., 2017). Similarly, ANXA4 contributes to membrane repair by binding oxidized phospholipids and stabilizing damaged regions during calcium flux, complementing ANXA2's function in maintaining membrane integrity.

AHNAK2, a cytoskeletal organizer, enhances this process by stabilizing membrane-cytoskeletal interactions and modulating calcium signaling, which is also essential for activating PDCD6. Under increasing of oxidative stress and ROS production,

leading to calcium influx into the cell. ANXA2P2 forms a complex with S100 proteins to regulate trafficking processes, while AHNAK2 supports the structural integrity needed for vesicle movement, to ensure precise calcium signaling and promotes faster recruitment of AHNAK proteins to the plasma membrane, then inducing more efficient membrane repair (Jaiswal et al., 2014; Zhang et al., 2023; Prislusky et al., 2024). This calcium signaling activates PDCD6, enabling it to function at the membrane. PDCD6, a calcium-binding protein commonly activated under oxidative stress conditions, plays a crucial role in membrane repair by coordinating vesicle trafficking machinery. Upon activation, PDCD6 facilitates endocytosis, which removes oxidized lipid components and other damaged molecules from the membrane. Simultaneously, it mediates exocytosis, incorporating new phospholipids to restore the structural and functional integrity of the damaged membrane (Zhen et al., 2021; Drescher et al., 2023). This dual role ensures efficient turnover of damaged components and maintains membrane homeostasis, particularly under conditions of oxidative stress. Together, these proteins form an integrated defense system, with ANXA2P2 and ANXA4 stabilizing membranes, AHNAK2 organizing the cytoskeleton, and PDCD6 promoting lipid turnover, ensuring resilience under oxidative stress induced by high n-3 PUFA levels. PRDX6 is essential for eliminating oxidized phospholipids due to its two enzymatic functions including phospholipase A2 (**PLA2**) and glutathione peroxidase-like activity. Its PLA2 function enables PRDX6 to target the sn-2 position of oxidized phospholipids, releasing the oxidized fatty acid and generating a lysophospholipid. Furthermore, PRDX6 neutralizes hydroperoxides (–OOH groups) in phospholipid molecules by converting them to their corresponding alcohols (–OH groups) using glutathione (GSH) as a cofactor. These combined actions play a crucial role in the detoxification of oxidized phospholipids, ensuring the integrity of cellular membranes and protecting them from oxidative stress.

In the TNP group, ANXA2P2 and PRDX6 expression were detected, suggesting that similar mechanisms observed in the positive control group, which exhibited slight phospholipid damage, are also active in this condition. Interestingly, the elevated expression of AHNAK2 highlights its pivotal role in adapting to the unique cellular demands induced by nanoparticle interactions. These demands include cytoskeletal reorganization and vesicle trafficking, which are critical for phospholipid redistribution and maintaining membrane integrity. While proteins directly involved in

nanoparticle uptake were not identified in this study, prior research with nanoparticles and glycosylated nanoparticles has demonstrated that endocytosis via transporters is fundamental to the cellular uptake process (Yeh et al., 2014; Foroozandeh et al., 2018; Wang et al., 2021; Qin et al., 2020; Baker et al., 2022), could be involved with protein coronas (Liu et al., 2020; Wang et al., 2024). This study underscores the importance of AHNK2 in facilitating cellular responses to nanoparticles, particularly in vesicle-mediated processes essential for membrane repair and adaptation.

The negative zeta potential of lipid nanoparticles (LNPs), indicative of a negatively charged surface, promotes interactions with positively charged or neutral regions of the cell membrane, as shown in previous studies (Grady et al., 2017; Yang et al., 2019a). This enhances adhesion and may facilitate fusion with the membrane. However, the negative zeta potential and particle size of LNPs also induce stress on the membrane, necessitating cytoskeletal components like AHNK2 to stabilize membrane-cytoskeletal interactions. AHNK2 has been implicated in vesicle trafficking and membrane repair (Ebstrup et al., 2021), which could help manage stress induced by LNPs.

Furthermore, AHNK2 might facilitate the integration and redistribution of n-3 PUFAs delivered by LNPs, contributing to the maintenance of membrane composition and function. Previous research has highlighted the role of the cytoskeleton in supporting vesicle-mediated processes, ensuring that LNPs are efficiently processed and their lipid cargo properly utilized by the cell (Li et al., 2020; Zhang et al., 2021; Brosio et al., 2023; Park et al., 2024). Intracellular vesicle transport depends on a well-organized cytoskeletal network, which is facilitated by Rab GTPases, motor adaptor proteins, and motor proteins like myosin (Cui et al., 2022). Supporting these processes, T-complex protein 1 subunit alpha (**TCP1**), as part of the chaperonin-containing TCP1 (CCT) complex, and Myosin-7 (MYO7L2) further supports mechanical efficiency by organizing myosin regulation (Mott et al., 2024), ensures the proper folding of structural proteins like actin and tubulin, which are critical for vesicle movement and cytoskeletal stability (Brackley et al., 2009; Dmitrieff & Nédélec, 2016; Mulens-Arias, 2021; Cui et al., 2022).

The coordinated of AHNK2, TCP1, and MYO7L2 may coordinate cytoskeletal reorganization and vesicle trafficking to optimize the cellular uptake and

processing of n-3 PUFAs. However, further laboratory investigations are necessary to provide evidence for this proposed coordination and its functional impact on LNP-mediated lipid delivery. The absence of a DAPs for ANXA4 and PDCD6 in the lipid nanoparticle condition suggests that their activation may less strongly induced as in the unencapsulated oil condition. This further supports a shift toward specialized processes involving lipid stabilization rather than active repair.

APOA1 and Fatty Acid-Binding Protein 3 (**FABP3**) play complementary roles in maintaining phospholipid homeostasis, mediating lipid transport, redistribution, and integration into membranes. These processes are particularly critical in the context of lipid nanoparticle delivery, ensuring the effective utilization of n-3 PUFAs. Lipid nanoparticles, composed of triglycerides and/or phospholipids, can induce APOA1 activity to remove excess phospholipids from overloaded membranes via its interaction with lipid transporters (Caracciolo et al., 2015; Chen et al., 2022). Meanwhile, FABP3 facilitates the intracellular handling of n-3 PUFAs by binding them. The research exhibits the over-expression in FABP3 delivering them to phospholipid remodeling pathways for membrane incorporation, by increasing the saturated phosphatidylcholine acyl chains (Lee et al., 2020) or directing them toward metabolic pathways such as β -oxidation for energy production (Huang et al., 2018).

5.4.10 Lipid nanoparticles promote enhanced muscle performance by prioritizing sarcomeric and calcium dynamics

n-3 PUFA supplementation increases the expression of Troponin C (**TNNC2**), Bridging integrator 1 (**BIN1**), and Myosin light chain 1 (**MYL1**) in both the unencapsulated oil and lipid nanoparticle conditions, reflecting shared mechanisms focused on maintaining contractile function, calcium sensitivity, and structural organization in skeletal muscle. However, the distinct differential protein responses between these conditions highlight adaptive priorities.

TNNC2, specific to fast-twitch fibers, ensures precise calcium binding and release, maintaining rapid, forceful contractions. Changed in Ca^{2+} concentration affect the structure regulated rapid skeletal muscle contraction (Yu et al., 2020). The DAPs expression observed in both conditions suggested that n-3 PUFA positively influences muscle contraction by the function of calcium binding protein like TNNC2.

In the NC vs PC group, oxidative stress from lipid peroxidation triggers an upregulation of proteins involved in muscle repair and stabilization. Sarcoplasmic/endoplasmic reticulum calcium ATPase 2 (**ATP2A2**) and Sarcalumenin (**SRL**) play key roles in regulating calcium homeostasis. ATP2A2 pumps calcium ions into the sarcoplasmic reticulum, preventing overload, while SRL buffers and stores calcium to maintain efficient contraction-relaxation cycles. Myomesin 1 (**MYOM1**) and Myomesin 2 (**MYOM2**) play essential roles in stabilizing myofibril organization by anchoring myosin filaments at the M-line, thereby maintaining structural integrity of the sarcomere (Hang et al., 2021). The high protein expression observed in the PC group suggests that these Myomesin proteins contribute to enhanced muscle contractility by ensuring proper filament alignment and supporting calcium homeostasis. This interaction facilitates efficient force generation during muscle contraction and recovery (Prill et al., 2019; Lamber et al., 2022). Simultaneously, proteins such as Myosin Light Chain Kinase 2 (**MYLK2**) and Myosin Light Chain Phosphorylation Factor (**MYLPF**) underscore the importance of calcium signaling in activating the actomyosin machinery. MYLPF, phosphorylated by MYLK2, enhances the interaction between myosin heads and actin filaments, thereby increasing contractile force and efficiency (Stull et al., 2011; Murgia et al., 2021). However, the observed elevated responses may be a result of oxidative stress induced by lipid oxidation by-product and calcium imbalance. Further research is necessary to elucidate the relationship between muscle function under n-3 PUFA supplementation, oxidative stress, and their combined effects on protein function.

Additionally, Myosin Light Chain 1 (**MYL1**) and Myosin Light Polypeptide 6 (**MYL6**) stabilize the actomyosin complex and actin cytoskeleton, ensuring sustained contractility during oxidative stress. At the Z-disc, LIM Domain Binding 3 (**LDB3**) reinforces sarcomeric stability, while BIN1 preserves T-tubule structure, ensuring synchronized electrical signaling and calcium release. These proteins form an integrated network to maintain contractile machinery, and enable skeletal muscle cells to recover and adapt.

In contrast, the TNP group focuses on optimizing contractile performance. PDZ and LIM domain protein 3 (**PDLIM3**), plays a critical role in mechanotransduction and sarcomeric stability, enabling skeletal muscle cells to adapt to the demands of enhanced contractile activity facilitated by n-3 PUFA delivery. PDLIM3, localized at the Z-disc, collaborates with LDB3 to stabilize actin filaments and reinforce sarcomeric

alignment during mechanical stress, serving as a hub for mechanotransduction (Krcmery et al., 2010). Beyond its structural role, PDLIM3 also promotes the proliferation and differentiation of skeletal muscle satellite cells, key players in muscle repair and regeneration (Yin et al., 2020). This regenerative capacity may be enhanced by n-3 PUFA supplementation, as suggested by findings that link n-3 PUFA to improved muscle cell function and adaptation (Jannas-Vela et al., 2023). TNNC2 complements this by fine-tuning calcium-mediated actomyosin interactions to enhance force generation. MYL1 improves myosin-actin cross-bridge dynamics for sustained performance. BIN1 preserves T-tubule integrity, synchronizing electrical signals with mechanical activity to ensure optimal contractility. The upregulation of myosin heavy chains including Fast myosin heavy chain HCIII (**MYH1A**), Myosin-1B (**MYH1D**), Myosin heavy chain 1E (**MYH1E**), and Myosin heavy chain 1F (**MYH1F**) further reinforces the contractile framework, enabling skeletal muscle cells to adapt to the demands of enhanced contractile activity (Lee et al., 2019). These findings demonstrate that lipid nanoparticles promote a streamlined, performance-oriented response, enhancing contractile efficiency and mechanical stability. In contrast, unencapsulated oil induces a broader response, emphasizing calcium homeostasis, structural repair, and oxidative stress adaptation.

5.4.11 Cellular stability and immune response modulation by tuna oil loaded in lipid nanoparticles

The DAPs profiles in the PC group indicate a dual response involving oxidative stress management and immune surveillance. Proteins such as calreticulin 3 (**CALR3**) and protein disulfide-isomerase A3 (**PDIA3**), CALR3 is a major Ca^{2+} binding protein in SR, plays role in control process during protein synthesis and folding (Michalak et al., 2024) and PDIA3 main function is to ensure and mediate the formation of the correct intramolecular disulfide bonds in nascent proteins (Antoniotti et al., 2022). These proteins typically occur in response to managing the sarcoplasmic reticulum (ER), particularly condition that challenge protein folding and calcium homeostasis. The upregulation of CALR3 and PDIA3 in the PC group refer to stress response in sarcoplasmic reticulum, highlight the activation of the unfolded protein response (UPR) to address misfolded and oxidatively damaged proteins, (Zhang et al., 2019; Antoniotti et al., 2022).

Concurrently, heat shock proteins 70 (**HSPA4**) and Hsp70 cognate 71 kDa protein (**HSPA8**) stabilize unfolded proteins, while proteasome subunit beta type-3 (**PSMB3**), a key proteasome component, facilitates protein turnover by degrading irreparably damaged proteins. These oxidative stress responses are complemented by immune regulation, with proteins like ubiquitin-40S ribosomal protein S27a (**RPS27A**), ubiquitin-60S ribosomal protein L40 (**UBA52**), and ubiquitin carboxyl-terminal hydrolase 14 (**USP14**) supporting ubiquitin-mediated antigen processing for MHC class I presentation, ensuring robust immune surveillance under stress conditions (Colberg et al., 2020). This study clearly demonstrates that the substrate driving the cellular response to oxidative stress is lipid oxidation by-product. This substrate was highly active in the PC group, indicating that the accumulation of unrecovered by-products may be a critical factor leading to calcium imbalance. Such an imbalance can disrupt protein folding within the sarcoplasmic reticulum (SR) lumen, further exacerbating cellular stress and impairing proper protein function.

Concurrently, heat shock proteins 70 (**HSPA4**) and Hsp70 cognate 71 kDa protein (**HSPA8**) stabilize unfolded proteins, while proteasome subunit beta type-3 (**PSMB3**), a key proteasome component, facilitates protein turnover by degrading irreparably damaged proteins. These oxidative stress responses are complemented by immune regulation, with proteins like ubiquitin-40S ribosomal protein S27a (**RPS27A**), ubiquitin-60S ribosomal protein L40 (**UBA52**), and ubiquitin carboxyl-terminal hydrolase 14 (**USP14**) supporting ubiquitin-mediated antigen processing for MHC class I presentation, ensuring robust immune surveillance under stress conditions (Colberg et al., 2020). This study clearly demonstrates that the substrate driving the cellular response to oxidative stress is lipid oxidation by-product. This substrate was highly active in the PC group, indicating that the accumulation of unrecovered by-products may be a critical factor leading to calcium imbalance. Such an imbalance can disrupt protein folding within the sarcoplasmic reticulum (SR) lumen, further exacerbating cellular stress and impairing proper protein function.

5.5 Conclusion

The comparative analysis highlights the distinct and overlapping molecular effects of tuna oil (PC) and lipid nanoparticle-loaded tuna oil (TNP). Both formulations

enhance energy metabolism, oxidative stress management, and immune function, underscoring their therapeutic potential for improving metabolic health and immunity. PC group predominantly supports broader metabolic processes, focusing on amino acid metabolism, antigen presentation, immune modulation, and protein synthesis. In contrast, TNP group offers a more targeted approach, prioritizing carbohydrate metabolism, muscle maintenance, lipid transport, and hormonal regulation.

Lipid nanoparticles demonstrate a stronger impact on skeletal muscle metabolism by optimizing oxidative phosphorylation, enhancing mitochondrial efficiency, and promoting aerobic energy production. They also stabilize lipids and minimize oxidative stress, streamlining metabolic responses. On the other hand, PC group induces a broader metabolic response, emphasizing calcium homeostasis, structural repair, and oxidative stress adaptation.

These findings reveal the specialized cellular mechanisms of each formulation, PC group facilitates broad-spectrum metabolic and immune processes, while TNP group provides a focused, performance-oriented response that enhances protein stability and energy efficiency. These insights highlight the potential for tailored applications of both formulations in supporting health and metabolic activity based on specific physiological needs. Further studies should focus on explore the effects of by-products resulting from lipid oxidation on muscle cell responses exposed to fish oil or nanoparticle form delivery. Key areas for investigation include the mechanisms of cellular uptake, the intracellular processing and utilization of nanoparticles, and the management of nanoparticle components within muscle cells. These studies will provide critical insights into optimizing the design and delivery of fish oil nanoparticles for improved cellular outcomes.

5.6 References

- Ahmed Laskar, A., & Younus, H. (2019). Aldehyde toxicity and metabolism: The role of aldehyde dehydrogenases in detoxification, drug resistance, and carcinogenesis. *Drug Metabolism Reviews*, 51, 42–64.
- Ahmed, A. A., Balogun, K. A., Bykova, N. V., & Cheema, S. K. (2014). Novel regulatory roles of omega-3 fatty acids in metabolic pathways: A proteomics approach. *Nutrition & Metabolism*, 11(1), 6.

- Ahsan, S. M., Rao, C. M., & Ahmad, M. F. (2018). Nanoparticle-protein interaction: The significance and role of protein corona. **Advances in Experimental Medicine and Biology**, 1048, 175–198.
- Allaman, I., Bélanger, M., & Magistretti, P. J. (2015). Methylglyoxal, the dark side of glycolysis. **Frontiers in Neuroscience**, 9.
- AlOkda, A., & Van Raamsdonk, J. M. (2023). Evolutionarily conserved role of thioredoxin systems in determining longevity. **Antioxidants**, 12(4), 944.
- Antoniotti, V., Bellone, S., Gonçalves Correia, F. P., Peri, C., Tini, S., Ricotti, R., ... Prodam, F. (2022). Calreticulin and PDIA3, two markers of endoplasmic reticulum stress, are associated with metabolic alterations and insulin resistance in pediatric obesity: A pilot study. **Frontiers in Endocrinology**, 13, 1003919.
- Baker, A. N., Hawker-Bond, G. W., Georgiou, P. G., Dedola, S., Field, R. A., & Gibson, M. I. (2022). Glycosylated gold nanoparticles in point-of-care diagnostics: From aggregation to lateral flow. **Chemical Society Reviews**, 51(16), 7238–7259.
- Barski, O. A., Tipparaju, S. M., & Bhatnagar, A. (2008). The aldo-keto reductase superfamily and its role in drug metabolism and detoxification. **Drug Metabolism Reviews**, 40(4), 553–624.
- Beijer, D., Agnew, T., Rack, J. G. M., Prokhorova, E., Deconinck, T., Ceulemans, B., ... Baets, J. (2021). Biallelic ADPRHL2 mutations in complex neuropathy affect ADP ribosylation and DNA damage response. **Life Science Alliance**, 4(11), e202101057.
- Bhattacharya, K., Weidenauer, L., Luengo, T. M., et al. (2020). The Hsp70-Hsp90 co-chaperone Hop/Stip1 shifts the proteostatic balance from folding towards degradation. **Nature Communications**, 11(1), 5975.
- Bin, L. (2016). Expression patterns and physiological roles of SH3BGR protein family as adaptor proteins. **Integrative Cancer Science and Therapeutics**, 3, 410–413.
- Bonilla, D. A., Kreider, R. B., Stout, J. R., Forero, D. A., Kerksick, C. M., Roberts, M. D., & Rawson, E. S. (2021). Metabolic basis of creatine in health and disease: A bioinformatics-assisted review. **Nutrients**, 13(4), 1238.
- Brackley, K. I., & Grantham, J. (2009). Activities of the chaperonin containing TCP-1 (CCT): implications for cell cycle progression and cytoskeletal organisation. **Cell Stress & Chaperones**, 14(1), 23–31.

- Brosio, G., Rossi, G., & Bochicchio, D. (2023). Nanoparticle-induced biomembrane fusion: Unraveling the effect of core size on stalk formation. **Nanoscale Advances**, 5(18), 4675–4680.
- Burillo, E., Jorge, I., Martínez-López, D., Camafeita, E., Blanco-Colio, L. M., Trevisan-Herraz, ... Martin-Ventura, J. L. (2016). Quantitative HDL proteomics identifies peroxiredoxin-6 as a biomarker of human abdominal aortic aneurysm. **Scientific Reports**, 6, 38477.
- Calder, P. C. (2015). Functional roles of fatty acids and their effects on human health. **JPEN Journal of Parenteral and Enteral Nutrition**, 39, 18S–32S.
- Caracciolo, G. (2015). Liposome-protein corona in a physiological environment: Challenges and opportunities for targeted delivery of nanomedicines. **Nanomedicine**, 11(3), 543–557.
- Chanburee, S., & Tiyafoonchai, W. (2017). Mucoadhesive nanostructured lipid carriers (NLCs) as potential carriers for improving oral delivery of curcumin. **Drug Development and Industrial Pharmacy**, 43(3), 432–440.
- Chang, C., & Nickerson, M. T. (2018). Stability and in vitro release behavior of encapsulated omega fatty acid-rich oils in lentil protein isolate-based microcapsules. **International Journal of Food Science and Nutrition**, 69(1), 12–23.
- Chen, L., Zhao, Z. W., Zeng, P. H., Zhou, Y. J., & Yin, W. J. (2022). Molecular mechanisms for ABCA1-mediated cholesterol efflux. **Cell Cycle**, 21(11), 1121–1139.
- Colberg, L., Cammann, C., Greinacher, A., & Seifert, U. (2020). Structure and function of the ubiquitin-proteasome system in platelets. **Journal of Thrombosis and Haemostasis**, 18(4), 771–780.
- Cui, L., Li, H., Xi, Y., Hu, Q., Liu, H., Fan, J., ... Lai, Y. (2022). Vesicle trafficking and vesicle fusion: mechanisms, biological functions, and their implications for potential disease therapy. **Molecular Biomedicine**, 3, 29.
- Dallacasagrande, V., & Hajjar, K. A. (2020). Annexin A2 in inflammation and host defense. **Cells**, 9(6), 1499.
- Defour, A., Medikayala, S., Van der Meulen, J. H., Hogarth, M. W., Holdreith, N., Malatras, A., ... Jaiswal, J. K. (2017). Annexin A2 links poor myofiber repair with

- inflammation and adipogenic replacement of the injured muscle. **Human Molecular Genetics**, 26(11), 1979–1991.
- Demonbreun, A. R., Quattrocelli, M., Barefield, D. Y., Allen, M. V., Swanson, K. E., & McNally, E. M. (2016). An actin-dependent annexin complex mediates plasma membrane repair in muscle. *Journal of Cell Biology*, 213(6), 705–718.
- Dmitrieff, S., & Nédélec, F. (2016). Amplification of actin polymerization forces. **Journal of Cell Biology**, 212(7), 763–766.
- Drescher, D. G., Drescher, M. J., Selvakumar, D., & Annam, N. P. (2023). Analysis of dysferlin direct interactions with putative repair proteins links apoptotic signaling to Ca^{2+} elevation via PDZD6 and FKBP8. **International Journal of Molecular Sciences**, 24(5), 4707.
- Ebstrup, M. L., Dias, C., Heitmann, A. S. B., Sønder, S. L., & Nylandsted, J. (2021). Actin cytoskeletal dynamics in single-cell wound repair. **International Journal of Molecular Sciences**, 22(19), 10886.
- Elechalawar, C. K., Hossen, M. N., McNally, L., Bhattacharya, R., & Mukherjee, P. (2020). Analysing the nanoparticle-protein corona for potential molecular target identification. **Journal of Controlled Release**, 322, 122–136.
- Foroozandeh, P., & Aziz, A. A. (2018). Insight into cellular uptake and intracellular trafficking of nanoparticles. **Nanoscale Research Letters**, 13, 339.
- Garibotto, G., Picciotto, D., Verzola, D., Valli, A., Sofia, A., Costigliolo, ... Esposito, P. (2023). Homocysteine exchange across skeletal muscle in patients with chronic kidney disease. **Physiological Reports**, 11(6), e15573.
- Gerling, C. J., Mukai, K., Chabowski, A., Heigenhauser, G. J. F., Holloway, G. P., Spriet, L. L., & Jannas-Vela, S. (2019). Incorporation of omega-3 fatty acids into human skeletal muscle sarcolemmal and mitochondrial membranes following 12 weeks of fish oil supplementation. **Frontiers in Physiology**, 10, 348.
- Gonzalez, C., Proudfoot, M., Brown, G., Korniyenko, Y., Mori, H., Savchenko, A., & Yakunin, A. (2006). Molecular basis of formaldehyde detoxification: Characterization of two S-formylglutathione hydrolases from *Escherichia coli*, FrmB and YeiG. **The Journal of Biological Chemistry**, 281(20), 14514–14522.

- Grady, M. E., Parrish, E., Caporizzo, M. A., Seeger, S. C., Composto, R. J., & Eckmann, D. M. (2017). Intracellular nanoparticle dynamics affected by cytoskeletal integrity. **Soft Matter**, 13(9), 1873–1880.
- Hajjar, K. A. (2015). The biology of annexin A2: From vascular fibrinolysis to innate immunity. **Transactions of the American Clinical and Climatological Association**, 126, 144–155.
- Hang, C., Song, Y., Li, Y., Zhang, S., Chang, Y., Bai, R., ... Cui, M. (2021). Knockout of MYOM1 in human cardiomyocytes leads to myocardial atrophy via impairing calcium homeostasis. **Journal of Cellular and Molecular Medicine**, 25(3), 1661–1676.
- Hang, T. T. T., Molee, W., & Khempaka, S. (2018). Linseed oil or tuna oil supplementation in slow-growing chicken diets: Can their meat reach the threshold of a “high in n-3 polyunsaturated fatty acids” product? **Journal of Applied Poultry Research**, 27(3), 389–400.
- Hitosugi, T., Zhou, L., Elf, S., Fan, J., Kang, H. B., Seo, J. H., ... Chen, J. (2012). Phosphoglycerate mutase 1 coordinates glycolysis and biosynthesis to promote tumor growth. **Cancer Cell**, 22(5), 585–600.
- Huang, L., Tepasamorndech, S., Kirschke, C. P., Newman, J. W., Keyes, W. R., Pedersen, T. L., & Dumnit, J. (2018). Aberrant fatty acid metabolism in skeletal muscle contributes to insulin resistance in zinc transporter 7 (znt7)-knockout mice. **Journal of Biological Chemistry**, 293(20), 7549–7563.
- Jackson, M. J., Pollock, N., Staunton, C., Jones, S., & McArdle, A. (2022). Redox control of signalling responses to contractile activity and ageing in skeletal muscle. **Cells**, 11(10), 1698.
- Jaiswal, J. K., Lauritzen, S. P., Scheffer, L., Sakaguchi, M., Bunkenborg, J., Simon, S. M., ... Nylandsted, J. (2014). S100A11 is required for efficient plasma membrane repair and survival of invasive cancer cells. **Nature Communications**, 5, 3795.
- Jannas-Vela, S., Espinosa, A., Candia, A. A., Flores-Opazo, M., Peñailillo, L., & Valenzuela, R. (2023). The role of omega-3 polyunsaturated fatty acids and their lipid mediators on skeletal muscle regeneration: A narrative review. **Nutrients**, 15(4), 871.

- Jones, P. D., & Ricke, S. C. (2021). Challenges in developing nano-delivery systems for enhancing the bioavailability of nutrients and phytochemicals: A review. **Critical Reviews in Food Science and Nutrition**, 61(5), 790-804.
- Karan, B. M., Little, K., Augustine, J., Stitt, A. W., & Curtis, T. M. (2023). Aldehyde dehydrogenase and aldo-keto reductase enzymes: Basic concepts and emerging roles in diabetic retinopathy. **Antioxidants**, 12(7), 1466.
- Katsnelson, G., & Ceddia, R. B. (2020). Docosahexaenoic and eicosapentaenoic fatty acids differentially regulate glucose and fatty acid metabolism in L6 rat skeletal muscle cells. **American Journal of Physiology-Cell Physiology**, 319, C1120-C1129.
- Khosinklang, W., Kubota, S., Riou, C., Kaewsatun, P., Molee, A., & Molee, W. (2023). Omega-3 meat enrichment and L-FABP, PPARG, and LPL genes expression are modified by the level and period of tuna oil supplementation in slow-growing chickens. **Journal of Animal Science**, 101, skad267.
- Klein, G., Wojtkiewicz, P., Biernacka, D., Stupak, A., Gorzelak, P., & Raina, S. (2020). Identification of substrates of cytoplasmic peptidyl-prolyl cis/trans isomerases and their collective essentiality in Escherichia coli. **International Journal of Molecular Sciences**, 21(12), 4212.
- Krcmery, J., Camarata, T., Kulisz, A., & Simon, H. G. (2010). Nucleocytoplasmic functions of the PDZ-LIM protein family: New insights into organ development. **BioEssays: News and Reviews in Molecular, Cellular and Developmental Biology**, 32(2), 100–108.
- Kusunoki, C., Yang, L., Yoshizaki, T., Nakagawa, F., Ishikado, A., Kondo, M., ... Maegawa, H. (2013). Omega-3 polyunsaturated fatty acid has an anti-oxidant effect via the Nrf-2/HO-1 pathway in 3T3-L1 adipocytes. **Biochemical and Biophysical Research Communications**, 430(1), 225-230.
- Lalia, A. Z., Dasari, S., Robinson, M. M., Abid, H., Morse, D. M., Klaus, K. A., & Lanza, I. R. (2017). Influence of omega-3 fatty acids on skeletal muscle protein metabolism and mitochondrial bioenergetics in older adults. **Aging (Albany NY)**, 9(4), 1096-1129.

- Lamber, E. P., Guicheney, P., & Pinotsis, N. (2022). The role of the M-band myomesin proteins in muscle integrity and cardiac disease. **Journal of Biomedical Science**, 29, 18.
- Laville, E., Sayd, T., Morzel, M., Blinet, S., Chambon, C., Lepetit, J., ... Hocquette, J. F. (2009). Proteome changes during meat aging in tough and tender beef suggest the importance of apoptosis and protein solubility for beef aging and tenderization. **Journal of agricultural and food chemistry**, 57(22), 10755–10764.
- Lee, L., Karabina, A., Broadwell, L., & Leinwand, L. (2019). The ancient sarcomeric myosins found in specialized muscles. **Skeletal Muscle**, 9(1), 7.
- Lee, S. M., Lee, S. H., Jung, Y., Lee, Y., Yoon, J. H., Choi, J. Y., ... Kwon, K. S. (2020). FABP3-mediated membrane lipid saturation alters fluidity and induces ER stress in skeletal muscle with aging. **Nature Communications**, 11, 5661.
- Li, N., & Liu, X. (2020). Phosphoglycerate Mutase 1: Its Glycolytic and Non-Glycolytic Roles in Tumor Malignant Behaviors and Potential Therapeutic Significance. **Onco Targets and Therapy**, 13, 1787-1795.
- Li, Q., & Hoppe, T. (2023). Role of amino acid metabolism in mitochondrial homeostasis. **Frontiers in Cell and Developmental Biology**, 11, 1127618.
- Li, X., Ni, Q., He, X., Kong, J., Lim, S. M., Papoian, G. A., ... Jiang, Y. (2020). Tensile force-induced cytoskeletal remodeling: Mechanics before chemistry. **PLOS Computational Biology**, 16(6), e1007693.
- Liu, N., Tang, M., & Ding, J. (2020). The interaction between nanoparticles-protein corona complex and cells and its toxic effect on cells. **Chemosphere**, 245, 125624.
- Makia, N. L., Bojang, P., Falkner, K. C., Conklin, D. J., & Prough, R. A. (2011). Murine hepatic aldehyde dehydrogenase 1a1 is a major contributor to oxidation of aldehydes formed by lipid peroxidation. **Chemico-Biological Interactions**, 191, 278–287.
- Manson, A., Winter, T., & Aukema, H. M. (2023). Phospholipase A2 enzymes differently impact PUFA release and oxylipin formation ex vivo in rat hearts. **Prostaglandins, Leukotrienes and Essential Fatty Acids**, 191, 102555.

- Michalak, M. (2024). Calreticulin: Endoplasmic reticulum Ca(2+) gatekeeper. **Journal of Cellular and Molecular Medicine**, 28, e17839.
- Mol, M., Regazzoni, L., Altomare, A., Degani, G., Carini, M., Vistoli, G., & Aldini, G. (2017). Enzymatic and non-enzymatic detoxification of 4-hydroxynonenal: Methodological aspects and biological consequences. **Free Radical Biology and Medicine**, 111, 328–344.
- Monastyrskaya, K., Babiychuk, E. B., & Draeger, A. (2009). The annexins: spatial and temporal coordination of signaling events during cellular stress. **Cellular and Molecular Life Sciences**, 66(16), 2623–2642.
- Mott, A. C., Blaschka, C., Mott, A., & Falker-Gieske, C. (2024). Transcriptional profiling of the M. complexus in naked neck chickens suggests a direct pleiotropic effect of GDF7 on feathering and reduced hatchability. *BMC Genomics*, 25, 1092.
- Mulens-Arias, V. (2021). Dissecting the inorganic nanoparticle-driven interferences on adhesome dynamics. **Journal of Nanotheranostics**, 2(3), 174–195.
- Murgia, M., Nogara, L., Baraldo, M., Reggiani, C., Mann, M., & Schiaffino, S. (2021). Protein profile of fiber types in human skeletal muscle: A single-fiber proteomics study. **Skeletal Muscle**, 11, 24.
- Nabuurs, C. I., Choe, C. U., Veltien, A., Kan, H. E., van Loon, L. J., Rodenburg, R. J., ... Heerschap, A. (2013). Disturbed energy metabolism and muscular dystrophy caused by pure creatine deficiency are reversible by creatine intake. **Journal of Physiology**, 591(2), 571–592.
- Nemoto, T. K., Fukuma, Y., Itoh, H., Takagi, T., & Ono, T. (2006). A disulfide bridge mediated by cysteine 574 is formed in the dimer of the 70-kDa heat shock protein. **Journal of Biochemistry**, 139(4), 677–687.
- Önal Acet, B., Gül, D., Stauber, R. H., Odabaşı, M., & Acet, Ö. (2024). A review for uncovering the “protein-nanoparticle alliance”: Implications of the protein corona for biomedical applications. **Nanomaterials**, 14(10), 823.
- Ou, W. B., Luo, W., Park, Y. D., & Zhou, H. M. (2001). Chaperone-like activity of peptidyl-prolyl cis-trans isomerase during creatine kinase refolding. **Protein Science**, 10(11), 2346–2353.

- Park, J., Wu, Y., Suk Kim, J., Byun, J., Lee, J., & Oh, Y. K. (2024). Cytoskeleton-modulating nanomaterials and their therapeutic potentials. **Advanced Drug Delivery Reviews**, 211, 115362.
- Penning, T. M. (2015). The aldo-keto reductases (AKRs): Overview. *Chemico-Biological Interactions*, 234, 236–246.
- Prill, K., Carlisle, C., Stannard, M., Windsor Reid, P. J., & Pilgrim, D. B. (2019). Myomesin is part of an integrity pathway that responds to sarcomere damage and disease. **PLOS ONE**, 14, e0224206.
- Prislusky, M. I., Lam, J. G. T., Contreras, V. R., Ng, M., Chamberlain, M., Pathak-Sharma, S., ... Seveau, S. (2024). The septin cytoskeleton is required for plasma membrane repair. **EMBO Reports**, 25, 3870–3895.
- Qin, M., Zhang, J., Li, M., Yang, D., Liu, D., Song, S., ... Zhang, Q. (2020). Proteomic analysis of intracellular protein corona of nanoparticles elucidates nano-trafficking network and nano-bio interactions. **Theranostics**, 10(3), 1213–1229.
- Schulze, A., Tran, C., Levandovskiy, V., Patel, V., & Cortez, M. (2016). Systemic availability of guanidinoacetate affects GABAA receptor function and seizure threshold in GAMT-deficient mice. **Amino Acids**, 48(8), 2041–2047.
- Seo, H., Lee, I., Chung, H. S., Bae, G. U., Chang, M., Song, E., & Kim, M. J. (2016). ATP5B regulates mitochondrial fission and fusion in mammalian cells. **Animal Cells and Systems**, 20(3), 157–164.
- Simopoulos, A. P. (2008). The importance of the omega-6/omega-3 fatty acid ratio in cardiovascular disease and other chronic diseases. **Experimental Biology and Medicine**, 233(6), 674–688.
- Singhal, S. S., Singh, S. P., Singhal, P., Horne, D., Singhal, J., & Awasthi, S. (2015). Antioxidant role of glutathione S-transferases: 4-Hydroxynonenal, a key molecule in stress-mediated signaling. **Toxicology and Applied Pharmacology**, 289, 361–370.
- Soulage, C. O., Pelletier, C. C., Florens, N., Lemoine, S., Dubourg, L., Juillard, L., & Guebre-Egziabher, F. (2020). Two toxic lipid aldehydes, 4-hydroxy-2-hexenal (4-HHE) and 4-hydroxy-2-nonenal (4-HNE), accumulate in patients with chronic kidney disease. **Toxins (Basel)**, 12(9), 567.

- Stull, J. T., Kamm, K. E., & Vandenboom, R. (2011). Myosin light chain kinase and the role of myosin light chain phosphorylation in skeletal muscle. **Archives of Biochemistry and Biophysics**, 510(2), 120–128.
- Suh, J. H., Ho, C.-T., & Wang, Y. (2017). Evaluation of carbonyl species in fish oil: An improved LC–MS/MS method. **Food Control**, 78, 463–468.
- Sun, Y., Zhou, Y., Rehman, M., Wang, Y. F., & Guo, S. (2024). Protein corona of nanoparticles: Isolation and analysis. **Chem & Bio Engineering**, 1(9), 757–772.
- Suzuki, R., Nagata, K., Yumoto, F., Kawakami, M., Nemoto, N., Furutani, M., ... Tanokura, M. (2003). Three-dimensional solution structure of an archaeal FKBP with a dual function of peptidyl-prolyl cis-trans isomerase and chaperone-like activities. **Journal of Molecular Biology**, 328, 1149–1160.
- Syed, N. A., Bhatti, A., & John, P. (2023). Molecular link between Glo-1 expression and markers of hyperglycemia and oxidative stress in vascular complications of Type 2 diabetes mellitus. **Antioxidants (Basel)**, 12(9), 1663.
- Tachtsis, B., Camera, D., & Lacham-Kaplan, O. (2018). Potential roles of n-3 PUFAs during skeletal muscle growth and regeneration. **Nutrients**, 10(3), 309.
- Tian, C., Asghar, S., Wu, Y., Chen, Z., Jin, X., Yin, L., ... Xiao, Y. (2017). Improving intestinal absorption and oral bioavailability of curcumin via taurocholic acid-modified nanostructured lipid carriers. **International Journal of Nanomedicine**, 12, 7897–7911.
- Tomczyk, M. (2024). Omega-3 fatty acids and muscle strength—Current state of knowledge and future perspectives. **Nutrients**, 16(23), 4075.
- Tunki, L., Kulhari, H., Vadithe, L. N., Kuncha, M., Bhargava, S., Pooja, D., & Sistla, R. (2019). Modulating the site-specific oral delivery of sorafenib using sugar-grafted nanoparticles for hepatocellular carcinoma treatment. **European Journal of Pharmaceutical Sciences**, 137, 104978.
- Vistoli, G., De Maddis, D., Cipak, A., Zarkovic, N., Carini, M., & Aldini, G. (2013). Advanced glycoxidation and lipoxidation end products (AGEs and ALEs): An overview of their mechanisms of formation. **Free Radical Research**, 47, 3–27.
- Wakabayashi, Y., Tamura, Y., Kouzaki, K., Kikuchi, N., Hiranuma, K., Menuki, K., ... Nakazato, K. (2020). Acetaldehyde dehydrogenase 2 deficiency increases mitochondrial reactive oxygen species emission and induces mitochondrial

- protease Omi/HtrA2 in skeletal muscle. **American Journal of Physiology-Regulatory, Integrative and Comparative Physiology**, 318(4), R677–R690.
- Wang, H., Zhang, Z., Guan, J., Lu, W., & Zhan, C. (2021). Unraveling GLUT-mediated transcytosis pathway of glycosylated nanodisks. **Asian Journal of Pharmaceutical Sciences**, 16(1), 120–128.
- Wang, R., He, J., Xu, Y., & Peng, B. (2024). Impact of protein coronas on lipid nanoparticle uptake and endocytic pathways in cells. **Molecules**, 29(20), 4818.
- Wang, Y., Jin, S., Li, N., Liu, J., Shi, H., & Li, Y. (2015). Systematic study of stress-inducible protein 1 (Stip1) in the male reproductive system and its expression during stress response. **Gene**, 554(1), 58–63.
- Wangngae, S., Chansaenpak, K., Yostawonkul, J., Mahingsadet, K., Jinaphon, T., Molee, W., & Kamkaew, A. (2023). Lipid-based nanoparticles as a targeted delivery system in Korat chicken. **ACS Food Science & Technology**, 3(11), 1913–1919.
- Xu, H., Xu, Y., Liang, X., Wang, Y., Jin, F., Liu, D., Ma, Y., ... Zeng, W. (2013). Porcine skeletal muscle differentially expressed gene ATP5B: Molecular characterization, expression patterns, and association analysis with meat quality traits. **Mammalian Genome**, 24, 142–150.
- Yang, S., Wang, Y., & Zhang, X. (2019a). Curvature sculptured growth of plasmonic nanostructures by supramolecular recognition. **Physical Review Materials**, 3, 116002.
- Yang, X., Song, J., & Yan, L. J. (2019b). Chronic inhibition of mitochondrial dihydrolipoamide dehydrogenase (DLDH) as an approach to managing diabetic oxidative stress. **Antioxidants (Basel)**, 8(2), 32.
- Yeh, Y. C., Kim, S. T., Tang, R., Yan, B., & Rotello, V. M. (2014). Insulin-based regulation of glucose-functionalized nanoparticle uptake in muscle cells. **Journal of Materials Chemistry B**, 2, 4610–4614.
- Yin, H., Zhao, J., He, H., Chen, Y., Wang, Y., Li, D., & Zhu, Q. (2020). Gga-miR-3525 targets PDLIM3 through the MAPK signaling pathway to regulate the proliferation and differentiation of skeletal muscle satellite cells. **International Journal of Molecular Sciences**, 21(15), 5573.
- Yu, C., Zhan, X., Liu, C., Zhang, Z., Jiang, J., Xu, G., & Xue, J. (2020). A mechanism underlying sex-associated differences in ankylosing spondylitis: Troponin C2,

- fast skeletal type (TNNC2), and calcium signaling pathway. **Medical Science Monitor**, 26, e925179.
- Zhang, S., Cai, Z., & Li, H. (2023). AHNAK's roles in physiology and malignant tumors. **Frontiers in Oncology**, 13, 1258951.
- Zhang, Y., Li, L., & Wang, J. (2021). Role of ligand distribution in the cytoskeleton-associated endocytosis of ellipsoidal nanoparticles. **Membranes**, 11(12), 993.
- Zhang, Z., Zhang, L., Zhou, L., Lei, Y., Zhang, Y., & Huang, C. (2019). Redox signaling and unfolded protein response coordinate cell fate decisions under ER stress. **Redox Biology**, 25, 101047.
- Zhao, J. M., & Qi, T. G. (2021). The role of TXNL1 in disease: Treatment strategies for cancer and diseases with oxidative stress. **Molecular Biology Reports**, 48, 2929–2934.
- Zhen, Y., Radulovic, M., Vietri, M., & Stenmark, H. (2021). Sealing holes in cellular membranes. **The EMBO Journal**, 40(7), e106922